Influence of arbuscular mycorrhizal fungi and the expression of K^+/Cs^+ transporters on the accumulation of caesium by plants

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Abstract

Radiocaesium (^{134}Cs, ^{137}Cs) is of environmental concern because of its incorporation into the food chain and prolonged emission of harmful radiation. Plants take up caesium *via* cation transporters which cannot discriminate between radioactive and stable caesium (^{133}Cs). Around 80% of angiosperms live in symbiosis with arbuscular mycorrhizal (AM) fungi that deliver mineral nutrients to their hosts. Contrasting effects of AM fungi on caesium accumulation by plants have been reported. The ultimate aim of this thesis was to determine whether AM fungi reduced caesium accumulation in *Medicago truncatula* by down regulating the expression of plant genes encoding specific potassium transporters through improving potassium nutrition of their hosts.

Accumulation of potassium and stable caesium by non mycorrhizal and mycorrhizal *Medicago truncatula* was studied, and the effects of caesium and AM fungi on plant gene expression were investigated. In these experiments, shoot potassium concentrations of non mycorrhizal and mycorrhizal plants were identical. However, in some experiments AM associations decreased shoot caesium concentrations. These observations were also true for five other plant species studied. Colonisation of *Medicago truncatula* with *Glomus* sp. influenced expression of some genes encoding cation transport proteins, but the expression profile did not suggest improved potassium nutrition. The presence of caesium also affected the expression of several putative cation transporters, but the consequences of these changes are unknown. A reduced colonisation rate of *Medicago truncatula* by *Glomus intraradices* was observed at caesium concentrations that exist in the rhizosphere.

In conclusion, in these experiments, AM fungi did not improve plant potassium nutrition, and there was no evidence that AM fungi reduced caesium accumulation by down regulating expression of plant genes encoding potassium transporters. Although colonisation by AM fungi can reduce shoot caesium concentrations, this was not always observed. Thus, fungal inoculation cannot be relied upon to deliver crops with reduced radiocaesium concentrations.

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Declaration

I hereby declare that the following thesis is based on the results of investigation conducted by myself, and that this thesis is my own composition. This thesis has not in whole or any part been previously presented for a higher degree. Work other than my own is clearly stated in the text with references to the relevant researchers or their publications.

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Chapter 1:
General Introduction

1.1 CATION CHANNELS AND THE ACCUMULATION OF CAESIUM BY NON MYCORRHIZAL PLANTS

This Section 1.1 is based on White, P.J., Wiesel, L. and Broadley, M.R. (2010) Cation channels and the uptake of radiocaesium by plants. In: Demidchik, V. and Maathuis, F. (eds) Ion Channels and Plant Stress Responses, Springer, Dordrecht, pp 47–67.

1.1.1 Caesium

Caesium (Cs) is an alkali metal element with chemical properties similar to rubidium (Rb) and potassium (K). It is found naturally as the stable isotope ^{133}Cs, which may reach concentrations of 25 $\mu g\ g^{-1}$ dry soil and low micromolar concentrations in the soil solution (White and Broadley, 2000). Caesium is not required by plants and, although Cs^+ can perturb cellular biochemistry by competing with K^+ (Cline and Hungate, 1960; Kordan, 1987; Sheahan et al., 1993; Hasegawa, 1996; Hampton et al., 2004; Le Lay et al., 2006; Qi et al., 2008), it is rarely present at toxic concentrations in the natural environment (White and Broadley, 2000). However, two anthropogenic radioisotopes of Cs (^{134}Cs and ^{137}Cs) produced in nuclear reactors and thermonuclear explosions are of environmental concern (White and Broadley, 2000). These radioisotopes migrate rapidly in an aqueous environment, emit harmful β and γ radiation during their decay, have relatively long half-lives (2.06 and 30.17 years, respectively) and are rapidly incorporated into biological systems (White and Broadley, 2000). They enter the terrestrial food chain through plants, and their presence in foodstuffs impacts upon both health and commerce.

Large areas in Europe (Figure 1.1) and worldwide were contaminated with ^{137}Cs fallout from the accident at the nuclear power plant in Chernobyl in 1986 (Smith and Beresford, 2005). Agricultural land in Belarus, Russia and Ukraine is still contaminated by ^{137}Cs originating from the Chernobyl accident (Smith et al., 2000; Beresford et al., 2001). Two strategies are available to return this land to safe agricultural production. The first is to cleanse the soil of radiocaesium. The second is

to grow crops that do not accumulate radiocaesium in their edible portions. Since Cs accumulation by plants is a heritable trait (Payne et al., 2004), plants with extreme phenotypes could be developed in breeding programmes. Growing plants with an increased ability to accumulate ^{137}Cs accelerates the cleansing of contaminated soils (Entry et al., 1996; Dushenkov, 2003; White et al., 2003), whilst plants accumulating less ^{137}Cs in their edible tissues can be used to develop 'safer' crops (White and Broadley, 2000; White et al., 2003, 2004). Cultivation of safer crops complements other agricultural countermeasures to reduce the radiation dose to populations inhabiting areas contaminated by ^{137}Cs (Alexakhin, 1993; Beresford et al., 2001). A recent survey of over 130 potential countermeasures for managing land contaminated with radiocaesium suggested that selective crop breeding was one of only six strategies worthy of further exploration (http://www.strategy-ec.org.uk).

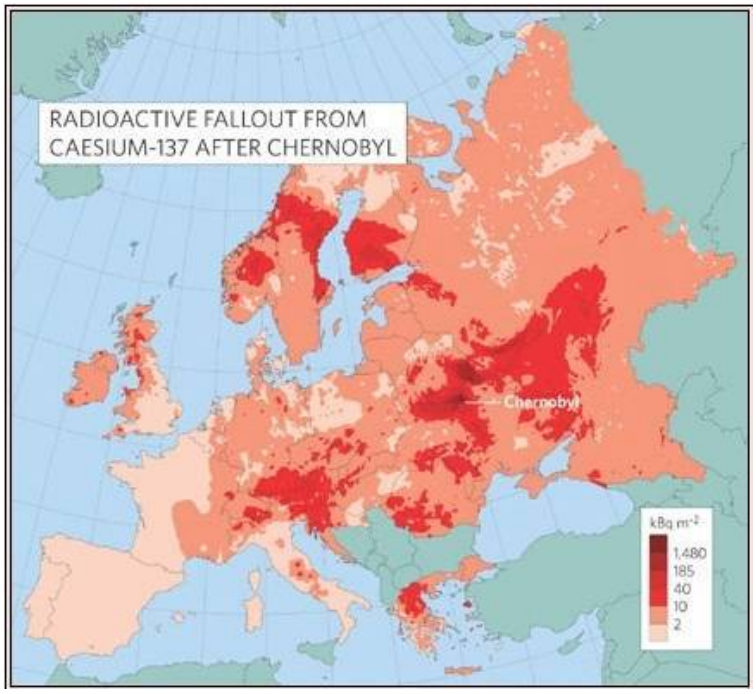

Figure 1.1: Radioactive fallout of ^{137}Cs in Europe after the accident at the nuclear power plant in Chernobyl in 1986 (with permission of Smith and Beresford, 2005).

Plants acquire Cs from the soil solution. It is taken up by epidermal and cortical cells of the root as the monovalent cation, Cs^+, which is transported symplastically, through the interconnected cytoplasms of root cells, across the root to the stele, where it is loaded into the xylem (White and Broadley, 2000; White et al., 2004). Only about 20% of the Cs delivered to the shoot *via* the xylem is retained by the shoot, and most is returned to the root *via* the phloem for recirculation within the

plant (Buysse et al., 1995; Hampton, 2005). Thus, it is argued that the physiological processes impacting most on Cs accumulation by plants are the uptake of Cs from the rhizosphere and the delivery of Cs to the xylem (White and Broadley, 2000; Hampton et al., 2005). These processes are catalysed by transport proteins in the plasma membrane of root cells, and control of their activities are, therefore, fundamental to the development of safer crops for soils contaminated by radiocaesium.

1.1.2 Historical studies

It has long been known that the fluxes of monovalent cations across lipid membranes must be catalysed by transport proteins. Based upon the chemical similarity of Cs^+, Rb^+ and K^+, the concentration-dependencies for their uptake, and competition between these cations for uptake by plant roots, researchers proposed that they shared the same uptake mechanisms: high affinity mechanisms at micromolar rhizosphere concentrations and low affinity mechanisms at millimolar rhizosphere concentrations (Collander, 1941; Epstein and Hagen, 1952; Menzel, 1954; Menzel and Heald, 1955; Sutcliffe, 1957; Bange and Overstreet, 1960; Middleton et al., 1960; Handley and Overstreet, 1961; Shaw and Bell, 1989, 1991; Zhu et al., 1999; Zhu and Smolders, 2000). In addition, it was proposed that the high-affinity mechanisms catalyzing Cs^+ uptake were unconditionally energy-dependent, whereas the low affinity mechanisms catalyzing Cs^+ uptake could occur through nonspecific cation channels utilizing the Cs^+ electrochemical gradient alone (Bange and Overstreet, 1960; Shaw and Bell, 1989). However, molecular mechanisms of cation transport cannot be inferred solely from kinetic parameters: it is well known that inward-rectifying K^+ channels catalyse K^+ influx to plant cells from solutions with extremely low K^+ concentrations, provided there is a supporting electrochemical gradient, and that H^+/K^+ cotransporters contribute to K^+ influx to plant cells across a wide range of extracellular K^+ concentrations (White and Broadley, 2000; Gierth and Mäser, 2007; Britto and Kronzucker, 2008; Karley and White, 2009). The uptake of Cs^+ by plant roots is not only reduced by the presence of monovalent cations in the rhizosphere, with an apparent effectiveness of $K^+ \geq Rb^+ > NH_4^+ > Na^+ \geq Li^+$ (Bange and Overstreet, 1960; Handley and Overstreet, 1961; Shaw and Bell, 1989; Hampton et al., 2004), but is also partially inhibited by millimolar concentrations of divalent cations, with an apparent effectiveness of $Ba^{2+} > Mg^{2+} > Ca^{2+}$ (Bange and Overstreet,

1960; Handley and Overstreet, 1961; Resnik et al., 1969; Sze and Hodges, 1977; Smolders et al., 1997; Broadley et al., 2001; Hampton et al., 2004) and trivalent cations, such as La^{3+} and Gd^{3+} (Broadley et al., 2001; Hampton et al., 2004).

1.1.3 Caesium transport proteins in root cells

Several types of transport protein are able to catalyse Cs^+ transport across the plasma membrane of root cells (Table 1.1). Inward-rectifying K^+ channels (KIRCs), voltage insensitive cation channels (VICCs), voltage-dependent Ca^{2+} channels (HACCs and DACCs) and 'high-affinity' K^+/H^+ symporters (KUPs) can catalyse Cs^+ influx to root cells, whilst outward-rectifying cation channels (KORCs and NORCs) can catalyse Cs^+ efflux from root cells (White and Broadley, 2000; White et al., 2004; Hampton et al., 2005; Qi et al., 2008). These transport proteins have contrasting abilities to discriminate between Cs^+ and K^+ and their relative abundance and activities vary with cell type, plant species and environmental conditions. It has, therefore, been postulated that differences in the complement of these transport proteins can account for the observations that both Cs^+ uptake and shoot Cs/K quotients vary (i) with plant species and (ii) with plant K status (White and Broadley, 2000; White et al., 2003, 2004; Hampton et al., 2005; Qi et al., 2008; Wiesel et al., 2008). Since Cs is not an essential element, nor is toxic to plants at the concentrations found in the natural environment, it is unlikely that there has been any evolutionary pressure to select for protein structures that permit or exclude Cs^+ transport. Thus, differences in the Cs/K selectivity of transport proteins are likely to have arisen serendipitously, as a consequence of the requirements for the transport of other, physiologically important, cations.

Several K^+ selective channels belonging to the 'Shaker' superfamily are present in the plasma membrane of *Arabidopsis* root cells (Table 1.1). These include the KIRCs AtAKT1, which appears to be the dominant K^+ channel involved in K^+ nutrition (Hirsch et al., 1998; Spalding et al., 1999; Broadley et al., 2001; Gierth et al., 2005; Xu et al., 2006), AtKC1/AtKAT3/AtAKT4, which appears to be a regulatory subunit for AtAKT1 (Reintanz et al., 2002; Pilot et al., 2003; Fizames et al., 2004), and (possibly) AtKAT1, and the KORCs AtSKOR, which is implicated in loading K^+ into the xylem for transport to the shoot (Gaymard et al., 1998), and AtGORK, which is present in cells throughout the root, where it is thought to be involved in osmotic regulation and the maintenance of a negative cell membrane potential (Ivashikina et al., 2001;

Reintanz et al., 2002; Fizames et al., 2004). Orthologs of genes encoding these channels have been found in roots of all plant species studied to date (Zimmermann and Chérel, 2005; Ashley et al., 2006; Gambale and Uozumi, 2006; Lebaudy et al., 2007). Although KIRCs are permeable to Cs^+, they transport little Cs^+ into root cells because increasing extracellular Cs^+ reduces cation permeation through them (Wegner and Raschke, 1994; Maathuis and Sanders, 1995; White and Lemtiri-Chlieh, 1995; Bregante et al., 1997; White, 1997; White and Broadley, 2000). The KORCs are also permeable to Cs^+ and are relatively insensitive to inhibition by extracellular Cs^+ (Maathuis and Sanders, 1995; Roberts and Tester, 1995, 1997; White and Lemtiri-Chlieh, 1995; Vogelzang and Prins, 1995; White, 1997; Gaymard et al., 1998), although there is evidence that KORCs are inhibited by cytoplasmic Cs^+ in a voltage-dependent manner (Maathuis and Sanders, 1995).

Caesium-permeable VICCs in the plasma membrane of root cells have been characterized using a variety of electrophysiological techniques. These channels are a subset of the non-specific cation channels (NSCCs; Demidchik et al., 2002b; Demidchik and Maathuis, 2007). They were first observed as a 'leak conductance' in the plasma membrane of green algae (Yurin et al., 1991; Demidchik et al., 1997). Their counterparts in higher plants were initially characterized following incorporation of plasma membrane vesicles from rye roots into artificial planar lipid bilayers (White and Tester, 1992) and their presence was subsequently confirmed in protoplasts from rye roots (White and Lemtiri-Chlieh, 1995). Since then they have been observed in plasma membrane fractions from wheat roots (Davenport and Tester, 2000; White, 2005) and appear to be ubiquitous in protoplasts from plant roots (White, 1997, 1999; Roberts and Tester, 1997; Buschmann et al., 2000; Maathuis and Sanders, 2001; Demidchik and Tester, 2002; Demidchik et al., 2002a,b; Volkov and Amtmann, 2006; Demidchik and Maathuis, 2007). It is thought that VICCs are encoded by members of the cyclic-nucleotide gated channel (*CNGC*) and glutamate receptor (*GLR*) gene families (White and Broadley, 2000; Davenport, 2002; Demidchik et al., 2002b; White et al., 2002, 2004; Talke et al., 2003; Hampton et al., 2005; Demidchik and Maathuis, 2007; Kaplan et al., 2007; Roy et al., 2008), most of which are expressed in roots (Table 1.1). Direct evidence that AtCNGCs and AtGLRs transport Cs^+ is scarce. However, both AtCNGC2 and AtCNGC4 mediated cyclic-nucleotide-dependent Cs^+ influx when expressed in oocytes (Leng et al., 2002; Balagué et al., 2003), the expression of *AtCNGC10* in *Escherichia coli* LB650 (*ΔtrkH*, *ΔtrkG*) resulted in Cs toxicity (Li et al.,

2005), and the addition of cAMP to inside-out membrane patches from protoplasts of *Arabidopsis* root cells reduced the activity of VICCs permeable to Cs^+ (Maathuis and Sanders, 2001). Similarly, AtGLR3.4 mediated Cs^+ influx when expressed in oocytes (Meyerhoff et al., 2005) and glutamate-activated, voltage-independent Cs^+ currents sensitive to quinine, La^{3+} and Gd^{3+} have been recorded in protoplasts from *Arabidopsis* root cells (Demidchik et al., 2002b, 2004). This pharmacological profile is consistent with that of GLR-mediated phenomena in plants (White et al., 2002).

Although NORCs transport Cs^+ (Wegner and Raschke, 1994) they are unlikely to contribute significantly to Cs^+ efflux across the plasma membrane, since they open only at extremely positive membrane potentials and unphysiologically high cytosolic Ca^{2+} concentrations (Wegner and Raschke, 1994; White, 1997; Wegner and De Boer, 1997). Similarly, although DACCs are permeable to Cs^+ (White, 2000, 2005; White et al., 2002), it is thought that the Ca^{2+} concentrations found in the rhizosphere will prevent Cs^+ permeating these channels (White and Broadley, 2000). No genes encoding DACCs are known for certain. One candidate in some plant species appears to be *TPC1* (Hashimoto et al., 2005), although AtTPC1 is present in the tonoplast of *Arabidopsis* (Peiter et al., 2005; Ranf et al., 2008; Gradogna et al., 2009). Several HACCs have been recorded in the plasma membrane of root cells (White, 2000; Demidchik et al., 2002a, 2007; White et al., 2002; Foreman et al., 2003; Miedema et al., 2008). The HACCs are thought to be encoded by members of the annexin gene family, all of which are expressed in roots (Clark et al., 2001; White et al., 2002; Mortimer et al., 2008). The permeability to Cs^+ of neither HACCs nor annexins appears to have been confirmed. However, a Cs^+-permeable, hyperpolarisation-activated NSCC activated by reactive oxygen species has been observed in the plasma membrane of protoplasts of *Arabidopsis* root cells (Demidchik et al., 2003).

The 'high-affinity' K^+/H^+ symporters present in the plasma membrane of root cells are encoded by members of the KUP gene family (Table 1.1). These transporters augment K^+ uptake by roots of K-starved plants (Zimmermann and Chérel, 2005; Amtmann et al., 2006; Gierth et al., 2005; Ashley et al., 2006; Rodríguez-Navarro and Rubio, 2006; Gierth and Mäser, 2007; Grabov, 2007). In *Arabidopsis*, most genes encoding AtKUPs are expressed in roots, with *AtHAK5* and, occasionally, *AtKUP3* being induced by K starvation (Kim et al., 1998; Maathuis et al., 2003; Ahn et al., 2004; Armengaud et al., 2004; Hampton et al., 2004, 2005; Shin and Schachtman, 2004; Gierth et al., 2005; Zimmermann and Chérel, 2005; Qi et al.,

2008). Although the membrane locations of most KUPs are unknown, it has been observed that AtHAK5 is present in the plasma membrane of root cells (Qi et al., 2008). Plant KUPs are expected to transport Cs^+, as do their homologues from fungi and bacteria (White and Broadley, 2000), but this has rarely been tested. However, the expression of a modified *AtHAK5*, with a leucine changed to a histidine at position 776, in a mutant yeast strain (CY162: Δ*trk1*,Δ*trk2*) with reduced K^+ uptake allows it to accumulate both K^+ and Cs^+ (Rubio et al., 2000; Qi et al., 2008), and Cs uptake and accumulation by *Arabidopsis* parallels the expression of *AtHAK5* (Hampton et al., 2004; Qi et al., 2008). Similarly, heterologous expression of barley, rice or pepper orthologs of *AtHAK5* (*HvHAK1*, *OsHAK1*, *CaHAK1*) promotes Cs^+ uptake in yeast, and their expression in roots of K-starved plants is correlated with increased high-affinity Cs^+ uptake (Santa-María et al., 1997; Rubio et al., 2000; Bañuelos et al., 2002; Martínez-Cordero et al., 2005). It has been shown recently, that *AtKUP/HAK/KT9* expressed in a K transport-deficient mutant of *E. coli* mediated Cs^+ uptake (Kobayashi et al., 2010). The 'high-affinity' K^+/H^+ symporters are characteristically inhibited by NH_4^+ (Spalding et al., 1999; Santa-María et al., 2000; Bañuelos et al., 2002; Martínez-Cordero et al., 2005; Nieves-Cordones et al., 2007; Fulgenzi et al., 2008; Qi et al., 2008), and monovalent cations compete for transport sites (White and Broadley, 2000).

Caesium influx to the vacuole is likely to be catalysed by cation/H^+ antiporters, whereas Cs^+ release from vacuoles probably occurs through Cs^+-permeable cation channels. Members of the CPA cation/H^+ antiporter family, which in *Arabidopsis* comprises eight *AtNHX* genes, 28 *AtCHX* genes, six *AtKEA* genes and two *AtNHD* genes resembling *NhaD*, are likely to catalyse Cs^+ transport into vacuoles of root cells, although this has not been proven (Sze et al., 2004; Zimmermann and Chérel, 2005; Ashley et al., 2006; Pardo et al., 2006; Gierth and Mäser, 2007). In *Arabidopsis*, AtNHX1, AtNHX2, AtNHX3, AtNHX4 and AtNHX5 have been located in the tonoplast of root cells (Aharon et al., 2003; Sze et al., 2004; Pardo et al., 2006), AtCHX17 is located in the endomembranes of epidermal and cortical cells of the mature root and its expression is upregulated by K-starvation (Cellier et al., 2004; Sze et al., 2004; Pardo et al., 2006), AtCHX20 is located in endomembranes of the root cap (Padmanaban et al., 2007), and *AtKEA1* is also expressed in roots (Sze et al., 2004). Indirect assays based on the ability of cations to dissipate a pH gradient held in liposomes containing AtNHX1, which is found in the tonoplast of root cells,

or LeNHX2, an ortholog of AtNHX5 that is present in Golgi and pre-vacuolar compartments of tomato roots, suggest that these proteins transport Cs^+ but at lower rates than K^+ (Venema et al., 2002, 2003).

The electrical activities of several distinct Cs^+-permeable cation channels have been recorded in vacuoles from root cells, of which the two most frequently observed are the tonoplast SV and FV channels (Lebaudy et al., 2007; Pottosin and Schönknecht, 2007). The gene encoding the *Arabidopsis* SV channel appears to be *AtTPC1* (Peiter et al., 2005; Ranf et al., 2008; Gradogna et al., 2009), but an intracellular location of AtTPC1 orthologs is not observed in all plant species (Hashimoto et al., 2005). The SV channel has a significant permeability to Cs^+ (White, 2000). The genetic identity of the FV channel is not yet known (Demidchik and Maathuis, 2007). It is possible that Cs^+ fluxes across the tonoplast might be mediated by cation channels encoded by members of the tandem pore K^+ channel (*TPK/KCO*) and Kir-like (*KCO3*) gene families (Véry and Sentenac, 2003; Zimmermann and Chérel, 2005; Lebaudy et al., 2007). In *Arabidopsis*, *AtTPK1*, *AtTPK2*, *AtTPK3* (=*AtKCO6*), *AtTPK5* and *AtKCO3* are all expressed in roots and located at the tonoplast (Schönknecht et al., 2002; Zimmermann and Chérel, 2005; Voelker et al., 2006; Latz et al., 2007). However, *AtTPK1* appears to encode a channel that has little permeability to Cs^+ and resembles the K^+-selective, VK channel (Bihler et al., 2005; Gobert et al., 2007; Latz et al., 2007; Lebaudy et al., 2007). Intriguingly, some KUPs, such as OsHAK5, are also found in the tonoplast (Bañuelos et al., 2002), and it has been suggested that these cation/H^+ symporters might catalyse the efflux of monovalent cations from the vacuole (Rodríguez-Navarro and Rubio, 2006).

Table 1.1: Transport proteins able to catalyse Cs^+ fluxes across the plasma membranes of root cells. The putative *Arabidopsis* genes encoding these transporters, and evidence for their expression in root cells and regulation by K starvation (in parentheses), are indicated.

Transporter	Selectivity	Gene family	Expression pattern in root	Key references
KIRC	KIRC: P_{Cs}/P_K = 0.07-0.43	Shaker (7 members)	AtAKT1: Epidermis, cortex, endodermis. (Unaffected) AtKAT1: Vasculature. AtKC1: Root hairs, epidermis, cortex, endodermis. (Unaffected)	Wegner and Raschke (1994), Maathuis and Sanders (1995), White and Broadley (2000), Reintanz et al. (2002), Véry and Sentenac (2003), Maathuis et al. (2003), Pilot et al. (2003), White et al. (2004), Zimmermann and Chérel (2005)
KORC	KORC: P_{Cs}/P_K = 0.12-0.31 AtSKOR: P_{Cs}/P_K = 0.15	Shaker (2 members)	AtSKOR: Pericycle, xylem, parenchyma. (Transient decrease) AtGORK: Root hairs. (Unaffected)	Maathuis and Sanders (1995), Roberts and Tester (1997), Gaymard et al. (1998), White and Broadley (2000), Ivashikina et al. (2001), Reintanz et al. (2002), Very and Sentenac (2003), Pilot et al. (2003), Maathuis et al. (2003), White et al. (2004), Zimmermann and Chérel (2005), Volkov and Amtmann (2006)
NORC	$P_{Cs} = P_K$	Unknown	-	Wegner and Raschke (1994)
DACC	ScDACC: P_{Cs}/P_K = 0.85	Unknown	-	White (2000, 2005), White and Broadley (2000), White et al. (2002)
HACC	Unknown	Annexins (7 members)	All expressed in roots. AnnAt1: Throughout root. AnnAt2: Collet endodermis, initiating laterals, tip epidermis.	Clark et al. (2001), White et al. (2002), Mortimer et al. (2008)
H^+/K^+ symport	AtHAK5 [Cs]	AtKUP (13 members)	AtKUP1, AtKUP4, AtKUP5, AtKUP6, AtKUP7, AtKUP8, AtKUP10: Root. (Unaffected) AtKUP2: Growth zones. (Unaffected) AtKUP3: Root. (Increased) AtHAK5: Root. (Increased)	Rubio et al. (2000), Rigas et al. (2001), Elumalai et al. (2002), Maathuis et al. (2003), Ahn et al. (2004), Armengaud et al. (2004), Fizames et al. (2004), Hampton et al. (2004, 2005), White et al. (2004), Gierth et al. (2005), Zimmermann and Chérel (2005), Amtmann et al. (2006), Ashley et al. (2006), Qi et al. (2008)

Table 1.1 (continued)

Transporter	Selectivity	Gene family	Expression pattern in root	Key references
VICC	ScVICC: P_{Cs}/P_K = 0.85 AtVICC: P_{Cs}/P_K ≈ 0.85 ThVICC: P_{Cs}/P_K = 0.43 AtCNGC2: G_{Cs}/G_K = 0.64	AtCNGC (20 members)	AtCNGC1, AtCNGC2, AtCNGC3, AtCNGC5, AtCNGC6, AtCNGC8, AtCNGC9, AtCNGC10, AtCNGC12, AtCNGC13, AtCNGC14, AtCNGC15, AtCNGC17, AtCN GC18, AtCNGC19: Root. (Unaffected)	White and Tester (1992), White and Lemtiri-Chlieh (1995), White and Broadley (2000), Maathuis and Sanders (2001), Demidchik and Tester (2002), Demidchik et al. (2002b), Leng et al. (2002), White et al. (2002, 2004), Balagué et al. (2003), Talke et al. (2003), Hampton et al. (2005), White (2005), Zimmermann and Chérel (2005), Volkov and Amtmann (2006), Gobert et al. (2006), Borsics et al. (2007), Christopher et al. (2007), Frietsch et al. (2007), Urquhart et al. (2007)
		AtGLR (20 members)	All expressed in roots. AtGLR1.1: Collet, lateral roots. (Unaffected) AtGLR1.2, AtGLR1.3: Root (Upregulated) AtGLR2.1: Throughout root, except tip. (Unaffected) AtGLR2.3, AtGLR2.4, AtGLR2.8: Root. (Unaffected) AtGLR3.1: Vasculature. AtGLR3.2: Stele. AtGLR3.3, AtGLR3.5, AtGLR3.6: Root. (Unaffected)	Zhu et al. (2001), Chiu et al. (2002), Davenport (2002), Demidchik et al. (2002b, 2004), White et al. (2002, 2004), Hampton et al. (2004, 2005), Meyerhoff et al. (2005), Roy et al. (2008)

1.1.4 Molecular mechanisms for Cs uptake by roots of non mycorrhizal plants

1.1.4.1 K-replete plants

The kinetic parameters of proteins able to transport Cs^+ across the plasma membrane have been incorporated into a theoretical model to predict their contributions to Cs^+ influx to a stereotypical root cell (White and Broadley, 2000; Hampton et al., 2005). This model suggests that, under K-replete conditions, (i) Cs^+ influx through KIRCs is negligible, (ii) VICCs mediate most (30 to 90%) Cs^+ influx, with KUPs mediating the remainder, and (iii) KORCs load Cs^+ into the xylem. These predictions have been tested using *Arabidopsis*. First, the pharmacology of Cs^+ influx to roots of intact *Arabidopsis* was compared with that of transport proteins that could mediate Cs^+ influx (White and Broadley, 2000; Broadley et al., 2001; Hampton et al., 2004; Qi et al., 2008). Second, Cs accumulation by mutants lacking specific transport proteins was assayed, with the expectation that mutants lacking transport proteins mediating Cs^+ influx to roots would have reduced Cs^+ uptake and shoot Cs concentrations (Broadley et al., 2001; White et al., 2004; Hampton et al., 2005; Qi et al., 2008). Third, genetic loci impacting on Cs^+ accumulation in K-replete plants were identified (Payne et al., 2004; Kanter et al., 2010).

The prediction that VICCs catalyse significant Cs^+ influx to root cells is supported by the observation that both VICCs and Cs^+ uptake by roots of K-replete plants, are partially inhibited by submillimolar concentrations of Gd^{3+}, La^{3+}, Ba^{2+}, Mg^{2+} and Ca^{2+}, but not by tetraethylammonium (TEA^+) or 10 μM Br-cAMP (White and Lemtiri-Chlieh, 1995; White, 1997, 1999; White and Broadley, 2000; Broadley et al., 2001; Demidchik et al., 2002a,b; Hampton et al., 2004, 2005; Volkov and Amtmann, 2006). The prediction, that Cs^+ influx to root cells through KIRCs is negligible, is supported by the observations that both Cs^+ influx to roots and shoot Cs concentrations of *Arabidopsis* lacking AtAKT1 are often greater that those of wild-type plants (Broadley et al., 2001; White et al., 2004; Qi et al., 2008). Two explanations for the increased Cs uptake in *akt1* mutants have been suggested: (a) that the expression of genes encoding Cs^+-permeable transporters contributing to cellular K-homeostasis, such as AtHAK5, are upregulated in plants lacking AtAKT1, which is consistent with transcriptional analyses of *akt1* mutants (Zimmerman and Chérel, 2005; Qi et al., 2008), and (b) that the loss of AtAKT1 results in a more

negative cell membrane potential and, thereby, increases the activity of other Cs^+-permeable transporters (White et al., 2004).

Evidence that AtCNGCs underlie the VICC-mediated Cs^+ influx to roots of K-replete plants is based on measurements of Cs accumulation by *Arabidopsis* mutants lacking individual AtCNGCs. However, although some *Arabidopsis* mutants lacking AtCNGCs, such as *cngc2*, *cngc3*, *cngc16*, *cngc19* and *cngc20*, have lower shoot Cs concentrations than wild-type plants, mutants lacking other AtCNGCs, such as *cngc1*, *cngc9*, *cngc10* and *cngc12*, have greater shoot Cs concentrations than wild-type plants (White et al., 2004; Hampton et al., 2005). Again, it has been suggested that increased Cs accumulation in *Arabidopsis* mutants lacking particular AtCNGCs is a consequence of functional compensation in gene expression (White et al., 2004; Hampton et al., 2005). Thus, the expression of genes encoding plasma membrane K^+ transporters might be altered to compensate for the absence of AtCNGCs that contribute significantly to cellular K homeostasis and/or the expression of genes encoding Ca^{2+} transporters might be altered to compensate for the absence of AtCNGCs that contribute to cytoplasmic Ca^{2+}-homeostasis or intracellular Ca^{2+} signalling (White et al., 2004; Hampton et al., 2005). This hypothesis is consistent with the upregulation of genes encoding AtKUPs in the *cngc4* mutant (Hampton, 2005), and the observation that a greater fraction of Cs^+ influx to roots of *cngc1* and *cngc4* mutants is inhibited by extracellular NH_4^+ than in wild-type plants (Hampton et al., 2005). Interestingly, only the lack of AtCNGC1 decreased shoot K concentration significantly, which may attest to functional compensation by other K^+ transport proteins to maintain K^+ homeostasis in mutants lacking other AtCNGCs, and shoot Ca concentration was not affected by the absence of any AtCNGC (Hampton et al., 2005). *Arabidopsis* mutants lacking AtHAK5 (*hak5-1*, *hak5-2*) or AtKUP4 (*trh1*), and *Arabidopsis* mutants with aberrant AtKUP2 activity (*shy3.1*), have lower shoot Cs concentrations than wild-type plants (White et al., 2004; Qi et al., 2008). This is consistent with the prediction that KUPs catalyse Cs^+ influx to root cells. The prediction that Cs^+ is delivered to the xylem by a KORC, AtSKOR, is supported by the observation that shoot Cs concentrations are generally reduced in the *skor* mutant (White et al., 2004).

When the L*er* x Col genetic mapping population of *Arabidopsis* was grown on agar containing subtoxic levels of Cs, four chromosomal loci (QTL) impacting on shoot Cs concentration were identified, accounting for >80% of the genetic

contribution to the trait variation (Payne et al., 2004). These QTL were located on Chromosomes I, II, IV and V. Significantly, the QTL on the top of Chromosomes I and V co-localised with QTL impacting shoot Cs concentration in the L*er* x CVI genetic mapping population of *Arabidopsis* (Payne et al., 2004). A cursory glance at these chromosomal regions reveals the presence of genes encoding putative Cs^+ transporters. For example, genes encoding a putative plasma membrane K^+ channel (*AtTPK4*) on Chromosome I and a putative plasma membrane VICC (*AtGLR3.1*) on Chromosome II are found within a genomic region of 100,000 bp (c. 25 genes) on either side of the marker where a significant allelic effect on shoot Cs concentration was observed in the L*er* x Col population (Payne et al., 2004). Using an F_2 population of the accessions Sorbo and Sq-1 from *A. thaliana* QTL impacting on shoot Cs concentration on chromosomes I and V were identified (Kanter et al., 2010). Genes encoding putative Cs^+ transport proteins (*AtCHX16* on chromosome I and *AtCNGC1* on chromosome V) lay within QTL peaks (Kanter et al., 2010). The alleles of *AtCNGC1* of the accessions Sorbo and Sq-1 show polymorphism because they differ in five amino acids. Sequencing of the top ten accessions with high and low Cs^+ concentrations revealed that six high accumulating accessions and one low accumulating accession had the high accumulator Sorbo-type *CNGC1* allele (Kanter et al., 2010). The authors concluded that these results gave independent support for the involvement of CNGC1 in Cs^+ accumulation but other genes must play important roles too (Kanter et al., 2010).

1.1.4.2 K-starved plants

The intrinsic cationic selectivity of KIRCs, KUPs and VICCs differs (Table 1.1), and the fluxes of Cs^+ and K^+ that they catalyse are influenced uniquely by both the absolute and relative concentrations of these cations in the rhizosphere (White and Broadley, 2000). The expression of genes encoding these transporters is also affected differently by plant K status (Table 1.1). White et al. (2004) suggested that these phenomena could account for: (i) the lack of correlation between the shoot Cs:K ratio and the Cs^+:K^+ ratio in the soil solution when plants were grown in media with contrasting K^+ concentrations (Cline and Hungate, 1960; Smolders et al., 1996a,b), (ii) differences in the relative uptake of Cs^+ and K^+ by plants of different K-status (e.g. Qi et al., 2008) and (iii) increased Cs^+ uptake and accumulation by K-starved plants (e.g. Zhu and Smolders, 2000; Hampton et al., 2004; Qi et al., 2008).

In *Arabidopsis*, K-starvation, but not Cs-toxicity, increases the expression of *AtHAK5*, occasionally *AtKUP3*, and both *AtGLR1.2* and *AtGLR1.3* in roots (Kim et al., 1998; Maathuis et al., 2003; Ahn et al., 2004; Armengaud et al., 2004; Hampton et al., 2004, 2005; Shin and Schachtman, 2004; Gierth et al., 2005; Cao et al., 2008; Qi et al., 2008). Potassium starvation also reduces the expression of *AtSKOR* (Maathuis et al., 2003; Pilot et al., 2003), but rarely affects the expression of genes encoding KIRCs, CNGCs or TPK/KCOs in *Arabidopsis* roots (Maathuis et al., 2003; Pilot et al., 2003; Hampton et al., 2004, 2005; Shin and Schachtman, 2004; White et al., 2004; Zimmermann and Chérel, 2005). The increased expression of *AtKUP*s, and in particular *AtHAK5*, results in an increased capacity for Cs^+ uptake, and changes in the pharmacology of Cs^+ uptake by roots of K-starved plants (Hampton et al., 2004, 2005; Qi et al., 2008). The fraction of Cs^+ uptake inhibited by NH_4^+ is greater in K-starved *Arabidopsis* than in K-replete *Arabidopsis*, which is consistent with the pharmacology of KUPs (Bañuelos et al., 2002; Martínez-Cordero et al., 2005; Nieves-Cordones et al., 2007; Fulgenzi et al., 2008; Qi et al., 2008) and the hypothesis that AtKUPs mediate more Cs^+ influx to roots of K-starved plants (Hampton et al., 2004, 2005; Qi et al., 2008). Thus, during K-starvation, K^+ uptake by *Arabidopsis* roots changes from being dominated by AtAKT1 to being dominated by AtHAK5, whilst Cs^+ uptake changes from being dominated by VICCs to being dominated by AtHAK5. This results not only in greater Cs accumulation, but also in a greater Cs/K quotient, in tissues of K-starved plants.

1.2 ARBUSCULAR MYCORRHIZA AND THEIR INFLUENCE ON CAESIUM ACCUMULATION BY PLANTS

1.2.1 Arbuscular mycorrhiza

Arbuscular mycorrhiza (AM) is a symbiosis between a wide range of host plants and obligate symbiotic fungi. Arbuscular mycorrhiza is the most common form of mycorrhiza and is developed by members of most families of angiosperms and gymnosperms as well as sporophytes of Pteridophyta and Lycopodiophyta (Smith and Read, 2008). Some free-living gametophytes of Pteridophyta and of Marchantiophyta are also known to form AM symbioses (Bonfante and Genre, 2008; Smith and Read, 2008). Although AM are mainly found in herbaceous species they are also found in tree species. An analysis of mycorrhizal literature with data of 336

plant families revealed that AM occurs in 74% of all angiosperm species and 8% of the angiosperms belong to families that have both AM and non mycorrhizal species (Brundrett, 2009). Angiosperm families that contain mainly non mycorrhizal plants are Amaranthaceae, Brassicaceae, Capparaceae, Caryophyllaceae, Chenopodiaceae, Cyperaceae, Molluginaceae, Papaveraceae, Polygonaceae, Portulacaceae, Urticaceae and Zygophyllaceae (Smith and Read, 2008; Brundrett, 2009). Arbuscular mycorrhiza can be found in nearly all ecosystems including deserts and tropical rainforest (Strack et al., 2003; Smith and Read, 2008). The fungi forming AM belong to the monophyletic phylum of Glomeromycota and probably diverged from the same common ancestor as Ascomycota and Basidiomycota (Schüßler et al., 2001). Glomeromycota are haploid, asexual fungi that reproduce clonally (Harrison, 1999; Bonfante and Genre, 2008; Smith and Read, 2008). Anastomosis of coenocytic hyphae allows the exchange of nuclei, but has not been observed between different geographic isolates of the same species or between different species (Parniske, 2008; Smith and Read, 2008). Glomeromycota are multinucleate and contain between 700 and 35000 nuclei per spore (Hosny et al., 1998). Around 150 Glomeromycota species have been described (Schüßler et al., 2001) but high variability in genetic composition within and between spores of a single fungal species exist (Harrison, 1999; Smith and Read, 2008). Arbuscular mycorrhiza probably evolved around 400–460 million years ago and played a crucial role in the colonisation of land by plants (Pirozynski and Malloch, 1975; Redecker et al., 2000). A wide range of plant and fungus combinations exist (Smith and Read, 2008). Klironomos (2000) tested several plant and fungal combinations for compatibility and concluded that AM fungi are not host specific. However, many fungal species cannot be successfully raised in pot cultures, possibly because the appropriate host plant species or environmental requirements have not been met (Smith and Read, 2008) and host preferences seem to play an important role in natural ecosystems (Parniske, 2008).

Arbuscular mycorrhizal fungi can colonise roots *via* hyphae, *via* infected root fragments or *via* spores (Requena et al., 2007; Smith and Read, 2008). Infection *via* an existing symbiosis seems to be the main method of colonisation in most habitats (Smith and Read, 2008). However, infection *via* spores also occurs frequently. Under appropriate soil conditions of hydration and temperature, fungal spores germinate even if no host plant is present. Limited hyphal growth occurs for two to three weeks and several nuclei move into the developing hyphae. If no host root is found, hyphal

growth stops after two to four weeks and most of the cytoplasm of the hyphae including nuclei is retracted into the spore (Bécard et al., 2004; Requena et al., 2007; Smith and Read, 2008). It has been demonstrated that spores of *Gigaspora gigantea* can germinate up to nine times in the absence of a host plant (Koske, 1981). If a host plant is present increased hyphal growth occurs. The development of the AM symbiosis requires an exchange of signalling molecules between plant and fungus (Figure 1.2). Plant roots produce 'branching factors' that induce fungal genes involved in mitochondrial activity and branching of hyphae (Bécard et al., 2004; Parniske, 2008; Smith and Read, 2008). Some of these compounds have recently been identified as strigolactones (Besserer et al., 2006; Gianinazzi-Pearson et al., 2006; Reinhardt, 2007; Requena et al., 2007; Parniske, 2008). These precolonisation events depend on plant phosphorus (P) status. Exudates from plants of low P status stimulate hyphal growth and branching whereas exudates from plants of high P status reduce stimulation of spore germination and hyphal growth (Vierheilig, 2004). Flavonoid levels have been proposed to be responsible for the different responses. At high P levels, alfalfa (*Medicago sativa*) accumulated medicarpin that has been reported to inhibit germination and hyphal growth (Guenuone et al., 2001; Vierheilig, 2004). As response to plant root exudates, the fungus produces Myc factors that trigger host gene expression (Bécard et al., 2004; Parniske, 2008). Certain elements of the signal transduction are common to both AM and rhizobial symbioses (Parniske, 2008; Smith and Read, 2008). Myc factors seem to be similar to Nod factors which are involved in the development of the rhizobial symbiosis in legumes (Imaizumi-Anraku et al., 2005; Gianinazzi-Pearson et al., 2006).

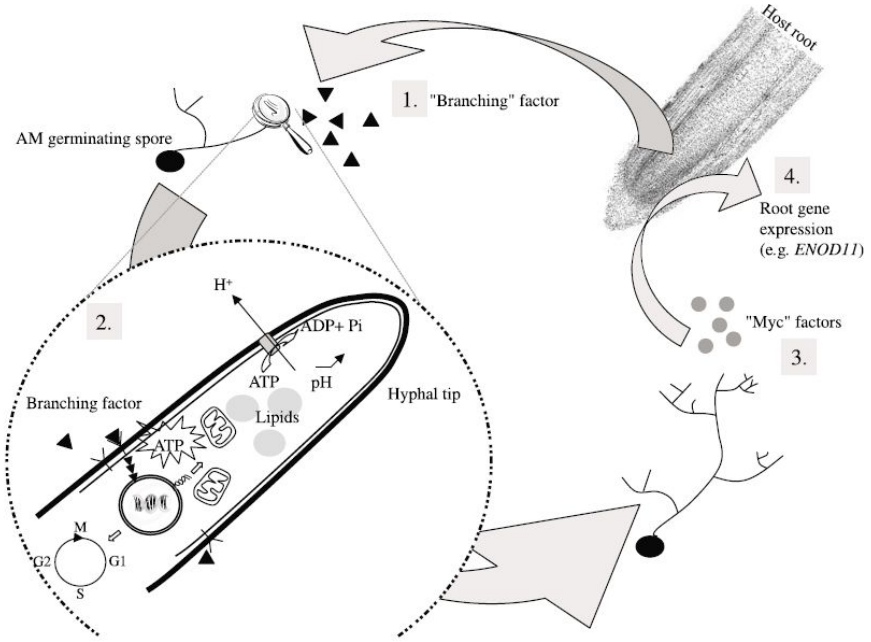

Figure 1.2: Model of early stages of the development of the arbuscular mycorrhizal symbiosis. The host root produces 'branching factors' [1] which trigger growth and branching of AM fungal hyphae. Fungal metabolism is activated [2] and the AM fungus produces Myc factors [3]. These factors lead to the activation of root gene expression necessary for the development of the symbiosis [4] (Bécard et al., 2004). Reproduced with permission © 2008 NRC Canada or its licensors.

When a fungal hypha comes in contact with a host root an appressorium is formed (Smith and Read, 2008). Appressoria of AM fungi are inflated multinucleate structures which vary in shape and size depending on the fungal species (Giovannetti et al., 1993). Appressoria formation requires topographical and biochemical signals (Bécard et al., 2004) which lie within the epidermal cell wall (Harrison, 1999; Smith and Read, 2008). Appressoria are only formed on roots of host plants (Giovannetti et al., 1993; Harrison, 1997, 1999; Smith and Read, 2008). Arbuscular mycorrhizal fungi produce cell wall degrading enzymes, which might help penetrating the host cell wall (Harrison, 1999). After the formation of an appressorium by the fungus, the plant cell produces a prepenetration apparatus (PPA) due to chemical or mechanical stimulation (Genre et al., 2005; Bonfante and Genre, 2008). Nuclear migration is initiated from a position directly below the appressorium and the nucleus migrates across the epidermal cell creating a column of cytoplasm (Figure 1.3). The column defines the future path taken by the infection hyphae (Genre et al., 2005; Bonfante and Genre, 2008). Arbuscular mycorrhizal fungi generally colonise cortical cells but do not colonise the vascular system or root meristems (Smith and Read, 2008).

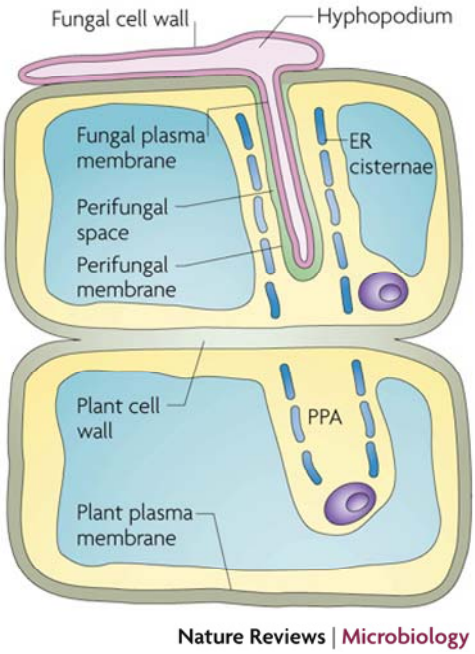

Figure 1.3: Model of the formation of a prepenetration apparatus (PPA) in a plant root. At contact of a fungal appressorium (hyphopodium) on a root cell, the cell forms a PPA by nuclear migration. The PPA is lined with endoplasmic reticulum cisternae and the fungal hypha follows the PPA column through the plant cell (Parniske, 2008). Reproduced with permission © 2008 Macmillan Publishers Ltd: NATURE REVIEWS Microbiology.

Two main morphological growth patterns of AM occur, the *Arum*- and *Paris*-type mycorrhiza (Harrison, 1999; Smith and Read, 2008). In the *Arum*-type of association, the fungus grows relatively rapidly by intercellular hyphae into the root cortex. In the root cortex, short side branches of the hyphae penetrate the plant cells and produce highly branched arbuscules (Smith and Read, 2008). In the *Paris*-type of association, the fungus colonises cortical cells by extensive development of intracellular coiled hyphae which sometimes develop arbuscule-like branches. The coiled hyphae spread directly from cell to cell and hardly any intercellular growth occurs (Smith and Read, 2008). The *Arum*-type has long been regarded as the most common type of association but recent studies have shown that the *Paris*-type is also very common (Smith and Read, 2008). However, most research has focused on *Arum*-type mycorrhiza. In the *Arum*-type of association, when a fungal hypha penetrates a cortical cell it branches heavily to form an arbuscule and the fungal cell wall becomes thinner (Bonfante and Peretto, 1995; Smith and Read, 2008). The plasma membranes of fungal and plant cells remain intact. The plant plasma

membrane invaginates and undergoes changes to become the periarbuscular membrane. Pumplin and Harrison (2009) used two marker protein fusions, MtPt4-green fluorescent protein (GFP) and GFP-MtBcp1, to investigate the protein composition of the periarbuscular membrane in *Medicago truncatula* roots. The protein MtPt4 is a mycorrhiza specific plant phosphate transporter (Harrison et al., 2002) whereas MtBcp1 is a mycorrhiza specific predicted glycosylphosphatidylinositol-anchored blue copper-binding protein from plants (Valot et al., 2006). It was demonstrated that the MtPt4-GFP marker was expressed exclusively in the periarbuscular membrane around hyphal branches whereas the GFP-MtBcp1 marker was expressed in the plasma membrane of cortical cells containing arbuscules and in the periarbuscular membrane surrounding arbuscule trunks but not branches (Pumplin and Harrison, 2009). The expression pattern suggests that the modification to the periarbuscular membrane may not occur until the arbuscule develops branches (Pumplin and Harrison, 2009). The apoplastic space between fungal and plant cell is called the periarbuscular space. The periarbuscular space contains components of the primary plant cell wall, but these do not assemble (Harrison, 1997; Peterson and Massicotte, 2004; Parniske, 2008).

The plant cell containing arbuscules undergoes several modifications. The vacuole decreases in size and becomes fragmented, the cytoplasm increases in volume, cell organelles proliferate, and the nucleus migrates to the centre of the cell and increases in size (Alexander et al., 1989; Smith and Smith, 1990; Gianinazzi-Pearson, 1996; Harrison, 1997, 1999; Strack et al., 2003; Reinhardt, 2007; Smith and Read, 2008). The increase in nucleus size is associated with an increase in the amount of decondensed chromatin, which indicates greater transcriptional activity (Gianinazzi-Pearson, 1996; Smith and Read, 2008). It has recently been shown that cells containing young arbuscules contain not only vacuole fragments but also a large continuous vacuole that is invaginated during arbuscule development (Pumplin and Harrison, 2009). Furthermore, extensive remodelling of the microtubule cytoskeleton during arbuscule development occurs (Harrison, 1999; Blancaflor et al., 2001). The cortical cells adjacent to those containing arbuscules also reorganise their microtubules, suggesting that cortical cells might initiate modification of their cytoskeleton before fungal penetration (Blancaflor et al., 2001). Plant and fungal membranes associated with young arbuscules show increased ATPase activity (Smith and Smith, 1990; Gianinazzi-Pearson et al., 1991; Harrison, 1999) and changes in

gene expression that are described in Chapter 6 of this thesis. The lifetime of an arbuscule is around seven days in many plant species (Parniske, 2008; Smith and Read, 2008). The reason is unknown but it has been suggested that the lifetime of an arbuscule depends on the ability to deliver P, and possibly other nutrients, to the plant cell (Parniske, 2008). In mature mycorrhiza, AM fungi sometimes develop vesicles that are presumed to act as storage organs (Smith and Read, 2008). After successful colonisation, the AM fungus starts to produce extraradical mycelium. This mycelium explores the soil in search of nutrients and other plant hosts for colonisation. The extraradical mycelium of AM fungi also produces spores (Dodd, 1994; Harrison, 1999; Bago, 2000; Requena et al., 2007; Smith and Read, 2008).

To date, most plant mutants unable to form AM are legumes. Arbuscular mycorrhiza defective mutants have been identified in *Pisum sativum*, *Vicia faba*, *Medicago truncatula*, *M. sativa*, *Lotus japonicus*, *Glycine max* and *Phaseolus vulgaris* (Smith and Read, 2008). These AM defective mutants have been classified in two groups. In myc^{-1} (or pen^{-}) mutants fungal appressorium formation is blocked at an early stage and the root epidermis is not penetrated (Duc et al., 1989; Gollotte et al., 1993; David-Schwartz et al., 2001; Smith and Read, 2008). In peas, the myc^{-1} mutation occurs from a single gene mutation and results in cell wall modifications with deposition of β-1,3-glucans underneath the appressoria (Golotte et al., 1993). In myc^{-2} (or ard^{-}) mutants root penetration and growth of intercellular hyphae occurs but the mycorrhizal development is stopped before arbuscule formation (Gianinazzi-Pearson, 1996; David-Schwartz et al., 2001; Smith and Read, 2008). A myc^{-} mutant of tomato has been described which can be infected *via* extraradical hyphae from a symbiosis with a wild-type tomato and develop arbuscules, but cannot be infected by fungal spores (David-Schwartz et al., 2001).

1.2.2 Functions of the arbuscular mycorrhizal symbiosis

Extraradical hyphae of AM fungi are not able to absorb hexoses from the soil (Bago et al., 2002) and, as obligate symbionts, the fitness of AM fungi depends entirely on carbon (C) supplied by their plant hosts (Jakobsen et al., 2002; Smith and Read, 2008). Up to 4–20% of photosynthetically synthesised C is delivered by plants to their fungal partner (Jakobsen et al., 2002; Parniske et al., 2008; Smith and Read, 2008). The intraradical mycelium takes up glucose or fructose and the fungus rapidly converts these to trehalose and glycogen. Storage lipids are also synthesised within

the intraradical mycelium, and these lipids and smaller amounts of glycogen are transported to the extraradical mycelium of the fungus (Bago et al., 2000, 2002; Pfeffer et al., 2001). The specific location of hexose uptake by the fungus is not yet clear. Uptake could occur from the periarbuscular space or *via* the intercellular hyphae, where hexoses would be taken up from the apoplast (Fitter, 2006; Smith and Read, 2008). Uptake into fungal cells could be passive, following a concentration gradient developed by the rapid conversion of hexoses by the fungus, or active, mediated by proton gradients created by H^+-ATPases (Ferrol et al., 2002; Smith and Read, 2008).

In return for the C supply, the fungi deliver nutrients, especially phosphorus (P), to their plant hosts. Inorganic P (P_i, consists of $H_2PO_4^-$ and HPO_4^{2-} ions) in soil is often poorly available for plants (Smith and Read, 2008; White and Hammond, 2008) and direct uptake of P_i by roots leads to a P_i depletion zone around roots (Bucher, 2007; Smith and Read, 2008). The extraradical hyphae of AM fungi are able to grow beyond the P_i depletion zone and access P_i that is unavailable to plant roots (Jakobsen et al., 1994). Phosphorus is taken up in the form of P_i by AM fungi and P_i is incorporated into the cytosolic P_i pool which is kept at a constant concentration to maintain various cell functions (Ezawa et al., 2002). If sufficient P_i is available, it is converted into polyphosphate (polyP, a linear polymer of variable numbers of P_i residues, linked by high energy phosphoanhydride bonds) and both P_i and polyP are stored in vacuoles (Ezawa et al., 2002; Smith and Read, 2008). PolyP seems to be transported by the tubular vacuolar system to the intraradical mycelium (Nielsen et al., 2002; Uetake et al., 2002; Viereck et al., 2004; Smith and Read, 2008). Within the intraradical mycelium polyP is hydrolysed and P_i is released into the periarbuscular space (Viereck et al., 2004; Javot et al., 2007). Plant transporters are responsible for P_i uptake from the periarbuscular space (see Chapter 3 of this thesis; Smith and Read, 2008).

The AM fungi also deliver nitrogen to their plant hosts. It was demonstrated that *Glomus intraradices* can take up nitrate (NO_3^-) and ammonium (NH_4^+) but seems to prefer NH_4^+ over NO_3^- (Johansen et al., 1992). The uptake of organic nitrogen in the form of ^{15}N-glycine and ^{15}N-glutamic acid by *Glomus mosseae* has also been shown (Hawkins et al., 2000). Nitrogen compounds are used to synthesise arginine in the extraradical mycelium of the AM fungi, which is then transported to the intraradical mycelium probably in fungal vacuoles (Jin et al., 2005; Cruz et al.,

2007). In the intraradical mycelium arginine is broken down to NH_4^+ which is then released into the periarbuscular space and taken up by the plant hosts (Jin et al., 2005; Cruz et al., 2007). However, the transfer of N by AM fungi does not necessarily lead to greater plant growth (Johansen et al., 1992; Hawkins et al., 2000).

The AM fungi seem to play a minor role in the acquisition of other major nutrients, such as K, but it has been shown that AM fungi can deliver K to their host plants. Since accumulation of K can be influenced by ammonium, nitrate and sodium as well as by the synthesis of polyphosphates, Smith and Read (2008) suggested that these factors must be taken into account when studying K uptake by AM fungi and its transport to plants. Micronutrients such as zinc and copper have also been shown to be delivered by AM fungi (Smith and Read, 2008).

Apart from their major role in nutrient transfer, AM fungi provide several more benefits to their plant hosts. Arbuscular mycorrhizal fungi can improve the drought tolerance of plants (Augé, 2001). Although there is no clear evidence of water transport by hyphae, AM fungi typically increase the water use efficiency of plants (for review see Augé, 2001). Mycorrhizal plants often show not only increased plant size and improved nutritional status but also greater root growth, less wilting, increased soil water extraction and a quicker recovery under drought stress (Augé, 2001; Al-Karaki et al., 2004; Khalvati et al., 2005).

Arbuscular mycorrhizal fungi have the ability to protect plants from heavy metals (HM) by binding HM and reducing the uptake of HM into plants (Joner et al., 2000; Hildebrandt et al., 2007). Maximum sorption of Cd from solution by *Glomus mosseae* was achieved after 30 min with no additional sorption during six hours of incubation indicating non-metabolic binding of Cd to cell walls (Joner et al., 2000). It has been demonstrated that AM fungi increase P and K concentrations in plant tissues, whilst reducing concentrations of Cd, Cr, Mn, Cu, Mo, Fe and Ni (Vivas et al., 2005). Indigenous fungi were more efficient than an introduced fungus in doing this (Vivas et al., 2005). Glomalin, a glycoprotein produced by AM fungi, has been shown to bind HM (González-Chávez et al., 2004). Due to their potential to accumulate HM and decrease HM uptake by plants, AM fungi have been suggested to be beneficial for revegetation and for phytoremediation of HM contaminated soils (Gaur and Adholeya, 2004; Göhre and Paszkowski, 2006; Turnau et al., 2008).

Arbuscular mycorrhizal fungi can also protect plants from soil-borne pathogens, especially fungi, and can alter host plant susceptibility to insect

herbivores (Harrier and Watson, 2003; Whipps, 2004; Gosling et al., 2006). The fungi also interact with other soil microorganisms. Mycorrhiza-helper bacteria promote AM symbioses by stimulating hyphal growth or by producing compounds that enhance the production of plant root exudates thereby indirectly enhancing mycorrhizal formation (Barea et al., 2005). Furthermore, AM fungi interact with nitrogen fixing bacteria and plant growth promoting bacteria benefiting host plant development and nutrition (Harrier and Watson, 2003; Barea et al., 2005; Gosling et al., 2006).

Extraradical hyphae of AM fungi improve soil structure by helping to bind soil particles together into stable microaggregates (Miller and Jastrow, 2000; Harrier and Watson, 2003; Barea et al., 2005; Bedini et al., 2009). Glomalin is a very stable hydrophobic glycoprotein and has been shown to play an important role in stabilising soil aggregates and thereby counteracting soil erosion (Miller and Jastrow, 2000; Morgan et al., 2005; Bedini et al., 2009). Arbuscular mycorrhizal fungi show high functional diversity, and plant growth responses to AM fungi vary widely from parasitic to mutualistic (Klironomos, 2003; Jones and Smith, 2004). Klironomos (2000) tested several plant and fungi combinations and concluded that AM fungi are functionally variable because the fungi had different abilities to improve P uptake or to protect the plant against fungal pathogens. Munkvold et al. (2004) showed that different *Glomus* species, and even different isolates of the same species, led to differences in shoot dry weights and P content of cucumber plants. The influence of AM fungi on the expression of phosphate transporters in plants also varies with fungal strain (Burleigh et al., 2002).

1.2.3 Caesium accumulation by mycorrhizal plants

Because of the role of AM fungi in plant nutrition, it has been suggested that they might affect Cs accumulation by plants (Entry et al., 1996). It has been demonstrated that AM fungi were able to take up and transport radiocaesium to host plants when only the fungi had access to the Cs supply (Declerck et al., 2003; Dupré de Boulois et al., 2006). Dupré de Boulois et al. (2006) used an *in vitro* system where ^{134}Cs was supplied to the fungal hyphae and showed that 10% of the Cs that was taken up by the fungal hyphae was transported to shoots of *Medicago truncatula*. It could be shown that AM fungi can transfer Cs from one plant to another when two plants were connected *via* hyphae of their AM fungal partners (Meding and Zasoski,

2008; Gyuricza et al., 2010c). However, Joner et al. (2004) conducted three experiments demonstrating that the contribution of AM fungi to transfer of radiocaesium from soil to plants was not significant. Joner et al. (2004) used three different growth systems where only the fungi had access to the Cs supply. *Trifolium subterraneum* was grown in association with *Glomus mosseae* BEG 69, *Zea mays* was grown in association with *Glomus intraradices* BEG 157 and *Medicago truncatula* was grown in association with *Glomus intraradices* BEG 87 in different soil mixtures contaminated with ^{137}Cs or ^{134}Cs (Joner et al., 2004). In all three cases the amounts of Cs transported by the AM fungi to their plant hosts were minimal and concentrations of Cs in shoots of mycorrhizal plants were not significantly different from non mycorrhizal plants (Joner et al., 2004).

Under field conditions in radiocaesium contaminated areas, not only the fungal hyphae but also the plant roots are in contact with the radionuclide. Several studies have investigated the influence of AM fungi on Cs accumulation by plants (Rogers and Williams, 1986; Dighton and Terry, 1996; Entry et al., 1999; Berreck and Haselwandter, 2001; Joner et al., 2004; Rosén et al., 2005; Dubchak et al., 2010; Gyuricza et al., 2010b). In these studies, both plant roots and fungal hyphae had access to the Cs supply. The influence of AM fungi on Cs concentrations in shoots of plants was not consistent (Table 1.2). The inconsistent effects of AM fungi on Cs accumulation by their plant symbionts might be explained by a number of factors. First, the availability of Cs to organisms depends on the physical and chemical properties of the substrate (Entry et al., 1996). Second, both plant and fungal species differ in their ability to access different soil Cs pools (Berreck and Haselwandter, 2001) and plant species differ in their ability to accumulate Cs (see Chapter 2; Andersen, 1967; Broadley et al., 1999a). Third, the accumulation of Cs by organisms depends on the concentrations of K and Cs in the substrate (Buysse et al., 1996; Berreck and Haselwandter, 2001). The experiments listed in Table 1.2 were performed using different growth substrates, such as soil (Rogers and Williams, 1986; Entry et al., 1999; Rosén et al., 2005), sand or sand-sandy loam soil (Berreck and Haselwandter, 2001; Joner et al., 2004; Dubchak et al., 2010) or solidified growth medium (Gyuricza et al., 2010b). Furthermore, plant and fungal species differed, as did the concentrations of K and Cs in the substrate. Another factor that could have influenced the results obtained in the studies listed in Table 1.2 is the difference in growth periods. The organisms were in contact with Cs for one week

(Gyuricza et al., 2010b), four weeks (Joner et al., 2004), or for longer than 16 weeks (Entry et al., 1999; Rosén et al., 2005).

Table 1.2: Effects of arbuscular mycorrhizal fungi on Cs accumulation in plant shoots. In these studies different plant and fungal species were used, and both plant roots and fungal hyphae had access to the Cs supply.

Influence	Plant species	Fungal species	Reference
decrease	*Agrostis tenuis*	*Glomus mosseae*	Berreck and Haselwandter (2001)
decrease	*Trifolium repens*	Soil fungi	Dighton and Terry (1996)
increase	*Helianthus annuus*	*G. intraradices*	Dubchak et al. (2010)
increase	*Paspalum notatum*	*G. mosseae / G. intraradices*	Entry et al. (1999)
increase	*Sorghum halepense*	*G. mosseae / G. intraradices*	Entry et al. (1999)
increase	*Panicum virgatum*	*G. mosseae / G. intraradices*	Entry et al. (1999)
decrease	*Medicago truncatula*	*G. intraradices*	Gyuricza et al. (2010b)
no effect	*Medicago truncatula*	*G. intraradices*	Joner et al. (2004)
increase	*Melilotus officinalis*	*Glomus* species	Rogers and Williams (1986)
no effect	*Sorghum sudanense*	*Glomus* species	Rogers and Williams (1986)
increase	*Allium porrum*	Soil fungi	Rosén et al. (2005)
no effect	*Lolium perenne*	Soil fungi	Rosén et al. (2005)

1.2.4 Arbuscular mycorrhiza in agriculture

Since many crop plants are mycorrhizal, the potential benefit of AM fungi in agriculture has been of widespread interest (Hamel, 1996; Harrier and Watson, 2003; Plenchette et al., 2005; Gosling et al., 2006; Smith and Read, 2008; Facelli et al., 2009). Several agricultural practices in intensive farming, such as fertilisation with P, tillage and the application of biocides, have negative effects on the survival of AM fungi (Hamel, 1996; Harrier and Watson, 2003; Plenchette et al., 2005; Gosling et al., 2006). Therefore, many studies have compared survival of AM, and benefits of AM fungi to crop plants, in intensive and low-input farming. High P concentrations in soil due to fertilisation lead to lower colonisation rates by AM fungi and lower spore numbers (Mäder et al., 2000; Galvez et al., 2001; Harrier and Watson, 2003; Kahiluoto et al., 2009). Reduced P fertilisation in low-input farming not only promotes survival and growth of AM fungi but also reduces the loss of P to the environment (Grant et al., 2005; Kahiluoto et al., 2009). Tillage reduces the survival of hyphae, and deep ploughing leads to a transport of spores and infected root

fragments to deeper soil layers (McGonigle and Miller, 2000; Kabir, 2005). Plants grown in undisturbed soil had higher P concentrations in their shoots than plants grown in disturbed soil (McGonigle and Miller, 2000), and spore densities in low-input systems decreased with soil depths (Oehl et al., 2005). Modern crop varieties have been bred for high yields under optimal mineral fertilisation and might therefore have become less dependent on AM fungi for their nutrition (Ryan and Graham, 2002; Harrier and Watson, 2003; Smith and Read, 2008). The influence of non mycorrhizal crops grown prior to mycorrhizal crops on AM formation is very complex and does not necessarily influence colonisation rate or yield (Sorensen et al., 2005; Vestberg et al., 2005). Despite these adverse influences, AM fungi are still able to increase the uptake of essential micronutrients such as zinc, to influence other soil biota, and to lead to improved soil structure in agricultural systems (Bethlenfalvay and Schüepp, 1994; Ryan and Graham, 2002; Smith and Read, 2008; Facelli et al., 2009).

The influence of agricultural practices on AM fungal diversity has also been studied. Spore abundance and species diversity assessed with molecular methods showed higher diversity of AM fungi in natural ecosystems and low-input farming than in intensive farming (Helgason et al., 1998; Jansa et al., 2002; Oehl et al., 2004, 2005, 2010; Hijri et al., 2006; Alguacil et al., 2008). Species of the genus *Glomus* seem to be generalists because they are dominant in agricultural soils whereas species of the genera *Acaulospora* and *Scutellospora* are more abundant in organic farming systems and natural ecosystems (Helgason et al., 1998; Jansa et al., 2002; Oehl et al., 2004, 2005, 2010; Hijri et al., 2006; Alguacil et al., 2008). However, some agricultural soils show high AM fungal diversity and low-input management and crop rotation have the potential to preserve AM fungal diversity over the long term (Hijri et al., 2006).

Arbuscular mycorrhizal fungi show differences in their ability to promote growth and increase nutrient content of their host plants (Hamel, 1996). Therefore, the potential of different AM inocula in agriculture has been studied. Inoculation of crop plants with AM fungi can improve mycorrhizal development and reduce the requirement for P, but the positive influence depends on inoculum type and P status of the soil and does not necessarily lead to improved plant growth (Gazey et al., 2004; Sorensen et al., 2008). The amount of colonisation of plants by the fungal species of the inoculum also depends greatly on the abundance and infectivity of

indigenous fungi (Abbott and Robson, 1982; Abbott et al., 1983; Plenchette et al., 2005). Furthermore, large scale production of AM inocula is difficult and the inocula must (1) provide a cocktail of different species that are adapted to different soils, (2) have positive effects on plant health, (3) be free of pathogens and agents that could negatively influence AM development and (4) have a significant shelf life under realistic storage conditions (Marx et al., 2002; Gianinazzi and Vosátka, 2004).

1.3 AIMS

The overarching aim of the thesis was to obtain a better understanding of the influence of arbuscular mycorrhiza on caesium accumulation by plants. The model plant *Medicago truncatula* was grown in symbiosis with *Glomus* sp. under *in vitro* conditions for most of the experiments performed in this thesis. However, a glasshouse experiment was also conducted in which several plant species were grown in soil. The aim of this glasshouse experiment was to determine whether conclusions from *in vitro* experiments were consistent with those obtained from experiments performed under semi natural conditions. Six different plant species (*Hordeum vulgare*, *Beta vulgaris*, *Brassica napus*, *Medicago truncatula*, *Solanum tuberosum* and *Helianthus annuus*) were grown in non sterile and sterile soil, and caesium concentrations in tissues of mycorrhizal and non mycorrhizal plants were measured (Chapter 2). Two hypotheses were tested (1) that plant species show genetic variation in caesium accumulation and (2) that arbuscular mycorrhiza reduce caesium accumulation in plants. For the *in vitro* experiments, growth conditions to achieve mycorrhizal *Medicago truncatula* plants were first optimised (Chapter 3). Phosphorus concentrations in the media and light intensity were key factors influencing mycorrhizal colonisation. Caesium toxicity to *Medicago truncatula* plants was also investigated to determine non toxic caesium concentrations for further experiments (Chapter 4). Subsequently, mycorrhizal and non mycorrhizal *Medicago truncatula* plants were grown in the presence or absence of caesium to test the hypothesis that arbuscular mycorrhizal fungi reduce caesium accumulation in this plant species (Chapter 5). Finally, differences in gene expression in *Medicago truncatula* tissues in response to caesium and to arbuscular mycorrhiza were determined using micro arrays (Chapter 6).

Chapter 2

Differences in caesium accumulation among plant species and the influence of mycorrhiza on plant caesium accumulation

2.1 INTRODUCTION

There is considerable variation among plant species in their ability to take up caesium (Cs) and accumulate it in their shoots (e.g. Andersen, 1967; Evans and Dekker, 1968; Buysse et al., 1996; Broadley and Willey, 1997; Gouthu et al., 1997; Broadley et al., 1999a,b; Fuhrmann et al., 2003; White et al., 2003; Willey et al., 2005; Willey and Tang, 2006; Cook et al., 2007, 2009; Wasserman et al., 2008; Choi et al., 2009; Waegeneers et al., 2009). In general, among the angiosperms, monocot species (e.g. Liliales, Poales) have lower shoot Cs concentrations than species from the eudicot orders Asterales, Brassicales and Caryophyllales (Broadley et al., 1999a; White et al., 2003; Willey et al., 2005). This suggests that the abundance or kinetics of Cs-transport proteins differ between plant species. In addition, the Cs/K ratios in shoots of different plant species grown under identical conditions vary widely (Andersen, 1967; White et al., 2004). Since different transport proteins have contrasting abilities to discriminate between Cs^+ and K^+ (Figure 2.1), these varying shoot Cs/K ratios suggest that a different complement of transport proteins is present in different plant species (White et al., 2004; Hampton et al., 2005; Wiesel et al., 2008). Shoot Cs concentrations and shoot Cs/K ratios were positively linearly correlated among plant species, suggesting that shoot Cs concentrations vary independently from shoot K concentrations (Figure 2.2). A plausible explanation for this observation is that all plants express constitutively an essential, selective K^+ transporter, such as the inward-rectifying K^+ channel (KIRC) AKT1, but differ in their complement of proteins catalysing the non specific uptake (or efflux) of Cs^+ and K^+, such as cyclic nucleotide gated channels (CNGCs, belonging to the family of voltage insensitive cation channels (VICCs)) or K^+/H^+ symporters (KUPs) (White et al., 2010). Thus, plants with higher shoot Cs concentrations and shoot Cs/K ratios are likely to have higher *CNGC*:*AKT1* and/or *KUP*:*AKT1* expression ratios than plants with lower shoot Cs concentrations and shoot Cs/K ratios.

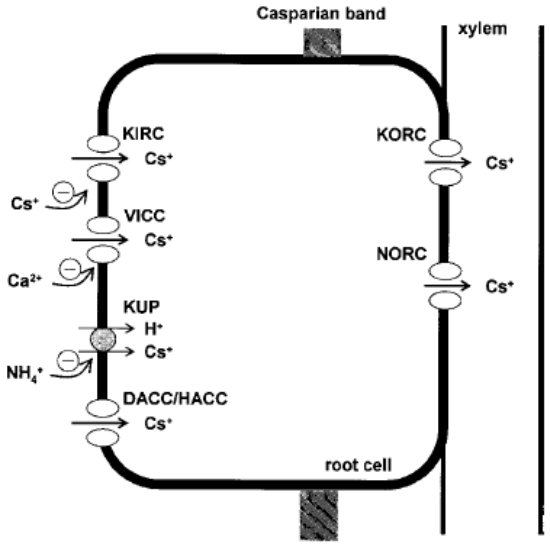

Figure 2.1: Cation transport proteins that contribute to Cs^+ movement across the plasma membrane (White and Broadley, 2000; Hampton et al., 2005). Inward rectifying K^+ channels (KIRCs), voltage insensitive cation channels (VICCs), K^+/H^+ symporters (KUPs) and voltage dependent Ca^{2+} channels (DACCs and HACCs) facilitate Cs^+ influx into root cells, and outward rectifying K^+ channels (KORCs and NORCs) facilitate Cs^+ efflux to the xylem.

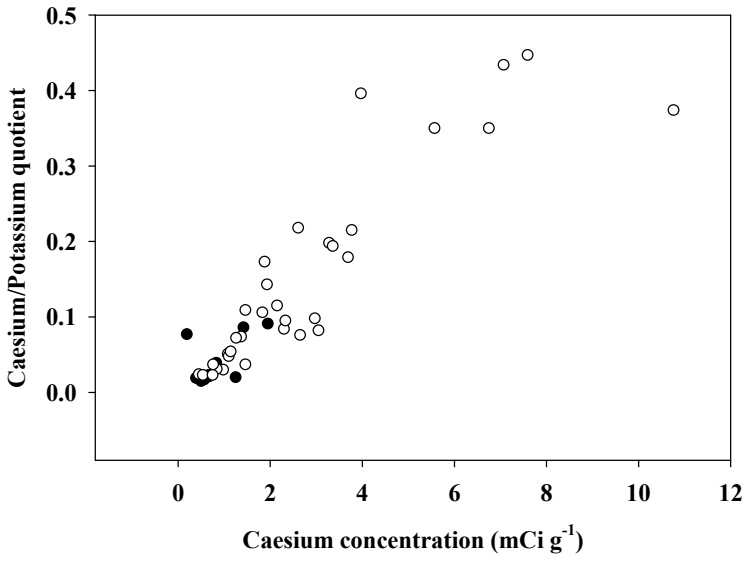

Figure 2.2: Relationship between Cs concentrations and Cs/K ratios in shoots of 44 plant species grown on fertilised soil contaminated with 10 µCi carrier-free ^{137}Cs (Andersen, 1967). Data for Poaceae (filled circles) and eudicots (open circles) species are shown.

Due to chemically similarities between K and Cs external K supply influences Cs uptake by plants. Fertilisation with K decreases Cs accumulation (Belli and Sansone, 1995; Ciuffo et al., 2003; Soudek et al., 2004). Furthermore, the greatest differences in Cs accumulation by different plant species occur at the lowest external K supply (Buysse et al., 1996). Waegeneers et al. (2001) investigated Cs uptake by five plant species on soils that differed in exchangeable K and Cs concentrations. They concluded that "High radiocaesium uptake occurs: i) if the plant species have a high intrinsic Cs uptake rate at low K concentrations, ii) if the species have a high biomass production, resulting in a large depletion of K in the bulk soil solution, iii) if the species have a high K uptake rate per unit root surface, resulting in a high rhizospheric K depletion".

The bioavailability of Cs strongly depends on soil characteristics (Ehlken and Kirchner, 2002). Caesium occurs in simple ionic form in soils and it does not undergo changes through redox reactions or complexation (Livens and Rimmer, 1988). However, Cs is strongly bound to clay particles (Coughtrey and Thorne, 1983) and the sorption of Cs to clay particles is affected by the mineralogy of clay minerals (Livens and Rimmer, 1988). Caesium is mobilised from soil sorption sites by ammonium (NH_4^+) and NH_4^+ is a more important mobiliser than K (Konopleva et al., 2009). Furthermore, rhizosphere processes influence the bioavailability of Cs. Uptake of K by plants from soil leads to potassium depletion in the rhizosphere. Reduced availability of K in the rhizosphere increases Cs uptake by plants, and plant roots and clay minerals act as competitive sinks for Cs in the rhizosphere (Delvaux et al., 2000).

Micro organisms in the rhizosphere, especially arbuscular mycorrhizal (AM) fungi, influence mineral acquisition by plants. Because of the role of AM fungi in plant nutrition, it has been suggested that they might affect Cs uptake by plants (Entry et al., 1996). However, there is no consistent information about the influence of mycorrhiza on Cs accumulation by plants (Table 1.2). Mycorrhizal associations could influence plant Cs accumulation directly, by altering the expression of genes encoding K-transporters, and/or indirectly by improving plant nutritional status. Although the role of AM fungi in K transport is not clear yet, it has been suggested that, under acidic soil conditions, AM fungi can improve K accumulation of plants (Clark and Zeto, 2000). An improved K status of the plants would lead to a decrease in Cs accumulation (Hampton et al., 2004).

2.1.1 Aims

The aim of this chapter was to investigate differences in Cs concentrations in roots and shoots of different plant species. Six species belonging to different plant families were chosen. A member of the Poaceae representing low accumulating Poales, a member of the Brassicaceae representing non mycorrhizal Brassicales, a member of the Chenopodiaceae representing high accumulating Caryophyllales and three additional eudicot species were selected. All species were arable crop plants because Cs enters the human food chain *via* plants. To assess any differences in Cs accumulation due to AM fungi, both mycorrhizal and non mycorrhizal plants were studied.

2.2 MATERIAL AND METHODS

2.2.1 Soil treatments

Caesium accumulation by different plant species, and the influence of mycorrhiza on this, was investigated in a pot experiment. Soil was taken from the Lower Pilmore field (56°27′08″N, 3°04′45″W), SCRI, Dundee, UK at the end of July 2008. It was a sandy, brown forest soil which had not been fertilised since 1996 (Bennett, J., personal communication). Grass had grown on the field for the previous three years. The soil was sampled to a depth of 10 cm and therefore represented rhizosphere soil. The soil was sieved to 4 mm and half of it was gamma-sterilised with a minimum dose of 25 kGy (Isotron plc., Swindon, UK). Four different treatments were applied. Non sterile and sterile soils without mycorrhizal inoculum, and non sterile and sterile soils with the addition of 10 g mycorrhizal inoculum (rootgrow^{TM}, friendly mycorrhizal fungi, Plantworks Ltd, Kent, UK). The pots containing 1 kg of soil each were watered before fertilisation with 80 kg ha^{-1} KNO_3 following the recommendations of RB209 (DEFRA, 2000) to avoid K and N limitations for plant growth. It was assumed that micronutrients were sufficient and the soil was not fertilised with P to allow for mycorrhizal colonisation of plants. Furthermore, 3 mg CsCl per kg soil were added. Therefore, each pot received 10 ml solution containing 115.2 mg KNO_3 and 3 mg CsCl.

2.2.2 Plant material and growth conditions

Seven plant species were grown: barley (*Hordeum vulgare* var. Optic; SCRI), beetroot (*Beta vulgaris*, Globe 2; Sutton Seeds, Paignton, UK), *Brassica napus* (SCRI), *Medicago truncatula* var. *truncatula* Jemalong A17 (SARDI, Genetic Resource Centre, Australia), onion (*Allium cepa*, Alisa Craig; Sutton Seeds, Paignton, UK), potato (*Solanum tuberosum* var. King Edward; SCRI) and sunflower (*Helianthus annuus*, Irish Eyes; Sutton Seeds, Paignton, UK). For barley, beetroot, *Brassica*, *Medicago*, onion and sunflower seeds were placed directly in pots and potato was grown from tubers. Each pot contained one plant. Ten replicates of each plant were grown for each treatment and five replicates of each treated soil without plants were maintained. The pots were set using a randomised block design (GenStat, 10^{th} edition, VSN International Ltd, Hemel Hempstead, UK). All pots were watered to weight with distilled water twice a week. The plants were grown in a glasshouse under daylight conditions (September–October 2008) without additional light supply and the temperature was maintained for twelve hours at 20°C and twelve hours at 15°C. After six weeks plant roots and shoots, and for potatoes newly developed tubers, were harvested, the fresh weights were measured and the material was frozen in liquid N_2 and stored at -80°C. Due to a very small biomass of all onion plants this species was excluded from further analysis.

2.2.3 Determination of mycorrhizal infection

2.2.3.1 Morphological analysis

Three plants of each species were used to determine mycorrhizal colonisation rates. Therefore, roots were harvested and stored in 70% ethanol (EtOH). The root samples were cut into 1 cm long pieces and were stained using an ink and vinegar technique (Vierheilig et al., 1998). The roots were cleared by boiling in 2.5% (wt/vol) KOH for 5 min for potato and sunflower roots, and for 3 min for the other root samples. After washing the roots in tap water the roots were stained by boiling for 5 min in a 5% ink-acetic acid solution. Black Shaeffer ink (Shaeffer, Ft. Madison, Iowa) was used. The stained roots were observed for mycorrhizal infection using an Axioplan 2 microscope (Carl Zeiss Ltd, Welwyn Garden City, UK). Photographs were taken using an Axiocam HRc camera (Carl Zeiss Ltd, Welwyn Garden City, UK).

To determine the percentage of mycorrhizal colonisation 200 intersections per root sample were checked for the presence or absence of arbuscules, vesicles, hyphae or spores. The percentage of mycorrhizal colonisation (% myc) was calculated (Equation 2.1).

% myc = number of colonised intersections / total number of intersections * 100 [eq. 2.1]

2.2.3.2 Molecular analysis

Genomic deoxyribonucleic acid (DNA) from roots of barley, beetroot, *Brassica*, *Medicago*, potato and sunflower plants was extracted with the NucleoSpin 96 Plant Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. Root materials were freeze dried (Alpha 1-2 LD plus, SciQuip Ltd., Shropshire, UK) and up to 20 mg of freeze dried roots were milled using acid washed steel balls (TissueLyser II, Qiagen Ltd., Crawley, UK). Afterwards, 700 µl extraction buffer were added. The samples were incubated at 56°C for 30 min and subsequently centrifuged at 6000 g for 20 min (4K15C, Sigma Aldrich, St. Louis, MO, USA). The supernatant was mixed with 375 µl lysis buffer plus 250 µl ethanol and transferred to a binding plate. A spin at 6000 g was followed by adding 500 µl wash buffer (CW) to each well. After another spin the flow through was discarded and 900 µl wash buffer (C5) was added. The samples were centrifuged at 6000 g for up to 15 min to remove all residues of the wash buffer. The DNA was eluted using 150 µl of pre-warmed elution buffer and stored at -80°C.

To determine if mycorrhizal DNA was present in the root samples a Polymerase Chain Reaction (PCR) using mycorrhiza specific AML primers (AML1: ATCAACTTTCGATGGTAGGATAGA; AML2: GAACCCAAACACTTTGGTTTCC; Lee et al., 2008) was performed. These primers result in the amplification of a region in the SSU rRNA gene of Glomeromycota of approximately 800 bp. The reaction solution contained 1.5 µl 10x HiFi buffer (Invitrogen Ltd., Paisley, UK), 0.6 µl of both 10 pmol AML1 and AML2 (VH bio Limited, Gateshead, UK), 0.6 µl of $MgSO_4$ (Invitrogen Ltd., Paisley, UK), 0.3 µl of 12.5 mM dNTP's (Promega, Southampton, UK), 0.3 µl of BSA (20 mg ml^{-1}; Roche Products Limited, Welwyn Garden City, UK) and 0.06 µl of Platinum® Taq DNA Polymerase High Fidelity (Invitrogen Ltd., Paisley, UK) for 2 µl of template DNA. After activation of the Platinum Taq at 94°C the DNA was amplified with 30 cycles of 94°C for 30 sec, 58°C for 40 sec and 68°C

for 55 sec. PCR products were separated on a 1% agarose gel and the amplified DNA stained using SYBR® Safe DNA gel stain (Invitrogen Ltd., Paisley, UK). A 1 kb DNA ladder (Promega, Southampton, UK) was run on the gel to give an estimate of product sizes.

2.2.4 Soil characterisation

Several soil characteristics were analysed. Before the start of the experiment untreated soil was collected and at harvest soil samples from each pot were taken and stored at 4°C. To resolve the organic matter (OM) content of the soil, soil that had been oven dried at 70°C over night was weighed and then incubated at 500°C over night. The amount of OM was defined as the amount of dried soil minus the amount of soil after combustion at 500°C. The pH of the soil solution was measured by adding 20 ml of 0.01 M $CaCl_2$ to 10 g of fresh soil. The samples were stirred and the pH measured using a pH meter (MP230, Mettler-Toledo Ltd., Leicester, UK). In order to measure nitrogen components and dissolved organic carbon (DOC) KCl extraction was carried out. For the extraction 40 ml of 1 M KCl were added to 10 g of fresh soil and mixed for 1 h. The solution was cleared using Whatman filter paper (diameter 125 mm, pore size 11 µm; VWR International Ltd., Poole, UK). The elemental analysis was undertaken using a Skalar SANplus Segmented Flow analyser (Skalar Analytical B.V., Breda, The Netherlands). A description of the procedure was provided by the company. To measure the amount of DOC in the soil extracts, the samples were acidified and then purged with a nitrogen gas stream to remove inorganic carbon (Menzel and Vaccaro, 1964). Buffered persulfate and hydroxylamine were added and the samples were irradiated in an UV destructor. The generated carbon dioxide diffused through a silicone membrane. A weakly buffered phenolphthalein indicator solution was used as the recipient stream, and the colour intensity of this solution decreased proportionally to the change in pH caused by the absorbed carbon dioxide gas. The colour intensity was measured at 550 nm by a photometric detector. To determine the amounts of nitrate (NO_3^-) and nitrite (NO_2^-) the sample was passed through a column containing granulated copper-cadmium to reduce the NO_3^- to NO_2^- (Bundy and Meisinger, 1994). The NO_2^- was measured by diazotizing with sulphanilamide and coupling with α-naphthylethylenediamine dihydrochloride to form a highly coloured azo dye which was measured at 540 nm. For determination of ammonium (NH_4^+) the element was chlorinated to

monochloramine which reacts with salicylate to form 5-aminosalicylate (Bundy and Meisinger, 1994). After oxidation and oxidative coupling a green coloured complex was formed and its absorption was measured at 660 nm. Carbon and nitrogen concentrations were determined using a CE-440 Elemental Analyzer (Exeter Analytical (UK) Ltd, Coventry, UK). A description of the method (CE-440 Theory of Operation) was provided by the company. For each sample 5 mg of soil were weighed out and put in tin capsules. The combustion of the soil occurred in pure oxygen at a temperature of 975°C. Helium was used to carry the combustion products through the analytical system, as well as for purging the system. The products of combustion were passed over reagents to assure complete oxidation and complete removal of undesirable by-products. In the reduction tube, oxides of nitrogen were converted to molecular nitrogen and residual oxygen was removed. The sample was then released into the thermal conductivity detector. Carbon dioxide was removed from the sample for measurement of the carbon content. The remaining gas now only consisted of helium and nitrogen. By comparison with pure helium the nitrogen concentration was determined. Acetanilide was used as conditioner and standard. It consists of 71.09% carbon, 6.71% hydrogen, 10.36% nitrogen and 11.84% oxygen. Benzoic acid was also used as a standard.

2.2.5 Determination of elemental concentrations

For determining total element concentrations in roots and shoots, plant material was oven dried at 40–50°C for several days. For determining total element concentrations in soil, the soil was oven dried at 70°C for 24 h. Following drying the material was milled using acid washed steel balls with a bead beater (TissueLyser II, Qiagen Ltd., Crawley, UK).

2.2.5.1 Microwave digestion

Prior to digestion, up to 50 mg of milled material was weighed out and placed in microwave digester tubes (50 ml; MARS, CEM Corporation, Matthews, NC, USA). Three milliliters of concentrated nitric acid (Aristar, VWR International Ltd., Poole, UK) were added to plant or soil powder and the samples left to digest in a fume hood for 15 min. Standards (1573a tomato leaves, National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA) were also processed to test the quality of the digestion and nitric acid was included as a blank sample to take

through the entire extraction procedure. The samples were placed in a microwave (MARS, CEM Corporation, Matthews, NC, USA) and digested using the STD-NO3 Digest programme (Table 2.1). After heating the samples up in steps to 180°C this temperature was held for 20 min to allow the complete digestion of the material. After a cooling step of 20 min the tubes were carefully opened in a fume hood and nitrogen oxide fumes were allowed to vent for at least 20 min. One milliliter of 30% hydrogen peroxide solution (Aristar, VWR International Ltd., Poole, UK) was added to each sample and the samples left to digest in a fume hood for 15 min. Subsequently, the samples were placed in the microwave again and cleared using the STD-NO3 Digest programme as described above (Table 2.1). Finally, Milli-Q water (Millipore (U.K.) Ltd., Watford, UK) was added to a final volume of 50 ml.

Table 2.1: STD-NO3 Digest programme for microwave acid digestion of plant and soil materials.

Ramp Time (min)	**Temperature (°C)**	**Hold Time (min)**
3	100	2
1	120	1
3	160	2
2	180	20

2.2.5.2 Inductively Coupled Plasma Mass Spectrometry

Elemental concentrations in plant tissues were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, ELAN DRC-e, PerkinElmerSCIEX, Massachusetts, USA). The instrument was fitted with ELAN software (PerkinElmerSCIEX, Massachusetts, USA). The digested plant or soil samples and nitric acid blanks were placed in the ICP-MS. For calibration, cation and anion standards were included. The concentrations for each ion in the standard stock solutions are listed in Table 2.2. Traditionally, element concentrations in plant tissues are reported in mg g^{-1}. Hence, the element concentrations in the standards are provided in mg l^{-1} to enable calibration.

Table 2.2: Element concentrations in cation (K, Mg, Na, Ca, Cs, Mn, Zn, Fe, Cu, Ni) and anion (P, Cl, S, Se) standard stock solutions.

Element	Salt	mg l^{-1} in stock
K	KNO_3	8000
Mg	$Mg(NO_3)_2$	4000
Na	$NaNO_3$	200
Ca	$Ca(NO_3)_2\,4H_2O$	4000
Cs	$CsNO_3$	0.001
Mn	$Mn(NO_3)_2\,4H_2O$	0.004
Zn	$ZnCl_2$	0.004
Fe	$FeCl_3\,6H_2O$	0.02
Cu	$CuCl_2$	20
Ni	$NiCl_2$	20
P	KH_2PO_4	1200
Cl	KCl	4000
S	K_2SO_4	3000
Se	Na_2SeO_4	200

In each run 1/200, 1/1000 and 1/2000 dilutions of the standard stock solutions were included. The standard with the lowest concentration was measured first. These standards were used to create calibration curves for each element. Figure 2.3 gives an example of a calibration curve for Cs.

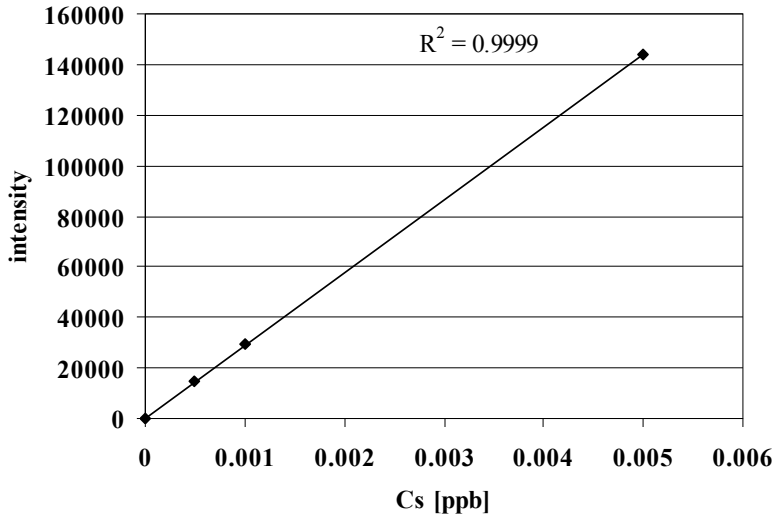

Figure 2.3: Calibration curve for Cs. Intensity values are plotted against Cs concentrations [ppb] in 1/200, 1/1000 and 1/2000 dilutions of the standard stock solution.

A description of the ICP-MS technique (The 30-Minute Guide to ICP-MS) was provided by PerkinElmerSCIEX. Briefly, liquid samples are converted into very small droplets by a nebuliser. These droplets are then injected into the plasma that is generated by passing argon through a series of concentric quartz tubes. The plasma ionises the elements in the droplets. An ion lens focuses the ions into the quadrupole region where a mass spectrometer separates ions from each other by their mass-to-charge ratio. A detector counts the ions. Subsequently, the software converts the counts for each ion to ppm or ppb values according to the standard curves. For analysis, the volume of the sample and the dry weight (DW) were used to calculate the amount of each ion in mg g^{-1} DW. All element concentrations were calibrated to the NIST tomato leaves standard (TLS). These calibrations provided the analytical error for each run. The actual readings for the TLS samples were used to determine the decrease in element concentrations in each step of the run. Using the concentration of each element in the TLS provided by NIST the recovery of the element concentrations was calculated. The decrease in element concentrations in each step of the run and the recovery of the element concentrations provided the basis for the drift correction in each run.

2.2.6 Statistical analysis

For comparison of soil characteristics in differently treated soils and of element concentrations in plant tissues and soil samples a general analysis of variance (ANOVA) was performed using GenStat (12^{th} Edition, VSN International, Hemel Hempstead, UK). The values of the element concentrations in plant tissues obtained by ICP-MS were log transformed to the base 10 to meet the ANOVA premises.

2.3 RESULTS

2.3.1 Definitions

Field sampled soil that was characterised before the start of the experiment is referred to as untreated soil. The soils that were used in the experiment were all fertilised with KNO_3 and sampled at harvest, six weeks after the start of the experiment. The term used to describe these four soils is treated soils. Two of the treated soils were sterilised and these soils are referred to as sterile soils and the other

two soils are referred to as non sterile soils. Two soils had a mycorrhizal inoculum added and are referred to as inoculated soils and the other two soils are referred to as uninoculated soils.

2.3.2 Soil characterisation

Soil characteristics of untreated soil and of treated soils were determined. Organic matter (OM) content, pH, the amounts of dissolved organic carbon (DOC), nitrate (NO_3^-) and nitrite (NO_2^-), ammonium (NH_4^+), and the ratio of total carbon and total nitrogen (C/N) were obtained (Table 2.3).

Table 2.3: Soil characteristics of untreated soil taken before the start of the experiment and of treated soils taken at harvest in the absence of plants. Mean values and 95% confidence intervals of pH, DOC, NO_3-NO_2^-, NH_4^+, C/N ratios and OM content are shown (n = 5 (pH, DOC, NO_3-NO_2^-, NH_4^+); n = 3 (C/N, OM)).

	Untreated	**Non Sterile**		**Sterile**	
		Inoculated	**Uninoculated**	**Inoculated**	**Uninoculated**
pH	5.02 ± 0.04	4.94 ± 0.05	4.88 ± 0.06	5.21 ± 0.21	5.42 ± 0.13
DOC [µg g^{-1}]	224.78 ± 26.59	34.29 ± 5.66	41.85 ± 5.74	66.36 ± 13.59	83.30 ± 18.30
NO_3-NO_2^- [µg g^{-1}]	2.47 ± 0.90	60.16 ± 10.40	64.07 ± 5.60	41.12 ± 14.61	12.95 ± 10.52
NH_4^+ [µg g^{-1}]	35.95 ± 6.34	5.38 ± 1.37	7.66 ± 5.77	63.19 ± 37.21	93.92 ± 22.87
C/N	17.58 ± 1.14	17.65 ± 0.35	17.99 ± 0.67	17.67 ± 0.46	17.73 ± 0.65
OM [g 100 g^{-1}]	13.02 ± 0.29	11.50 ± 0.41	11.67 ± 0.32	10.93 ± 0.23	11.05 ± 0.65

The pH of sterile soils was significantly higher than of non sterile soils (Table 2.3; General analysis of variance, $F_{1,24} = 46.35$, $p < 0.001$). The amount of DOC decreased significantly in treated soils in comparison with untreated soil (General analysis of variance, $F_{1,23} = 410.04$, $p < 0.001$) but remained significantly higher in sterile soils than in non sterile soils (General analysis of variance, $F_{1,23} = 24.45$, $p < 0.001$). The amount of NO_3-NO_2^- increased significantly in treated soils in comparison with untreated soil (General analysis of variance, $F_{1,23} = 55.76$, $p < 0.001$) and was even higher in non sterile than in sterile soils (General analysis of variance, $F_{1,23} = 48.36$, $p < 0.001$). The addition of the mycorrhizal inoculum increased the

amount of NO_3-NO_2^- in sterile soils whereas the amount of NO_3-NO_2^- remained unaffected by the inoculum in non sterile soils (General analysis of variance, $F_{1,23} = 10.11$, $p = 0.005$). The highest amounts of NH_4^+ occurred in sterile soils and non sterile soils had even lower amounts of NH_4^+ than untreated soil (General analysis of variance, $F_{1,23} = 48.13$, $p < 0.001$). The C/N ratios were not affected by any of the soil treatments. The OM content decreased in treated soils in comparison with untreated soil (General analysis of variance, $F_{1,14} = 91.98$, $p < 0.001$) but remained higher in non sterile soils than in sterile soils (General analysis of variance, $F_{1,14} = 13.46$, $p = 0.004$).

Furthermore, element concentrations of caesium (Cs), potassium (K), phosphorus (P), calcium (Ca) and magnesium (Mg) in untreated and treated soils in the absence of plants (Table 2.4 A) and in treated soils in the presence of potato or sunflower plants (Table 2.4 B) were determined.

The Cs concentrations of untreated soil were significantly lower than in treated soils (Table 2.4; General analysis of variance, $F_{3,38} = 3.35$, $p = 0.034$). Treated soils in the presence of potato or sunflower plants had significantly lower K concentrations than treated soils in the absence of plants (General analysis of variance, $F_{3,38} = 3.61$, $p = 0.026$). The P concentrations were highest in treated soils in the absence of plants (General analysis of variance, $F_{3,38} = 24.34$, $p < 0.001$). Inoculated soils had significantly higher Ca concentrations than uninoculated soils (General analysis of variance, $F_{3,38} = 13.52$, $p < 0.001$) and treated soils in the presence of plants had significantly lower Ca concentrations than in the absence of plants (General analysis of variance, $F_{3,38} = 3.81$, $p = 0.022$). The Mg concentrations in soils were not affected by any treatment.

Table 2.4: Concentrations of Cs, K, P, Ca and Mg in soil samples of untreated soil taken before the start of the experiment and of treated soils taken at harvest in the absence of plants (A) and in treated soils taken at harvest in the presence of potato and sunflower plants (B). Mean values and 95% confidence intervals (n = 3).

A	**Untreated**	**Non Sterile**		**Sterile**	
		Inoculated	**Uninoculated**	**Inoculated**	**Uninoculated**
Cs [mg g^{-1} DW]	0.003 ± 0.000	0.006 ± 0.002	0.006 ± 0.001	0.007 ± 0.004	0.007 ± 0.005
K [mg g^{-1} DW]	2.144 ± 0.507	2.269 ± 0.413	2.119 ± 0.405	2.495 ± 0.389	2.205 ± 0.294
P [mg g^{-1} DW]	1.910 ± 0.151	2.204 ± 0.176	2.408 ± 0.051	2.446 ± 0.012	2.105 ± 0.061
Ca [mg g^{-1} DW]	2.982 ± 0.213	3.437 ± 0.254	3.136 ± 0.060	3.738 ± 0.535	3.002 ± 0.208
Mg [mg g^{-1} DW]	5.579 ± 0.632	5.691 ± 0.328	5.816 ± 0.289	5.620 ± 0.103	5.886 ± 0.214

Table 2.4 (continued)

B	Non Sterile				Sterile			
	Inoculated		Uninoculated		Inoculated		Uninoculated	
	Potato	Sunflower	Potato	Sunflower	Potato	Sunflower	Potato	Sunflower
Cs [mg g^{-1} DW]	0.006 ± 0.002	0.006 ± 0.004	0.006 ± 0.002	0.006 ± 0.002	0.008 ± 0.000	0.006 ± 0.002	0.007 ± 0.001	0.006 ± 0.001
K [mg g^{-1} DW]	2.067 ± 0.827	2.017 ± 0.234	1.733 ± 0.273	1.989 ± 0.352	1.793 ± 0.339	2.065 ± 0.221	1.672 ± 0.364	1.779 ± 0.063
P [mg g^{-1} DW]	2.062 ± 0.287	1.943 ± 0.059	2.034 ± 0.150	1.960 ± 0.054	1.922 ± 0.126	1.969 ± 0.118	1.925 ± 0.049	1.948 ± 0.044
Ca [mg g^{-1} DW]	3.265 ± 0.174	3.318 ± 0.108	2.952 ± 0.033	2.974 ± 0.077	3.354 ± 0.132	3.296 ± 0.335	2.914 ± 0.211	2.893 ± 0.100
Mg [mg g^{-1} DW]	5.585 ± 0.657	5.783 ± 0.720	5.478 ± 0.121	5.455 ± 0.192	5.941 ± 0.371	5.744 ± 0.557	5.813 ± 0.431	5.431 ± 0.294

2.3.3 Plant status

Six different plant species were grown on soils containing Cs to determine phylogenetic differences in Cs accumulation.

2.3.3.1 Plant growth

After five weeks of growth, pictures of plants from each species were taken (Figure 2.4).

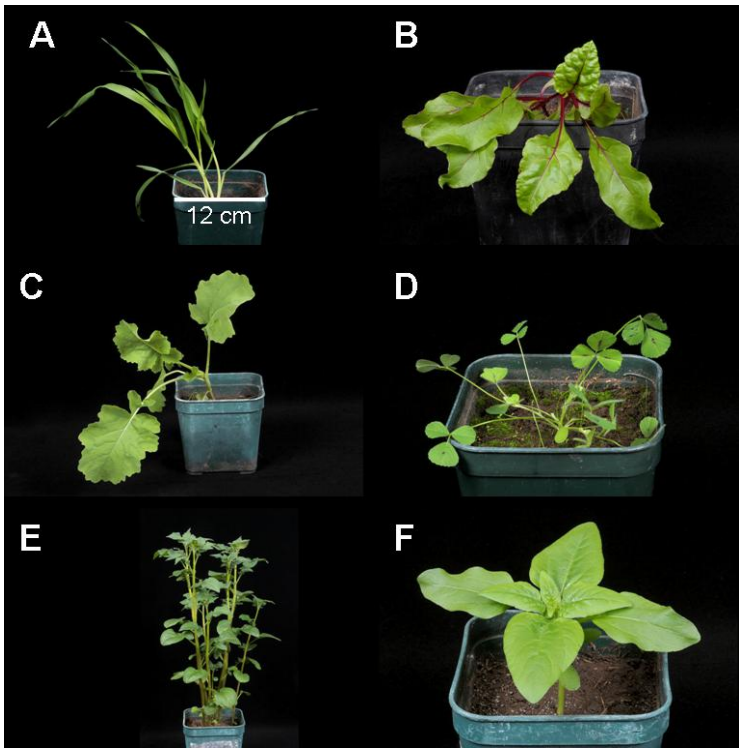

Figure 2.4: Five-week old (A) barley, (B) beetroot, (C) *Brassica*, (D) *Medicago*, (E) potato and (F) sunflower plants. All plants were grown in 12 cm by 12 cm pots.

The plants were harvested after a growth duration of six weeks and fresh weights (FWs) of roots and shoots were determined (Table 2.5). The plant materials were oven dried and dry weights (DWs) of roots and shoots were determined (Table 2.6).

Table 2.5: Fresh weights of barley, beetroot, *Brassica*, *Medicago*, potato and sunflower roots and shoots grown on non sterile or sterile soils with or without the addition of a mycorrhizal inoculum. Mean values and 95 % confidence intervals (n = 7).

Plant	**Tissue**	**Non Sterile**		**Sterile**	
		Inoculated	**Uninoculated**	**Inoculated**	**Uninoculated**
		Fresh weights [g]			
Barley	**Root**	1.490 ± 0.471	0.906 ± 0.268	1.208 ± 0.232	1.230 ± 0.137
Beetroot	**Root**	0.645 ± 0.215	0.598 ± 0.154	0.527 ± 0.165	0.326 ± 0.160
Brassica	**Root**	1.290 ± 0.285	0.806 ± 0.096	0.603 ± 0.283	0.684 ± 0.387
Medicago	**Root**	0.213 ± 0.083	0.260 ± 0.053	0.244 ± 0.141	0.283 ± 0.278
Potato	**Root**	19.034 ± 2.640	15.364 ± 6.016	13.324 ± 3.385	16.562 ± 3.774
Sunflower	**Root**	1.475 ± 0.651	1.797 ± 0.197	2.350 ± 1.100	2.600 ± 0.864
Barley	**Shoot**	12.810 ± 0.262	10.346 ± 2.144	13.560 ± 2.065	12.895 ± 5.106
Beetroot	**Shoot**	10.180 ± 3.440	9.724 ± 3.041	7.827 ± 2.303	4.856 ± 2.332
Brassica	**Shoot**	18.755 ± 2.703	17.430 ± 1.926	14.465 ± 4.984	15.570 ± 2.910
Medicago	**Shoot**	2.503 ± 1.012	2.843 ± 0.496	1.778 ± 1.130	1.080 ± 1.314
Potato	**Shoot**	47.618 ± 3.005	50.282 ± 4.071	55.790 ± 4.423	58.930 ± 3.990
Sunflower	**Shoot**	6.665 ± 2.434	8.085 ± 1.394	10.390 ± 4.132	9.632 ± 2.207

Table 2.6: Dry weights of barley, beetroot, *Brassica*, *Medicago*, potato and sunflower roots and shoots grown on non sterile or sterile soils with or without the addition of a mycorrhizal inoculum. Mean values and 95 % confidence intervals (n = 7).

Plant	**Tissue**	**Non Sterile**		**Sterile**	
		Inoculated	**Uninoculated**	**Inoculated**	**Uninoculated**
		Dry weights [g]			
Barley	**Root**	0.101 ± 0.060	0.044 ± 0.018	0.056 ± 0.016	0.068 ± 0.005
Beetroot	**Root**	0.030 ± 0.018	0.033 ± 0.021	0.028 ± 0.018	0.014 ± 0.010
Brassica	**Root**	0.053 ± 0.019	0.036 ± 0.009	0.033 ± 0.015	0.037 ± 0.013
Medicago	**Root**	0.014 ± 0.012	0.009 ± 0.006	0.009 ± 0.007	0.027 ± 0.023
Potato	**Root**	1.384 ± 0.289	1.401 ± 0.323	0.960 ± 0.534	0.961 ± 0.238
Sunflower	**Root**	0.062 ± 0.035	0.061 ± 0.012	0.061 ± 0.023	0.077 ± 0.028
Barley	**Shoot**	0.402 ± 0.101	0.357 ± 0.079	0.473 ± 0.084	0.414 ± 0.282
Beetroot	**Shoot**	0.280 ± 0.149	0.257 ± 0.097	0.255 ± 0.105	0.094 ± 0.060
Brassica	**Shoot**	0.772 ± 0.201	0.583 ± 0.091	0.509 ± 0.259	0.584 ± 0.166
Medicago	**Shoot**	0.187 ± 0.078	0.206 ± 0.039	0.107 ± 0.077	0.104 ± 0.144
Potato	**Shoot**	2.875 ± 0.362	3.220 ± 0.534	3.466 ± 0.450	3.661 ± 0.469
Sunflower	**Shoot**	0.312 ± 0.125	0.335 ± 0.136	0.347 ± 0.146	0.317 ± 0.105

For statistical analysis the FWs and DWs of roots were log transformed to the base 10 to meet the ANOVA premises. The FWs of roots of the different plant species were significantly different from each other (Table 2.5; General analysis of variance, $F_{5,119} = 212.52$, $p < 0.001$). *Medicago* roots were lightest, followed by beetroot, *Brassica*, barley and sunflower roots, and potato roots were heaviest. The FWs of shoots of the different plant species were significantly different from each other (General analysis of variance, $F_{5,120} = 865.16$, $p < 0.001$). *Medicago* shoots were lightest, followed by beetroot, sunflower, barley and *Brassica* shoots, and potato shoots were heaviest.

Fresh weights of roots and shoots were generally unaffected by any of the soil treatments. However, *Brassica* and beetroot roots from sterile soils had lower FWs than roots from non sterile soils (General analysis of variance, $F_{5,119} = 3.63$, $p = 0.005$). Furthermore, *Brassica* and beetroot shoots from sterile soils had lower FWs than shoots from non sterile soils whereas the FWs of potato shoots from sterile soils were higher than the FWs of potato shoots from non sterile soils (General analysis of variance, $F_{5,120} = 13.03$, $p < 0.001$).

The DWs of roots of the different plant species were significantly different from each other (Table 2.6; General analysis of variance, $F_{5,119} = 131.03$, $p < 0.001$). The DWs of shoots of the different plant species were also significantly different from each other (General analysis of variance, $F_{5,120} = 646.41$, $p < 0.001$). However, DWs of roots and shoots were generally unaffected by any of the soil treatments with the exception of potato shoots that had higher DWs on sterile soils than on non sterile soils (General analysis of variance, $F_{5,120} = 6.36$, $p < 0.001$).

Since DWs and FWs of all plant species were generally unaffected by any of the soil treatments (Tables 2.5 and 2.6) the values of DW or FW of each plant species from all four soil treatments were combined to calculate DW to FW ratios (Table 2.7). The plant species showed significant differences in the DW of roots to FW of roots ratios (Table 2.7; General analysis of variance, $F_{5,119} = 6.24$, $p < 0.001$) and in the DW of shoots to FW of shoots ratios (Table 2.7; General analysis of variance, $F_{5,119} = 99.19$, $p < 0.001$). The plant species also differed in their root to shoot ratios of FW (Table 2.7; General analysis of variance, $F_{5,119} = 100.85$, $p < 0.001$). However, the FWs of roots are only approximate values because roots got lost during the harvest process.

Table 2.7: Dry weight to FW ratios of roots and shoots of barley, beetroot, *Brassica*, *Medicago*, potato and sunflower plants, and FW of roots to FW of shoots ratios of barley, beetroot, *Brassica*, *Medicago*, potato and sunflower plants. Mean values and 95 % confidence intervals (n = 7).

Plant	DW_{roots}/FW_{roots}	DW_{shoots}/FW_{shoots}	FW_{roots}/FW_{shoots}
Barley	0.053 ± 0.008	0.034 ± 0.002	0.097 ± 0.012
Beetroot	0.046 ± 0.009	0.026 ± 0.004	0.067 ± 0.008
Brassica	0.062 ± 0.026	0.036 ± 0.004	0.047 ± 0.008
Medicago	0.054 ± 0.021	0.072 ± 0.009	0.145 ± 0.047
Potato	0.076 ± 0.013	0.062 ± 0.003	0.305 ± 0.042
Sunflower	0.033 ± 0.004	0.037 ± 0.005	0.230 ± 0.022

2.3.3.2 Mycorrhizal colonisation

Plant roots were stained to visualise mycorrhizal structures. Arbuscules in a sunflower root cell (A) and in a potato root cell (B, Figure 2.5) are shown.

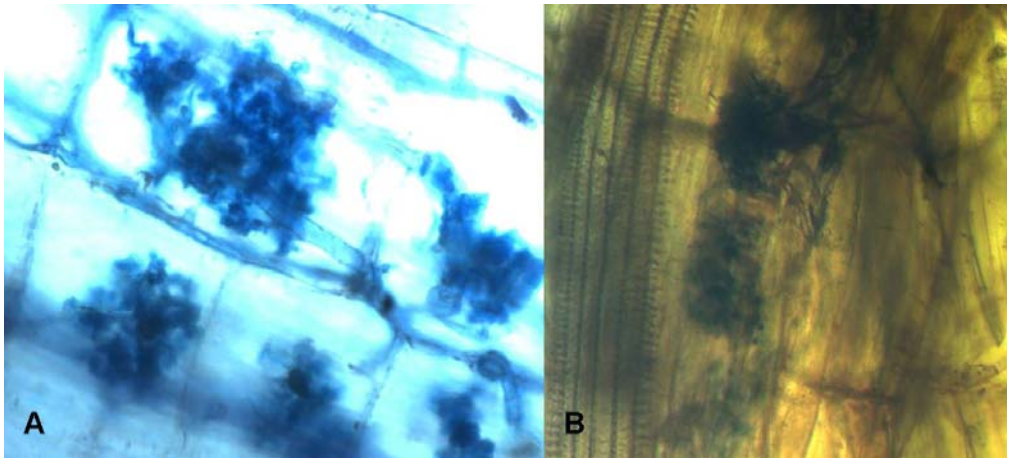

Figure 2.5: Arbuscules in a sunflower root cell (A, magnified 63 times) and in a potato root cell (B, magnified 20 times).

It was not possible to determine the percentage of mycorrhizal infection. Firstly, the staining failed for some root pieces. Secondly, large bits of the roots were too thick to be able to clearly decide if root cells were colonised. Thirdly, some roots were too dark to identify mycorrhizal structures. All the mycorrhizal structures that

could be identified clearly occurred in root cells of plants that had been grown on non sterile soils.

To clarify the mycorrhizal status of the plants, DNA was extracted from plant roots. The DNA was used to amplify a region of 800 bp of the small subunit ribosomal ribonucleic acid (SSU rRNA) gene (Lee et al., 2008) which is specific for Glomeromycota. The amplification was successful in roots of barley, *Medicago*, potato and sunflower plants which had been grown on non sterile soils with or without the addition of the mycorrhizal inoculum. None of the plants which had been grown on sterile soils, even if the mycorrhizal inoculum was applied, were mycorrhizal. *Brassica* and beetroot plants were generally non mycorrhizal. Figure 2.6 shows a gel image of PCR products amplified from potato roots.

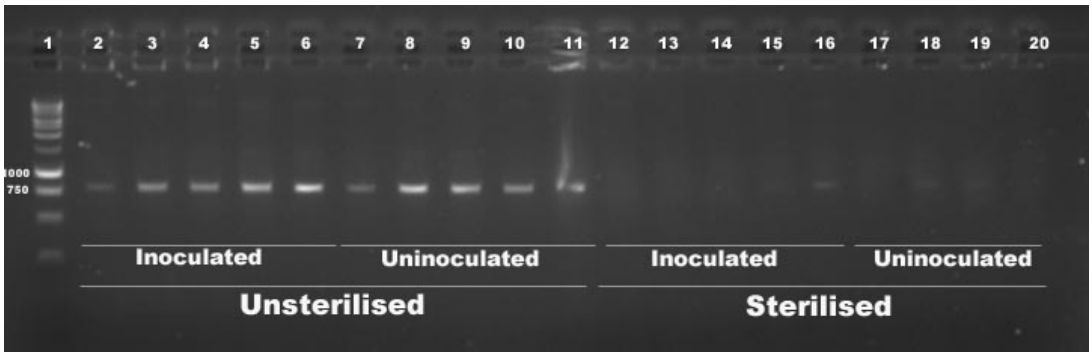

Figure 2.6: Gel image of amplified SSU rRNA gene fragments in potato roots. Lane 1 shows a 1 kb ladder. Lanes 2 to 6 show amplified products in roots that had been grown on inoculated, non sterile soils. Lanes 7 to 11 show amplified products in roots that had been grown on uninoculated, non sterile soils. Lanes 12 to 16 were loaded with PCR products from roots of plants that had been grown on inoculated, sterile soils. Lanes 12 to 16 were loaded with PCR products from roots of plants that had been grown on uninoculated, sterile soils.

2.3.4 Element concentrations in plant tissues

Concentrations of several elements were determined in roots and shoots of the six different plant species.

Caesium (Cs) concentrations in plant roots and shoots were investigated to determine the influence of mycorrhizal infection or sterilisation of soil on Cs accumulation (Table 2.8). Because Cs is transported by potassium (K) transporters the K concentrations in plant roots and shoots were measured (Table 2.9). Since phosphorus (P) is transported by mycorrhizal fungi to plants the P concentrations in

plant roots and shoots were measured (Table 2.10). Calcium (Ca) and magnesium (Mg) could influence Cs uptake. Therefore, the Ca (Table 2.11) and Mg (Table 2.12) concentrations in plant roots and shoots were measured.

Table 2.8: Caesium concentrations in roots and shoots of barley, beetroot, *Brassica*, *Medicago*, potato and sunflower plants grown on non sterile or sterile soils with or without the addition of a mycorrhizal inoculum. Mean values and 95 % confidence intervals (n = 7).

Plant	**Tissue**	**Non Sterile**		**Sterile**	
		Inoculated	**Uninoculated**	**Inoculated**	**Uninoculated**
		Cs concentrations [μg g^{-1} DW]			
Barley	**Root**	5.293 ± 4.226	6.643 ± 3.216	4.807 ± 2.128	8.195 ± 0.292
Beetroot	**Root**	5.030 ± 1.929	5.654 ± 2.577	5.392 ± 2.861	7.367 ± 0.967
Brassica	**Root**	5.159 ± 2.628	4.107 ± 1.819	4.536 ± 1.773	4.455 ± 2.747
Medicago	**Root**	10.246 ± 12.217	7.480 ± 1.626	5.269 ± 3.786	3.191 ± 0.903
Potato	**Root**	0.002 ± 0.001	0.002 ± 0.000	0.001 ± 0.000	0.002 ± 0.000
Sunflower	**Root**	10.146 ± 4.740	9.635 ± 3.222	6.519 ± 2.392	10.801 ± 3.486
Barley	**Shoot**	1.798 ± 0.416	2.967 ± 0.684	2.987 ± 0.430	3.891 ± 2.066
Beetroot	**Shoot**	2.094 ± 1.775	1.992 ± 1.249	2.177 ± 0.902	3.008 ± 1.281
Brassica	**Shoot**	2.260 ± 1.035	3.517 ± 1.043	4.296 ± 1.760	5.132 ± 0.938
Medicago	**Shoot**	0.726 ± 0.216	2.074 ± 1.564	1.029 ± 0.270	4.779 ± 1.849
Potato	**Shoot**	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
Sunflower	**Shoot**	1.643 ± 0.731	3.162 ± 0.908	2.307 ± 0.343	4.760 ± 0.857

Table 2.9: Potassium concentrations in roots and shoots of barley, beetroot, *Brassica*, *Medicago*, potato and sunflower plants grown on non sterile or sterile soils with or without the addition of a mycorrhizal inoculum. Mean values and 95 % confidence intervals (n = 7).

Plant	**Tissue**	**Non Sterile**		**Sterile**	
		Inoculated	**Uninoculated**	**Inoculated**	**Uninoculated**
		K concentrations [mg g^{-1} DW]			
Barley	**Root**	25.310 ± 4.311	28.741 ± 1.665	19.143 ± 3.793	16.242 ± 6.016
Beetroot	**Root**	70.398 ± 11.528	66.601 ± 6.958	55.216 ± 20.157	65.183 ± 26.559
Brassica	**Root**	36.047 ± 6.746	32.718 ± 3.583	21.449 ± 11.325	31.850 ± 4.136
Medicago	**Root**	31.005 ± 8.534	36.091 ± 12.133	24.546 ± 7.308	24.227 ± 13.328
Potato	**Root**	4.566 ± 1.131	4.410 ± 1.670	4.194 ± 0.590	4.453 ± 0.741
Sunflower	**Root**	49.849 ± 9.957	51.742 ± 11.373	59.423 ± 4.673	38.603 ± 14.754
Barley	**Shoot**	95.384 ± 13.921	90.607 ± 7.754	86.961 ± 8.273	88.993 ± 5.371
Beetroot	**Shoot**	74.429 ± 8.379	66.902 ± 13.676	73.465 ± 10.728	58.664 ± 15.166
Brassica	**Shoot**	57.951 ± 8.112	56.253 ± 11.339	61.870 ± 10.648	52.935 ± 6.874
Medicago	**Shoot**	55.120 ± 3.653	56.976 ± 4.734	54.167 ± 3.555	51.709 ± 9.330
Potato	**Shoot**	9.885 ± 1.432	10.282 ± 1.662	10.071 ± 0.590	10.556 ± 1.045
Sunflower	**Shoot**	56.920 ± 2.873	52.014 ± 5.034	51.461 ± 4.541	50.860 ± 6.650

Table 2.10: Phosphorus concentrations in roots and shoots of barley, beetroot, *Brassica*, *Medicago*, potato and sunflower plants grown on non sterile or sterile soils with or without the addition of a mycorrhizal inoculum. Mean values and 95 % confidence intervals (n = 7).

Plant	**Tissue**	**Non Sterile**		**Sterile**	
		Inoculated	**Uninoculated**	**Inoculated**	**Uninoculated**
		P concentrations [mg g^{-1} DW]			
Barley	**Root**	2.824 ± 0.337	3.132 ± 0.205	3.167 ± 0.239	2.970 ± 0.153
Beetroot	**Root**	4.054 ± 0.472	4.390 ± 0.872	4.924 ± 1.260	4.618 ± 1.058
Brassica	**Root**	5.805 ± 0.769	4.935 ± 0.521	4.296 ± 1.177	4.279 ± 0.999
Medicago	**Root**	3.662 ± 0.981	4.303 ± 1.956	3.517 ± 0.749	3.895 ± 1.188
Potato	**Root**	3.204 ± 0.451	2.972 ± 0.646	3.421 ± 0.430	4.224 ± 0.566
Sunflower	**Root**	3.544 ± 0.328	3.504 ± 0.201	7.007 ± 0.305	5.178 ± 1.018
Barley	**Shoot**	7.096 ± 1.319	7.171 ± 0.896	9.226 ± 1.511	9.577 ± 2.567
Beetroot	**Shoot**	3.792 ± 0.488	3.850 ± 0.399	4.814 ± 1.340	3.773 ± 0.431
Brassica	**Shoot**	5.961 ± 1.161	6.598 ± 1.486	7.453 ± 1.500	7.090 ± 1.698
Medicago	**Shoot**	3.566 ± 0.247	4.492 ± 0.215	6.366 ± 1.522	5.634 ± 1.809
Potato	**Shoot**	3.320 ± 0.682	3.639 ± 0.313	3.696 ± 0.501	4.296 ± 0.744
Sunflower	**Shoot**	6.052 ± 0.967	6.400 ± 0.735	7.195 ± 1.197	7.741 ± 0.486

Table 2.11: Calcium concentrations in roots and shoots of barley, beetroot, *Brassica*, *Medicago*, potato and sunflower plants grown on non sterile or sterile soils with or without the addition of a mycorrhizal inoculum. Mean values and 95 % confidence intervals (n = 7).

Plant	**Tissue**	**Non Sterile**		**Sterile**	
		Inoculated	**Uninoculated**	**Inoculated**	**Uninoculated**
		Ca concentrations [mg g^{-1} DW]			
Barley	**Root**	5.122 ± 2.181	3.761 ± 1.227	4.282 ± 2.137	2.542 ± 0.418
Beetroot	**Root**	3.592 ± 0.545	3.275 ± 0.347	3.196 ± 1.446	2.719 ± 1.024
Brassica	**Root**	7.642 ± 2.272	4.840 ± 1.173	3.047 ± 0.985	3.550 ± 1.019
Medicago	**Root**	5.849 ± 0.828	6.064 ± 1.477	4.137 ± 1.849	4.950 ± 1.280
Potato	**Root**	1.631 ± 0.231	1.392 ± 0.327	1.468 ± 0.344	1.348 ± 0.207
Sunflower	**Root**	7.761 ± 1.514	6.466 ± 1.059	5.878 ± 1.065	4.905 ± 0.732
Barley	**Shoot**	12.335 ± 1.740	12.296 ± 1.616	7.397 ± 0.775	7.154 ± 3.571
Beetroot	**Shoot**	16.172 ± 1.087	16.180 ± 2.463	10.464 ± 1.403	9.107 ± 1.728
Brassica	**Shoot**	23.816 ± 3.562	25.444 ± 2.637	16.019 ± 4.484	14.448 ± 0.722
Medicago	**Shoot**	25.920 ± 1.993	22.204 ± 1.018	21.225 ± 2.439	18.119 ± 6.682
Potato	**Shoot**	2.103 ± 0.112	2.044 ± 0.273	2.418 ± 0.131	2.264 ± 0.311
Sunflower	**Shoot**	32.723 ± 3.226	35.708 ± 3.170	25.357 ± 6.412	21.964 ± 5.383

Table 2.12: Magnesium concentrations in roots and shoots of barley, beetroot, *Brassica*, *Medicago*, potato and sunflower plants grown on non sterile or sterile soils with or without the addition of a mycorrhizal inoculum. Mean values and 95 % confidence intervals (n = 7).

Plant	**Tissue**	**Non Sterile**		**Sterile**	
		Inoculated	**Uninoculated**	**Inoculated**	**Uninoculated**
		Mg concentrations [mg g^{-1} DW]			
Barley	**Root**	3.173 ± 0.658	3.245 ± 0.852	2.544 ± 0.652	2.644 ± 0.004
Beetroot	**Root**	3.981 ± 0.671	3.472 ± 0.546	3.039 ± 0.825	3.124 ± 0.480
Brassica	**Root**	2.635 ± 0.510	1.798 ± 0.395	2.413 ± 0.314	2.002 ± 0.571
Medicago	**Root**	3.368 ± 0.771	3.511 ± 1.343	3.005 ± 0.836	3.276 ± 1.852
Potato	**Root**	3.370 ± 0.273	3.326 ± 0.379	3.241 ± 0.323	3.095 ± 0.327
Sunflower	**Root**	3.826 ± 0.662	3.280 ± 0.191	3.162 ± 0.217	3.337 ± 0.429
Barley	**Shoot**	3.574 ± 0.561	3.255 ± 0.371	2.152 ± 0.205	2.142 ± 0.326
Beetroot	**Shoot**	6.605 ± 2.588	7.029 ± 1.962	4.047 ± 1.543	3.501 ± 0.640
Brassica	**Shoot**	3.407 ± 0.450	3.643 ± 0.398	2.492 ± 0.273	2.206 ± 0.168
Medicago	**Shoot**	3.443 ± 0.266	3.187 ± 0.028	3.034 ± 0.543	2.624 ± 0.298
Potato	**Shoot**	2.482 ± 0.267	2.623 ± 0.563	2.341 ± 0.214	2.273 ± 0.377
Sunflower	**Shoot**	6.101 ± 0.549	5.645 ± 0.847	4.714 ± 0.666	3.890 ± 0.659

For statistical analysis element concentrations in roots and shoots were log transformed to the base 10 to meet the ANOVA premises.

The Cs concentrations in roots of the different plant species differed significantly (Table 2.8; General analysis of variance, $F_{5,113} = 864.81$, $p < 0.001$). Potato plants had significantly lower Cs concentrations in roots than the other five species and sunflower plants had significantly higher Cs concentrations in roots than the other five species. The Cs concentrations in roots of barley, beetroot, *Brassica* and *Medicago* plants did not differ significantly. None of the soil treatments influenced Cs concentrations in roots. The Cs concentrations in shoots of the different plant species differed significantly (Table 2.8; General analysis of variance, $F_{5,118} = 1434.24$, $p < 0.001$). Potato plants had significantly lower Cs concentrations in shoots than the other five species. Caesium concentrations in shoots increased in the order of *Medicago*, beetroot, sunflower, barley and *Brassica* plants. Generally, plants that had been grown on non sterile soils had significantly lower Cs concentrations in their shoots than plants that had been grown on sterile soils (General analysis of variance, $F_{1,118} = 30.90$, $p < 0.001$). In addition, the application of the mycorrhizal inoculum lowered the Cs concentrations in shoots (General analysis of variance, $F_{1,118} = 40.76$, $p < 0.001$). *Medicago* and sunflower plants were driving this effect because they showed much lower Cs concentrations in shoots when grown on inoculated soils than on uninoculated soils. Newly developed tubers from potato plants did not accumulate Cs.

Potassium concentrations in roots differed significantly between plant species (Table 2.9; General analysis of variance, $F_{5,119} = 172.93$, $p < 0.001$). Potato plants had the lowest K concentrations in roots followed by barley, *Medicago*, *Brassica* and sunflower plants with beetroot having the highest K concentrations in roots. Generally, plants grown on non sterile soils had higher K concentrations in their roots than plants grown on sterile soils (General analysis of variance, $F_{1,119} = 19.45$, $p < 0.001$). Potassium concentrations in shoots differed significantly between plant species (Table 2.9; General analysis of variance, $F_{5,120} = 750.53$, $p < 0.001$). Potato plants had significantly lower K concentrations in shoots than the other five species which showed more similar K concentrations in their shoots. Plants grown on inoculated soils had higher K concentrations in their shoots than those from uninoculated soils (General analysis of variance, $F_{5,120} = 4.03$, $p = 0.048$).

Tissue K to Cs ratios differed between plant species. There was no relationship between K and Cs concentrations in roots (Figure 2.7) or shoots (Figure 2.8) of the different plant species.

The P concentrations in roots of the different plant species differed significantly (Table 2.10; General analysis of variance, $F_{5,117} = 19.79$, $p < 0.001$). Phosphorus concentrations in barley roots were lowest and P concentrations in sunflower and *Brassica* roots were highest. Generally, plants grown on non sterile soils had lower P concentrations in their roots than plants grown on sterile soils (General analysis of variance, $F_{1,117} = 9.10$, $p = 0.003$). The influence of sterilisation on P concentrations in roots was plant species dependent because barley, potato and sunflower roots contained less P on non sterile soils whereas *Brassica* roots contained more P on non sterile soils and beetroot and *Medicago* roots did not show differences (General analysis of variance, $F_{5,117} = 10.63$, $p < 0.001$). The P concentrations in shoots of the different plant species differed significantly (Table 2.10; General analysis of variance, $F_{5,120} = 80.97$, $p < 0.001$). The lowest P concentrations occurred in potato and beetroot shoots and the highest P concentrations occurred in barley shoots. The P concentrations in shoots of plants grown on non sterile soils were lower than in shoots of plants grown on sterile soils (General analysis of variance, $F_{5,120} = 42.45$, $p < 0.001$).

The Ca concentrations in roots of the different plant species were significantly different from each other (Table 2.11; General analysis of variance, $F_{5,118} = 60.98$, $p < 0.001$). Potato plants had very low Ca concentrations in their roots and *Medicago* and sunflower plants had the highest Ca concentrations in their roots. Generally, plants grown on non sterile soils had significantly higher Ca concentrations in roots than plants grown on sterile soils (General analysis of variance, $F_{1,118} = 36.20$, $p < 0.001$). The addition of the mycorrhizal inoculum increased the Ca concentrations in roots (General analysis of variance, $F_{1,118} = 4.17$, $p = 0.044$) of all plant species except for *Medicago*. The Ca concentrations in shoots of the different plant species were significantly different from each other (Table 2.11; General analysis of variance, $F_{5,120} = 656.66$, $p < 0.001$). Like in their roots, potato plants had low Ca concentrations in shoots and sunflower plants had high Ca concentrations in shoots. Generally, plants grown on non sterile soils had significantly higher Ca concentrations in shoots than plants grown on sterile soils (General analysis of variance, $F_{1,120} = 136.32$, $p < 0.001$). The only exception was

potato which did not differ in shoot Ca concentrations when grown on sterile or non sterile soils.

The Mg concentrations in roots of the different plant species were significantly different (Table 2.12; General analysis of variance, $F_{5,118} = 11.87$, $p < 0.001$). The lowest Mg concentrations in roots occurred in *Brassica* plants and the highest in sunflower plants. Roots of all plant species contained more Mg when grown on non sterile soils than on sterile soils (Table 2.12; General analysis of variance, $F_{1,118} = 8.03$, $p = 0.006$). The Mg concentrations in shoots of the different plant species were significantly different (Table 2.12; General analysis of variance, $F_{5,118} = 54.57$, $p < 0.001$). In shoots, the Mg concentrations were lowest in potato plants and highest in sunflower plants. Generally, plants that had been grown on non sterile soils had higher Mg concentrations in shoots than plants that had been grown on sterile soils (General analysis of variance, $F_{1,118} = 100.07$, $p < 0.001$). Exceptions were Mg concentrations in *Medicago* and potato shoots which did not differ dependent on soil sterilisation (General analysis of variance, $F_{5,118} = 4.98$, $p < 0.001$).

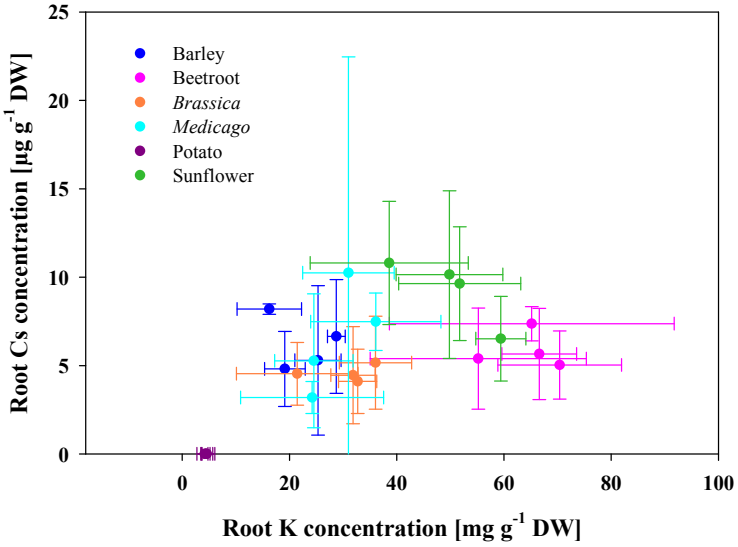

Figure 2.7: Caesium concentrations in roots *versus* K concentrations in roots of barley, beetroot, *Brassica*, *Medicago*, potato and sunflower plants grown on non sterile or sterile soils with or without the addition on a mycorrhizal inoculum. Data show mean values and 95% confidence intervals (Tables 2.8 and 2.9).

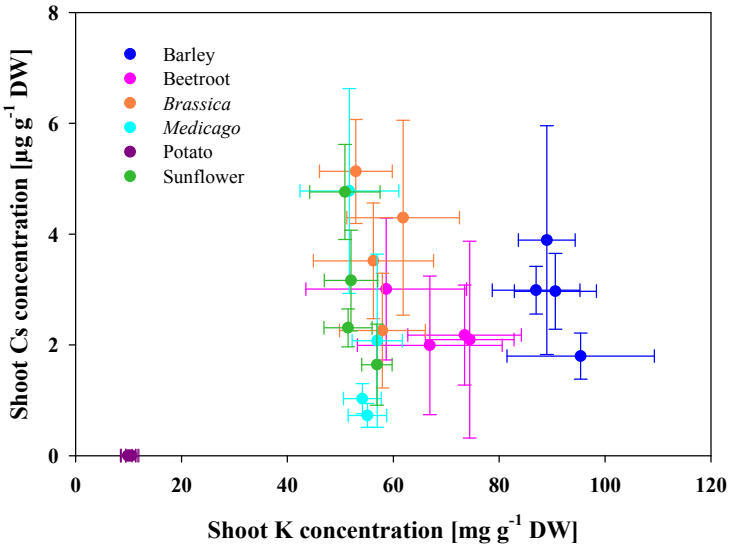

Figure 2.8: Caesium concentrations in shoots *versus* K concentrations in shoots of barley, beetroot, *Brassica*, *Medicago*, potato and sunflower plants grown on non sterile or sterile soils with or without the addition on a mycorrhizal inoculum. Data show mean values and 95% confidence intervals (Tables 2.8 and 2.9).

2.4 DISCUSSION

2.4.1 Soil characteristics were influenced by sterilisation, mycorrhizal inoculum and plant growth

Gamma-irradiation is a reliable method for soil sterilisation that causes only minimal alteration of physical properties of soil and leaves no chemical contamination (McLaren, 1969; McNamara et al., 2003). However, the process does have some effects on soil chemical properties. Gamma-sterilisation at 25 kGy kills all fungi and most, but not all, of the bacteria in soil (Ramsay and Bawden, 1983; McNamara et al., 2003).

The untreated soil was very dry at sampling and had not been fertilised since 1996 (Bennett, J., personal communication). The addition of KNO_3 as fertiliser increased the concentration of NO_3 in treated soils (Table 2.3). The amounts of dissolved organic carbon (DOC) and organic matter (OM) decreased in treated soils in comparison to untreated soil (Table 2.3). Since the treated soils were fertilised and watered during the experiment the growth rate of micro organisms might have increased and thereby the micro organisms might have taken up carbon or decayed OM. The amounts of OM remained higher in non sterile soils than in sterile soils but the amount of DOC was higher in sterile than in non sterile soils (Table 2.3). This could be due to destruction of OM and thereby release of DOC by gamma-sterilisation (Berns et al., 2008). Furthermore, DOC is readily utilised as a substrate by micro organisms (Marschner and Bredow, 2002). Therefore, a higher microbial activity in non sterile soils could have led to a lower amount of DOC in non sterile soils than in sterile soils. The process of gamma-sterilisation releases NH_4^+ from soils (Salonius et al., 1967; McLaren, 1969) but reduces the concentration of NO_3^- (Ramsay and Bawden, 1983; McNamara et al., 2003). These effects of sterilisation also occurred in the experiment performed here (Table 2.3). Additionally, the gamma-sterilisation increased the pH of the soils (Table 2.3). However, reports on the effect of sterilisation on pH are not consistent (McNamara et al., 2003). The concentration of NH_4^+ in non sterile soils was lower than in untreated soil (Table 2.3) which could be explained by uptake of NH_4^+ by micro organisms. The addition of the mycorrhizal inoculum increased the concentration of NO_3^- in sterile soils but had no effect in non sterile soils (Table 2.3). The total amounts of nitrogen, i.e. the sum of the concentrations of NO_3^- and NH_4^+, were the same in uninoculated, sterile soils and

in inoculated, sterile soils. In the process of nitrification, NH_4^+ is converted in NO_3^- by bacteria (Prosser, 1989). Therefore, the mycorrhizal inoculum might have contained nitrifying bacteria which then converted NH_4^+ to NO_3^- and thereby led to an increase of NO_3^- in inoculated, sterile soils.

The addition of CsCl to soil before the start of the experiment resulted in an increase of the Cs concentrations in treated soils (Table 2.4). Since the total plant uptake was less than 1% of the total available Cs, plant growth did not cause Cs concentrations to decrease in treated soils (Table 2.4). Neither sterilisation nor the addition of the mycorrhizal inoculum affected K or P concentrations in treated soils but plant growth reduced the concentrations of these elements in soils due to their uptake (Table 2.4). The addition of the mycorrhizal inoculum increased the Ca concentrations in inoculated soils (Table 2.4). Nevertheless, plants took up significant amounts of Ca and decreased the Ca concentration in soils with plants (Table 2.4).

Soil characteristics influence Cs sorption to soil particles and Cs uptake by plants. Rhodes (1957) showed that the adsorption of ^{137}Cs by calcareous soil from nutrient solutions was not affected by varying the pH between 4 and 10. Reports on the effect of pH on Cs uptake by plants are not consistent. Heredia et al. (2002) showed that pH in the range of 6.5 to 9 did not influence Cs uptake by the liverwort *Riccia fluitans* from nutrient solutions under K sufficient conditions. In contrast, Drissner et al. (1998) demonstrated higher transfer factors of Cs to fern (*Dryopteris carthusiana*) from forest soils with low pH. However, the pH of the forest soils was dependent on the kind of humus deposit and thickness of humus layer (Drissner et al., 1998). The authors concluded that the effect of pH and humus layer on transfer factors of Cs to fern could not be evaluated independently (Drissner et al., 1998). The higher concentrations of NH_4^+ in sterile soils than in non sterile soils (Table 2.3) could result in higher Cs accumulation by plants because NH_4^+ mobilises Cs from soil sorption sites (Konopleva et al., 2009). Caesium binds strongly to clay minerals (Coughtrey and Thorne, 1983; Livens and Rimmer, 1988) and the influence of OM on Cs uptake or sorption is thought to be minimal (Staunton and Levacic, 1999; Chibowski and Zygmunt, 2002) and only of importance in soil with an OM content above 95% (Rigol et al., 2002).

2.4.2 Growth differences and mycorrhizal colonisation of the different species

The six plant species barley, beetroot, *Brassica*, *Medicago*, potato and sunflower showed differences in fresh weights (FWs) of roots and in FWs of shoots. Potato roots and shoots were heaviest and *Medicago* roots and shoots were lightest (Table 2.5). Generally, the FWs of roots and shoots were unaffected by soil sterilisation or application of the mycorrhizal inoculum, but plant specific interactions with soil treatments occurred (Table 2.5). The dry weights (DWs) of roots and shoots of the six plants species were also not affected by any of the soil treatments (Table 2.6). The plant species differed in their root to shoot FW ratios (Table 2.7). *Brassica* and beetroot plants had the lowest root to shoot FW ratios and potato had the highest. The lower the ratio the less root is available to supply shoots with water and minerals. Difficulties in harvesting fine roots of *Brassica* and beetroot plants could have resulted in the low ratios for these species observed in the experiment performed here (Table 2.7).

The plants were examined for their mycorrhizal status. Successful colonisation occurred in plants growing on non sterile soils, except for *Brassica* and beetroot plants. *Brassica napus* belongs to the Brassicaceae which are generally non mycorrhizal (Tester et al., 1987; Brundrett, 2009). *Beta vulgaris* belongs to the Chenopodiaceae, whose members are also predominantly non mycorrhizal (Tester et al., 1987; Brundrett, 2009). None of the plants that had been grown on sterile soils were colonised. This shows that gamma-sterilisation was effective in elimination of arbuscular mycorrhizal (AM) fungi from soil which is consistent with literature (Ramsay and Bawden, 1983; McNamara et al., 2003). The application of the commercially available inoculum of mycorrhizal fungi did not result in mycorrhizal infection in any of the plant species. Gamma-sterilisation increases the concentration of exchangeable P in soil (McNamara et al., 2003). The concentrations of P in sterile soils might have prevented mycorrhizal colonisation of plant roots because the development of the symbiosis is sensitive to high P concentrations in soil (Thomson et al., 1986; Braunberger et al., 1991). Another reason for the lack of mycorrhizal colonisation of plants grown on inoculated, sterile soil could be that the inoculum did not contain any live AM fungal hyphae or spores.

2.4.3 Soil treatments and plant phylogeny influenced mineral accumulation of plants

Caesium was accumulated by all plant species over the growth duration of six weeks (Table 2.8). Generally, roots contained more Cs than shoots, which is in agreement with some other publications (Belli and Sansone, 1995; Waegeneers et al., 2001; Staunton et al., 2003). It is important to keep in mind that Cs concentrations measured in roots might be high due to soil contamination. Cook et al. (2007) demonstrated that, although roots were washed with distilled water after harvest, most of the measured Cs came from soil residues. They stated that many transfer factors for Cs obtained in plants sampled from natural sites are too high because soil contamination is not taken into account.

The plant species investigated here differed in their Cs accumulation (Table 2.8). Broadley et al. (1999a) reviewed Cs accumulation by different plant species and stated that dicots generally accumulated more Cs than monocots and that the highest Cs accumulations were found in Caryophyllales. Field experiments were excluded from the study because the authors stated that additional factors like foliar uptake could influence the data obtained for Cs accumulation (Broadley et al., 1999a). Furthermore, the meta-analysis contains data from long term accumulation experiments (e.g. Andersen, 1967) as well as short term exposure experiments (e.g. Broadley and Willey, 1997). Soil conditions can influence Cs accumulation by plants greatly (Andersen, 1967; Skarlou et al., 1996; Waegeneers et al., 2009) and plant specific interactions with soil type occur. It is therefore not surprising that there is no correlation (Figure 2.9) between shoot Cs concentrations in plant species measured here (Table 2.8) and relative Cs concentrations in shoots of plant species described by Broadley et al. (1999a). There is also no correlation (Figure 2.10) between Cs concentrations in shoots of the plant species measured in the experiment described here (Table 2.8) and Cs concentrations in plant shoots measured by Andersen (1967). Andersen (1967) investigated long term Cs accumulation by 44 plant species in pot experiments. The plants were grown on fertilised agricultural soil that was contaminated with ^{137}Cs.

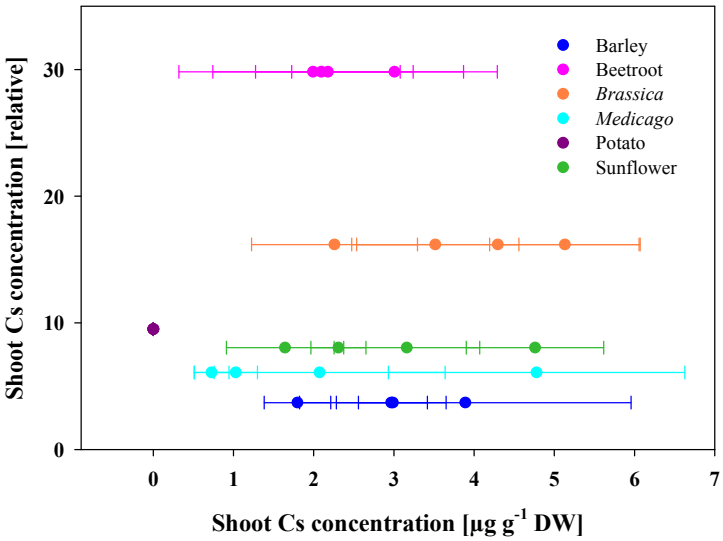

Figure 2.9: Caesium concentrations in shoots of the different plant species are not correlated with relative Cs concentrations given for the same species by Broadley et al. (1999a). Data show mean values and 95% confidence intervals (Table 2.8).

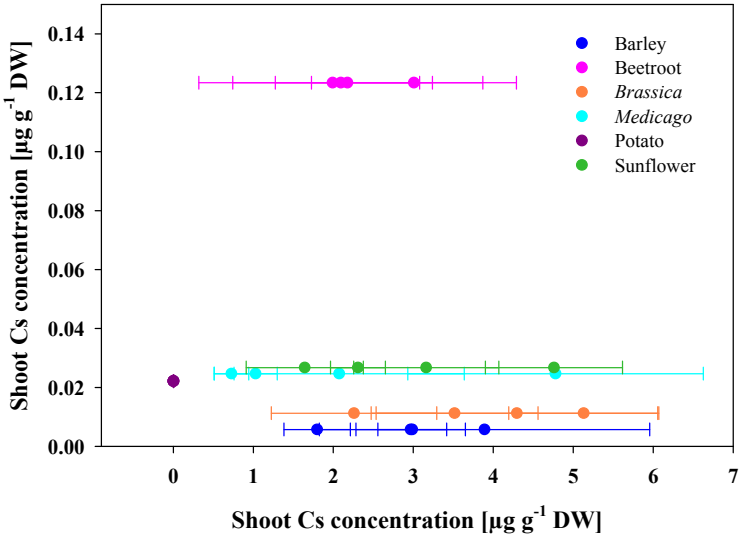

Figure 2.10: Caesium concentrations in shoots of the different plant species are not correlated with shoot Cs concentrations [µg g^{-1} DW] observed for the same species by Andersen (1967). Data show mean values and 95% confidence intervals (Table 2.8).

Andersen (1967) concluded that root crops tended to accumulate most Cs in their shoots but that no botanical group could be characterised by extremely high or low Cs concentrations. This is in agreement with Choi et al. (2009) who measured long term Cs accumulation by five plant species. Choi et al. (2009) demonstrated that tissue Cs concentrations varied during plant development in all plant species and therefore a conclusion about phylogenetic differences in Cs accumulation by plant species was not possible. Vegetables grown in private gardens in Bulgaria did not show any significant differences in tissue Cs concentrations at all (Djingova and Kuleff, 2002) and Cs concentrations in shoots of wild plants were generally very variable within plant families (Cook et al., 2007).

In many studies, monocot species accumulated less Cs in their shoots than dicot species (reviewed by Broadley et al., 1999a). However, in the experiment reported here, barley did not show the lowest root or shoot Cs concentrations (Table 2.8). Other researchers have shown that grass species had higher Cs concentrations in their shoots than dicot species grown under the same conditions (Belli and Sansone, 1995; Gouthu et al., 1997). Although beetroot belongs to the order of Caryophyllales, whose members had been shown to accumulate high concentrations of Cs in their shoots (Evans and Dekker, 1967; Gouthu et al., 1997; Broadley et al., 1999a), beetroot did not show the highest shoot Cs concentrations in the experiment reported here (Table 2.8). Tang and Willey (2003) investigated Cs accumulation by four Asteraceae species and two varieties of *Beta vulgaris* and measured higher Cs concentrations in shoots of the Asteraceae species. Potatoes had very low tissue Cs concentrations in the experiment presented here (Table 2.8). The Cs concentrations in potato roots and shoots were around 0.001 μg g^{-1} DW which is considerably less than reported for potato in other experiments (Andersen, 1967; Broadley et al., 1999a). However, potatoes accumulated Cs concentrations of 0.004–0.13 μg g^{-1} DW in their tubers from soil which contained 1–11 mg Cs per kg soil (Tsukada and Nakamura, 1999). This is a considerably lower concentration than found in any of the other plant species investigated in the experiment described here (Table 2.8). Additionally, the K concentrations found in potatoes were also very low at around 10 mg g^{-1} DW (around 1% DW) in shoots (Table 2.9). The critical value of K for plants is around 0.5–2% DW (Leigh and Wyn Jones, 1984; White and Karley, 2010). However, Tsukada and Nakamura (1999) found concentrations of K in tubers of potatoes of around 20 mg g^{-1} DW. While these are higher K concentrations than in

potatoes investigated in the experiment presented here (Table 2.9) the concentrations are still lower than in any other species investigated here which contained more than 50 mg g^{-1} DW of K (Table 2.9).

Furthermore, differences in Cs uptake by plant species are also dependent on K supply. It was demonstrated that the ranking of species for accumulation of Cs changes with changing K supply (Buysse et al., 1996). Nevertheless, there was no constant Cs to K ratio in roots or shoots for any of the plant species (Figures 2.10 and 2.11). This is in agreement with other publications which stated that there is no relationship between Cs and K concentrations in shoots (Andersen, 1967; Evans and Dekker, 1967; Buysse et al., 1996). Potassium concentrations in shoots measured in this experiment were correlated with K concentrations obtained by Andersen (1967). Figure 2.11 shows the relationship.

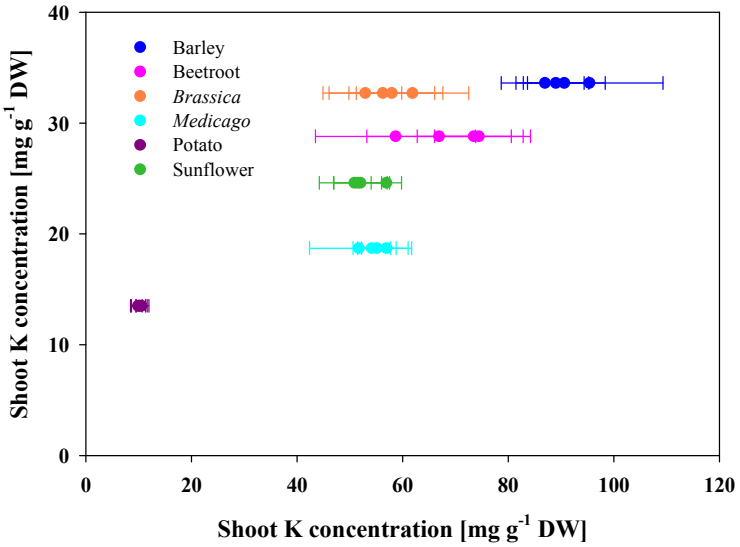

Figure 2.11: Potassium concentrations in shoots of the different plant species are correlated (Pearson correlation, $r = 0.84$, $p < 0.001$, d.f. = 24) with K concentrations [mg g^{-1} DW] observed for the same species by Andersen (1967). Data show mean values and 95% confidence intervals (Table 2.9).

If sufficient K is available plant species maintain their tissue K concentrations in a specific range. Generally, K sufficient plants contain between 2–10% DW K (Leigh and Wyn Jones, 1984; Ashley et al., 2006; White and Karley, 2010) and except for potato this was the case for the plants investigated in the experiment described here (Table 2.9). By contrast, Cs accumulation is dependent on soil properties and plant–soil interactions and because Cs is non essential for plants

(White and Broadley, 2000) it is not maintained at a specific concentration by plants. In conclusion, Cs does not behave as analogue of K.

The concentrations of Cs in shoots from plants grown on non sterile soils were lower than from plants grown on sterile soils (Table 2.8). Furthermore, the concentrations of K in roots were higher on non sterile soils than on sterile soils (Table 2.9). In acidic soils K acquisition can be enhanced by AM fungi but the effect depends on the fungal strain (Clark and Zeto, 2000). Although reports of the influence of AM fungi on Cs uptake by plants are not consistent (Table 1.2) it has been hypothesised that if AM fungi led to improved K status of plants then the uptake of Cs would decrease due to changes in the expression of genes encoding K transporters (Wiesel et al., 2008; White et al., 2010). Plants that were grown on non sterile soils were mycorrhizal whereas plants grown on sterile soils were non mycorrhizal. One explanation for decreased Cs accumulation in shoots of plants grown on non sterile soils could be that AM fungi decreased Cs accumulation in plant shoots. However, gamma-sterilisation of soil changes the chemical properties of soil and releases NH_4^+ (McLaren, 1969). Higher concentrations of NH_4^+ in sterile soils than in non sterile soils (Table 2.3) could have lead to the mobilisation of Cs from soil sorption sites (Konopleva et al., 2009). A greater availability of Cs to plants in sterile soils could have lead to higher concentrations of Cs in plant shoots grown on sterile soils than on non sterile soils. It therefore remains uncertain whether gamma-sterilisation of soil led to greater Cs accumulation by plants or if AM fungi decreased Cs accumulation by plants.

The addition of the mycorrhizal inoculum decreased Cs uptake (Table 2.8). The inoculum contained Ca (Table 2.4) and Ca inhibits Cs uptake under K-replete conditions (White and Broadley, 2000; Hampton et al., 2005). Under K-replete conditions Cs is transported by voltage insensitive cation channels (White and Broadley, 2000; Hampton et al., 2005) which are blocked by Ca. The Ca dependent uptake of Cs was also demonstrated in the liverwort *Riccia fluitans* which showed decreasing Cs accumulation due to increasing Ca supply under K-replete conditions (Heredia et al., 2002). In accordance with the decrease in Cs uptake an increase in K uptake occurred due to the addition of the mycorrhizal inoculum (Table 2.9). Due to the inhibition of voltage insensitive cation channels by Ca the transport of K *via* K^+/H^+ symporters might have been stimulated leading to higher K concentrations in shoots (Qi et al., 2008).

Potassium concentrations are related to water contents of plants because K has an important osmotic function in plants (Leigh and Wyn Jones, 1984). Broadley et al. (2004) reported that shoot K concentrations were positively related to shoot FW/DW ratios in several plant species. Plants investigated in the experiment presented here also showed a weak correlation between shoot K concentrations and FW/DW ratios of shoots (Pearson correlation, $r = 0.43$, $p = 0.0355$, d.f. = 24).

Phosphorus (P) concentrations in roots and shoots were higher in sterile soils than in non sterile soils (Table 2.10) because gamma-sterilisation increases the concentration of exchangeable P in soil (McLaren, 1969; McNamara et al., 2003). Critical concentrations of P in plants are around 0.3–0.5% DW (Marschner, 1995) but these values are highly species dependent (Broadley et al., 2004; White and Hammond, 2008). In the experiment conducted, all plants had P concentrations of above 0.3% DW (Table 2.10) and were therefore sufficient in P. One of the key functions of arbuscular mycorrhizal fungi is the supply of P to their host plants and high availability of P to plants can be disadvantageous for mycorrhizal colonisation (Smith and Read, 2008). However, it has been shown that total P concentrations in shoots are not always correlated with the contribution of AM fungi to plant P uptake (Smith et al., 2003, 2004). Flax (*Linum usitatissimum*), medic (*Medicago truncatula*) and tomato (*Lycopersicon esculentum*) had been grown in association with different species of *Glomus* on soils with limited P (Smith et al., 2003). A part of the soil that was only accessible to fungal hyphae was labelled with ^{33}P. Therefore, labelled P could only reach plants *via* hyphae of AM fungi (mycorrhizal pathway) whereas both roots and AM fungi could take up unlabeled P. Accumulation of P in mycorrhizal and non mycorrhizal plants was measured and the results showed that the mycorrhizal pathway can be exclusively responsible for plant P uptake (Smith et al., 2003). However, the contribution of the mycorrhizal P uptake pathway to total P uptake by plants varies with plant and fungal species (Smith et al., 2003, 2004). Phosphorus concentrations in shoots were not correlated to the percentage of P that was delivered *via* the mycorrhizal pathway (Smith et al., 2003, 2004). Therefore, it is possible that the plant species investigated here received P from their fungal partners although the percentage of mycorrhizal colonisation could not be determined and shoot P concentrations were higher in non mycorrhizal than in mycorrhizal plants (Table 2.10).

Magnesium (Mg) and calcium (Ca) concentrations in shoots differed among different species (Tables 2.11 and 2.12). The lowest Mg and Ca concentrations occurred in potato and barley plants and the highest Mg and Ca concentrations were measured in sunflower plants. Positive correlations between Mg and Ca concentrations in shoots across species have been reported (Broadley et al., 2004). Broadley et al. (2004) concluded that general correlations in Mg/Ca concentrations are likely due to chemical similarities between these elements and the resulting lack of selectivity during uptake. Shoot Mg/Ca ratios were correlated in the six plants species investigated here (Figure 2.12).

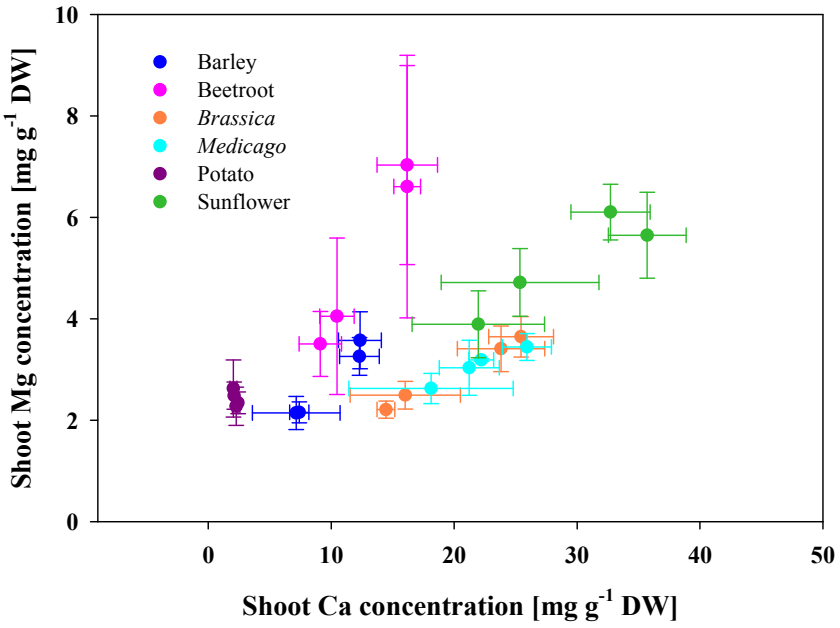

Figure 2.12: Magnesium concentrations in shoots of the different plant species are correlated with Ca concentrations in shoots (Pearson correlation, $r = 0.56$, $p = 0.0047$, d.f. = 24). Data show mean values and 95% confidence intervals (Tables 2.11 and 2.12).

Barley, as a member of the Poaceae, had low Mg and Ca concentrations in shoots (Figure 2.12), which has been ascribed to the lower pectin concentration in cell walls of monocots (White and Broadley, 2003). Sunflower shoots had slightly higher Mg and Ca concentrations (Figure 2.12) which is in agreement with Broadley et al. (2004). Beetroot, as a member of the order Caryophyllales, had high Mg concentrations in shoots but the Ca concentrations were similar to the other plant species (Figure 2.12). Since Caryophyllales are adapted to mineral rich soils it has

been hypothesised that this is the reason for high Mg accumulation (Broadley et al., 2004). Potatoes had unusually low Ca concentrations in their shoots and roots in the experiment reported here (Figure 2.12). Both Mg and Ca concentrations were higher in plants that had been grown on non sterile soils than in plants that had been grown on sterile soils (Tables 2.11 and 2.12). The amounts of these ions did not differ in sterile or non sterile soils (Table 2.4). One explanation for higher Mg and Ca concentrations in shoots of mycorrhizal plants could be that AM fungi transported these elements to their host plants. Clark and Zeto (2000) reviewed mineral acquisition by AM plants and reported that AM fungi can enhance Mg and Ca concentrations in plants mainly on acidic soils. Alternatively, the availability of these elements to plants was altered by the sterilisation process. In agreement with higher Ca concentrations in soils due to the application of the mycorrhizal inoculum, plants that had been grown on inoculated soils had higher Ca concentrations than ones that had been grown on uninoculated soils (Table 2.11). In conclusion, soil treatments and plant phylogeny influenced mineral acquisition by plants.

2.5 CONCLUSIONS

- *Beta vulgaris* and *Brassica napus* plants were non mycorrhizal
- *Helianthus annuus*, *Hordeum vulgare*, *Medicago truncatula* and *Solanum tuberosum* plants were colonised by AM fungi in non sterile soils but not in sterile soils irrespective of the addition of the mycorrhizal inoculum
- Caesium concentrations in roots and shoots were dependent on plant species
- Caesium concentrations in plant shoots observed in the experiment reported here were not correlated with published data of shoot Cs concentrations in the same plant species
- Potassium concentrations in plant shoots observed in the experiment reported here were correlated with published data by Andersen (1967) of K concentrations in shoots of the same plant species
- The Cs to K ratios were not constant for the different plant species
- Caesium concentrations in shoots were lower in plants grown on non sterile soils than in plants grown on sterile soils. Therefore, Cs accumulation by plants was either increased because of higher availability of Cs to plants due to gamma-sterilisation of soil or decreased due to colonisation with AM fungi

- Calcium in the mycorrhizal inoculum decreased Cs concentrations in plant shoots
- Magnesium concentrations in plant shoots were correlated to calcium concentrations in plant shoots

Chapter 3:

Optimisation of growth conditions for *Medicago truncatula* under *in vitro* conditions to achieve mycorrhizal infection with *Glomus* sp.

3.1 INTRODUCTION

3.1.1 *Medicago truncatula*

Medicago truncatula Gaertn. belongs to the Fabaceae, colloquially known as legumes. Fabaceae have more than 650 genera and 18,000 species (Doyle, 1994; Young et al., 2003) and are the third largest family of angiosperms (Doyle, 1994). They are one of the most important agricultural taxa worldwide and present a major source of protein for humans and animals (Cook, 1999). Due to rhizobial symbioses legumes are able to fix atmospheric nitrogen and therefore provide nitrogen for plant growth and soil improvement (Cook, 1999; Rose, 2008). Within the Fabaceae, *M. truncatula* is part of the subfamily Papilionoideae and the genus *Medicago* that contains 83 species (Small and Jomphe, 1988; Young et al., 2003). *Medicago truncatula* is an annual plant of Mediterranean origins (Small and Jomphe, 1988; Barker et al., 1990) and is well adapted to semi-arid conditions.

In 1990, Barker et al. proposed *M. truncatula* as a model plant for legumes. Within the Fabaceae, *Medicago sativa* is a forage crop of importance worldwide (Barker et al., 1990) but it is tetraploid with a genome of 800–900 million base pairs (bp). The advantage of *M. truncatula* is that it has a small, diploid genome (2n = 16) of around 500 million bp (Cook, 1999), which is roughly three to four times larger than the genome of *Arabidopsis thaliana* (Penmetsa and Cook, 2000). Furthermore, *M. truncatula* is self-fertilising, has efficient seed production and a rapid generation time (Cook, 1999). There exists considerable genetic variability within the species (Oldroyd and Geurts, 2001) but due to the self-fertilising character each ecotype is relatively homogeneous from the genetic point of view (Barker et al., 1990). The cultivar Jemalong is a commercial variety of *M. truncatula* which has been widely used in research. The genotype A17 is a single-seed descendent line from the cultivar Jemalong and is the reference genotype selected for sequencing of the genome (Kamphuis et al., 2007). A more detailed description of the genome of *M. truncatula* can be found in Chapter 6. Regeneration of plants is a requirement for transformation

(Rose, 2008). The genotype Jemalong 2HA is highly regenerable (Rose et al., 1999) and is able to be transformed by *Agrobacterium tumefaciens* and to regenerate *via* somatic embryogenesis (Chabaud et al., 1996).

Medicago truncatula forms symbioses with mycorrhizal fungi and with rhizobia which makes it an excellent model for studies of these interaction, allowing direct comparisons between mycorrhizal and rhizobial symbioses (Cook, 1999; Oldroyd and Geurts, 2001).

3.1.2 Phosphorus transporters in *Medicago truncatula*

Phosphorus (P) is an essential element for plant growth and development (Marschner, 1995; Chiou et al., 2001; White and Hammond, 2008). Plants absorb P as inorganic orthophosphate (P_i) from the soil solution (Hammond et al., 2004; Bucher, 2007; White and Hammond, 2008). The P_i concentration in soil solution can be as low as 1 μM (Versaw et al., 2002), because most of the P in soil exists in complex, insoluble, organic or inorganic forms and can therefore not be acquired by plants directly (White and Hammond, 2008). Hence, P is one of the most unavailable and inaccessible macronutrients in the soil and frequently limits plant growth (Chiou et al., 2001; Vance et al., 2003; Hammond et al., 2004). Plants developed strategies to increase P mobilisation which include secretion of phosphatases, organic acids and protons, enhanced root growth and changes in root architecture (Chiou et al., 2001; Versaw et al., 2002; Bucher, 2007; White and Hammond, 2008). In *M. truncatula*, root hair development occurred closer to the root tip and the length of root hairs increased under P-deficient conditions in comparison with nutrient sufficient plants (Bucciarelli et al., 2006).

Since the concentration of cytoplasmic P_i in roots is higher than the P_i concentration in soil, P_i uptake occurs against a large concentration gradient (Mudge et al., 2002). Proton-coupled P_i symporters transport P_i across the plasma membrane. Therefore, the driving force for P_i uptake is the H^+-gradient across the plasma membrane generated by H^+-ATPases (Bucher et al., 2001; Smith, F.W. et al., 2003). Plants contain two families of H^+-coupled P_i transporters, Pht1 and Pht2 (Bucher et al., 2001; Bucher, 2007). Members of the Pht1 family are mainly high-affinity transporters which are thought to be responsible for the soil-to-plant transfer of P (Bucher et al., 2001; Mudge et al., 2002; Shin et al., 2004), whereas members of the

Pht2 family are mainly low-affinity transporters which are thought to be responsible for the root-to-shoot transfer of P (Bucher et al., 2001; Mudge et al., 2002).

Medicago truncatula possesses both high and low affinity systems for P uptake. Two high affinity transporters MtPt1 and MtPt2 have been characterised in *M. truncatula* (Liu et al., 1998; Chiou et al., 2001). These transporters share 91.6% sequence identity and 98% protein identity. They are integral membrane proteins with 12 membrane-spanning domains and show high transcript levels under low P conditions. Both *MtPt1* and *MtPt2* are exclusively expressed in roots and their expression is induced in response to phosphate deprivation (Liu et al., 1998). However, the transcript level of *MtPt2* is more responsive to P deprivation than the transcript level of *MtPt1* (Liu et al., 1998). Western blot analysis revealed that the abundance of the MtPt1 protein mirrors the gene expression (Chiou et al., 2001) supporting the hypothesis that the phosphate starvation induced expression of the *MtPt1* gene is at least partly controlled at the transcriptional level. Fusion of a green fluorescent protein (GFP) gene to the *MtPt1* gene demonstrated that MtPt1 is located in the plasma membrane (Chiou et al., 2001). Furthermore, *in situ* hybridisation and immunolocalisation of MtPt1 showed that the transporter is specific to epidermal cells and root hairs (Chiou et al., 2001).

3.1.3 Phosphorus transporters in mycorrhizal *Medicago truncatula* plants

Many plants have developed another strategy to increase P uptake, a symbiotic association between fungi and plants called arbuscular mycorrhiza (AM). Fossil spores from the Ordovician which resemble spores of modern *Glomus* species (Redecker et al., 2000) and spores and vesicle like structures in early land plants from the Rhynie Chert in Scotland (Pirozynski and Malloch, 1975) suggest that an early form of the AM symbiosis evolved 450 million years ago and that the symbiosis was required for the colonisation of land by plants (Pirozynski and Malloch, 1975; Simon et al., 1993; Redecker et al., 2000; Schüßler et al., 2001; Bonfante and Genre, 2008). Within the symbiosis the fungi transport P to their host plants and gain carbohydrates in return (Harrison, 1997; Smith and Read, 2008). The acquisition of P from soil by AM fungi is significant because fungal hyphae can grow beyond the P depletion zone of host roots (Smith and Read, 2008). In some cases, AM colonisation can result in complete inactivation of the direct P_i uptake pathway *via* root hairs and epidermis and uptake of P_i then occurs solely *via* the

mycorrhizal pathway (Smith et al., 2003, 2004). Like plants, AM fungi have a low and a high affinity system for P_i uptake (Versaw et al., 2002). Within the fungal hyphae P is transported as polyphosphates. These are hydrolysed in arbuscules and P_i is transferred to the periarbuscular space (Javot et al., 2007). Plant transporters are responsible for the P_i uptake from the periarbuscular space (Karandashov and Bucher, 2005; Bucher, 2007). In *M. truncatula* a P_i transporter from the Pht1 family of plant P_i transporters MtPt4 has been characterised (Harrison et al., 2002). The sequence of *MtPt4* is significantly different from other plant P_i transporters and the *M. truncatula* genome does not contain other closely related sequences. The *MtPt4* gene encodes a low affinity phosphate transporter that is exclusively expressed in mycorrhizal roots. Western blot analysis demonstrated increasing levels of MtPt4 with increasing colonisation of roots with AM fungi and antibody staining confirmed that MtPt4 is located in the periarbuscular membrane (Harrison et al., 2002). Antibody staining suggested that the abundance of MtPt4 is coordinated with the life of arbuscules because the staining was strongest in mature arbuscules, decreased in degenerating arbuscules and was not detectable in young arbuscules (Harrison et al., 2002). Expressed in yeast, MtPt4 showed maximum activity at pH4 (Harrison et al., 2002) which is consistent with the acidic pH of the periarbuscular space (Guttenberger, 2000). Shoot P concentrations of *M. truncatula* RNAi lines lacking MtPt4 were not increased due to mycorrhizal colonisation in contrast to shoot P concentrations in wild type plants. This demonstrated that MtPt4 is responsible for P_i uptake in mycorrhizal roots of *M. truncatula* (Javot et al., 2007). Colonisation rates of RNAi lines lacking MtPt4 were significantly lower than of wild type plants. The loss of MtPt4 affected the development of the AM fungus *Glomus versiforme* within the root cells and the fungus did not develop extraradical hyphae (Javot et al., 2007). The absence of MtPt4 results in a block in P_i transfer from the arbuscule to the cortical cell which leads to premature death of arbuscules. The symbiosis is unable to develop further (Javot et al., 2007). These findings demonstrate that MtPt4 is responsible for P_i uptake in mycorrhizal roots which is in agreement with a down regulation of the expression of *MtPt1* and *MtPt2* during mycorrhizal colonisation (Liu et al., 1998; Versaw et al., 2002).

3.1.4 Phosphorus availability influences mycorrhizal colonisation

In general, the amount of mycorrhizal colonisation decreases when nutrients are in high abundance (Parniske, 2008). In particular, increasing P availability and plant P status decrease colonisation of plant roots by AM fungi (Thomson et al., 1986; Braunberger et al., 1991). The whole development of the symbiosis, in particular intraradical development of the fungus in the root cortex is reduced at high P supply (Javot et al., 2007). In addition, the growth of extraradical hyphae is reduced (Thomson et al., 1986). It was suggested that the lifespan of arbuscules is influenced by their ability to deliver P_i and probably other nutrients (Parniske, 2008). Plants appear to have the opportunity to maintain efficient arbuscules and to remove inefficient arbuscules by their early degradation.

3.1.5 Aim

The aim of this chapter was to determine the growth conditions required for successful colonisation of *M. truncatula* roots with *Glomus* sp. under *in vitro* conditions. Therefore, plants and fungi were grown in association on media containing different concentrations of P. The presence of mycorrhizal infection was judged by hyphal growth within the medium and by the development of new fungal spores.

3.2 MATERIAL AND METHODS

3.2.1 Organisms

Medicago truncatula Gaertn. var. *truncatula* Jemalong A17 was chosen as the experimental plant. The seeds were obtained from the South Australian Research and Development Institute (SARDI), Genetic Resource Centre, Australia.

The arbuscular mycorrhizal fungus *Glomus* sp. MUCL 43195 was used. The strain was obtained from the Glomeromycota In Vitro Collection (GINCO), Louvain-la-Neuve, Belgium. This strain was collected in Ontario, Canada, from indigenous soil in a sand dune habitat from the host plant *Ammophila breviligulata* (Dalpé et al., 1992).

3.2.2 Arbuscular Mycorrhizal–Plant (AM-P) *in vitro* culture system

The Arbuscular Mycorrhizal–Plant (AM-P) *in vitro* culture system was first described by Dupré de Boulois et al. (2006). The culture system consists of two compartments: a shoot compartment where the plant shoots grow in a centrifuge tube and a root compartment where the plant roots and the fungal hyphae grow in a Petri dish (Figure 3.1). The system was built by making a hole of 5 mm diameter in the lid of a sealed Petri dish (Sterilin Limited, Caerphilly, UK) and by cutting the bottom of a centrifuge tube (Sarstedt Aktiengesellschaft and Co., Nümbrecht, Germany) at a slight angle. A hole of 10 mm diameter was made in the lid of the centrifuge tube. The centrifuge tube was glued on to the Petri dish with hot melting glue (Bosch 11 x 200 mm, Metabowerke GmbH, Nürtingen, Germany) and an adhesive microfiltration disc (Tissue Quick Plant Laboratory, New Milton, UK) was placed over the hole in the lid of the centrifuge tube to allow gas exchange. The systems were then gamma-sterilised with a radiation dose of 25 kGy (Isotron plc, Swindon, UK).

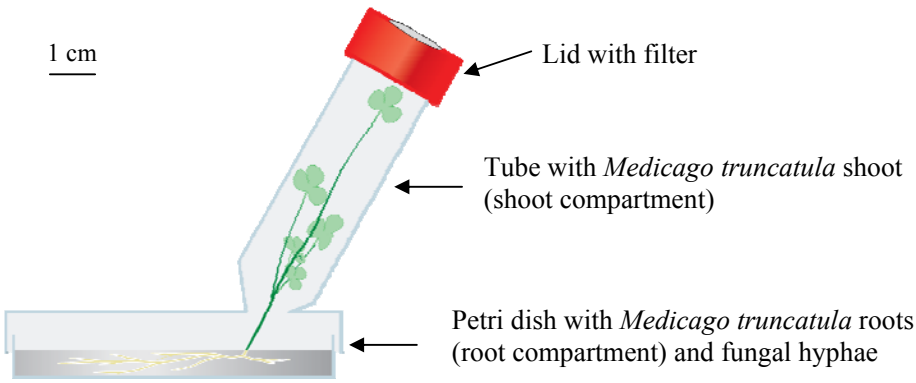

Figure 3.1: Arbuscular Mycorrhizal–Plant (AM-P) *in vitro* culture system to grow *Medicago truncatula*. Figure drawn by Ian Pitkethly, SCRI.

3.2.3 Media composition

Different growth media were used to grow *Medicago truncatula* in the AM-P *in vitro* culture system. One medium was modified Strullu-Romand (MSR) medium (Declerck et al., 1998, modified from Strullu and Romand, 1986; Table 3.1). The pH of the MSR medium was adjusted to 5.5 with NaOH and the MSR medium was solidified using 3 g l^{-1} Phytagel (Sigma Aldrich, St. Louis, MO, USA). Element analysis of Phytagel using ICP-MS showed that Phytagel contained 38 µM P which increased the absolute concentration of P in solidified MSR medium to 68 µM.

Table 3.1: Element composition of modified Strullu-Romand (MSR) medium.

component	mM
KNO_3	0.752
KCl	0.872
KH_2PO_4	0.030
$Ca(NO_3)_2 . 4H_2O$	1.520
$MgSO_4 . 7H_2O$	2.998
NaFeEDTA	0.020
	µM
H_3BO_3	29.920
$ZnSO_4 . 7H_2O$	0.974
$CuSO_4 . 5H_2O$	0.961
$Na_2MoO_4 . 2H_2O$	0.009
$MnSO_4 . H_2O$	11.005
$(NH_4)_6Mo_7O_{24} . 4H_2O$	0.028

Furthermore, variations of modified Hoagland's medium (Hoagland and Arnon, 1938) were used. Two different K concentrations of 1 and 5 mM were combined with three different P concentrations of 10, 20 and 50 µM. The increased cation concentration in the medium containing 5 mM K was counterbalanced by reducing the Ca concentration from 9 mM to 5 mM. The basal medium was called MH (1K, 20P) and the element concentrations are given in Table 3.2. A summary of the MH media with different K, P and Ca concentrations is given in Table 3.3. The pH of the MH media was adjusted to 5.5 with NaOH and the MH media were solidified using 6 g l^{-1} agar because agar does not contain P (A1296, Sigma Aldrich, St. Louis, MO, USA).

Table 3.2: Element composition of modified Hoagland's (MH (1K, 20P)) medium.

component	mM
KNO_3	1
$Ca(NO_3)_2 . 4H_2O$	9
$MgSO_4 . 7H_2O$	2
NH_4NO_3	1
NaFeEDTA	0.02
	µM
H_3BO_3	46.00
$ZnSO_4 . 7H_2O$	0.77
$CuSO_4 . 5H_2O$	0.32
$Na_2MoO_4 . 2H_2O$	0.50
H_3PO_4	20.00

Table 3.3: Overview about the different MH media with K, P and Ca concentrations. The accompanying anion to adjust K and Ca concentrations was NO_3^-.

MH	1K, 10P	1K, 20P	1K, 50P	5K, 10P	5K, 20P	5K, 50P
K [mM]	1	1	1	5	5	5
P [µM]	10	20	50	10	20	50
Ca [mM]	9	9	9	5	5	5

3.2.4 Cultivation of *Glomus* sp. on modified carrot roots

Since AM fungi are obligate symbionts they cannot be cultivated without a host plant (Chabaud et al., 2006). To obtain fungal spores to inoculate experimental plants, *Glomus* sp. was cultivated on modified carrot (*Daucus carota* L.) roots. These roots have a remarkable growth potential due to the transfer of root-inducing (Ri) plasmid genes of *Agrobacterium rhizogenes* to the plant (Mugnier and Mosse, 1987). This hairy root line was established by Bécard and Fortin (1988) and has become the most widespread host for monoxenic cultivation of AM fungi (Cranenbrouck et al., 2005).

The procedure for cultivating modified carrot roots is described in Cranenbrouck et al. (2005). Straight, white root apices were placed in sterile Petri plates containing MSR medium (Table 3.1). Since the roots were non autotrophic, 29.21 mM sucrose (Sigma Aldrich, St. Louis, MO, USA) and 1.88 µM calcium panthothenate, 0.004 µM biotin, 8.1 µM nicotinic acid, 4.38 µM pyridoxine, 2.96 µM thiamine, 0.29 µM cyanocobalamine (Sigma Aldrich, St. Louis, MO, USA) were added to the MSR medium. The apices were cut from three-week old roots and transferred to fresh plates. Two roots orientated in opposite directions were placed in each plate and the apices were pushed gently into the medium with sterile forceps (Figure 3.2). The Petri plates were sealed with Parafilm (Sigma Aldrich, St. Louis, MO, USA) and incubated at 27°C in darkness. Because the roots have negative geotropism the plates were stored in an inverted position to enable root growth within the medium. The procedure was repeated every three weeks to have a continuous culture of modified carrot roots.

Figure 3.2: Picture of modified carrot roots grown on MSR medium with sucrose and vitamins. Two carrot roots are shown which had been transferred to the medium three days previously.

The carrot roots were used for the cultivation of *Glomus* sp. The procedure for cultivating AM fungi on modified carrot roots is described in Cranenbrouck et al. (2005). One half of a split Petri dish was filled with MSR medium up to the height of the separating wall. The other half of the plate was filled with MSR medium containing sucrose and vitamins such that the medium formed a bulb (Figure 3.3). The side with MSR medium containing sucrose and vitamins served as the root compartment, where root and hyphal growth took place. The side with MSR medium served as the hyphal compartment, where only hyphal growth occurred.

A two-week old carrot root of 7–8 cm length was placed on the MSR medium with sucrose and vitamins and the apex was pushed gently into the medium. *Glomus* spores were extracted from older culture plates for inoculation of the carrot roots. Therefore, a piece of medium containing spores was cut out with a sterile scalpel and placed into an empty Petri dish. The gel was cut into small pieces and a citrate buffer containing 1.8 mM citric acid and 8.2 mM sodium citrate was added to dissolve the agar. The buffer was added to the gel by sterile filtration (Acrodisc Syringe Filter, 25 mm, 0.2 µm Supor membrane, Pall Corporation, Ann Arbor, MI, USA). After dissolution of the gel the spores were collected with a pipette (20–200 µl, Eppendorf UK Limited, Cambridge, UK) under a binocular microscope and then transferred to sterile distilled water. Afterwards, around 70–100 spores were placed on to the carrot root. The plates were incubated at 27°C in darkness in an inverted position. When successful infection took place the fungal hyphae crossed the wall and grew into the MSR medium because the fungus preferred sugar free medium for growth of extraradical mycelium. Root growth was repressed in the hyphal compartment by

cutting the root tips which crossed the wall. After a couple of months the fungus produced new spores (Figure 3.4).

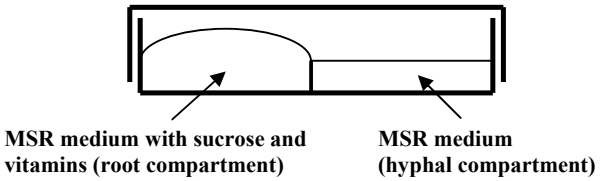

Figure 3.3: Split Petri dish with MSR medium containing sucrose and vitamins as the root compartment on one side and MSR medium as the hyphal compartment on the other side.

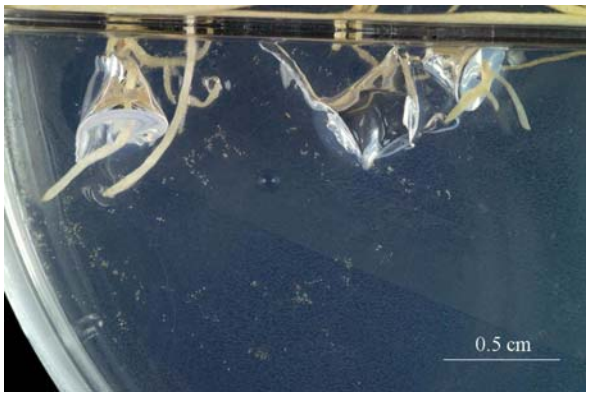

Figure 3.4: Picture of colonised modified carrot roots and newly developed *Glomus* sp. spores. The spores are formed on the extraradical mycelium of *Glomus* sp. that grows in the hyphal compartment (Figure 3.3).

3.2.5 Experimental setup

Medicago truncatula was grown under *in vitro* conditions with or without the symbiont *Glomus* sp. To sterilise the *Medicago* seeds they were washed with sodium hypochlorite solution (VWR International Ltd., Poole, UK) for 12 min followed by several washing steps with sterilised distilled water. The seeds were then placed on media under sterile conditions using sterile forceps. Seed germination occurred at 27°C in darkness. Four days later plates containing the seedlings were transferred to a growth cabinet held at 22°C with 16 h light and 8 h darkness and the seedlings were allowed to develop for three days. Seedlings whose roots had been growing inside the media, whose roots were at least 3–5 cm long and which had dark green cotyledons were chosen for the experiments.

The seedlings were placed into AM-P *in vitro* culture systems such that the shoots could develop in the shoot compartment and the roots would grow on medium

in the root compartment (Figure 3.1). Therefore the *in vitro* system was opened in a laminar flow cabinet and a Petri dish filled with medium was placed under the lid. Using sterile forceps the shoot of a plant seedling was pushed carefully through the hole in the lid of the Petri dish into the centrifuge tube. The root was placed on the medium and the apex was pushed gently into the medium. For the mycorrhizal plants at least 50 *Glomus* spores per plant were added to the roots using a pipette. The systems were sealed with Parafilm.

3.2.5.1 Growth of Medicago truncatula *plants on MSR medium*

Medicago truncatula plants were grown on MSR medium (Table 3.1). Half of the plants were inoculated with *Glomus* sp. spores and the remaining plants were used as non mycorrhizal controls. The plants were grown for six weeks in a growth cabinet with a photoperiod of 16 h light and 8 h darkness and a constant temperature of 22°C. The light intensity was between 125 and 200 μmol m^{-2} s^{-1}. The experiment was performed four times with more than ten replicates in each treatment.

3.2.5.2 Growth of Medicago truncatula *plants on MH media*

Medicago truncatula plants were grown on MH media (Tables 3.2, 3.3). On MH (1K, 20P) 12 plants were grown as non mycorrhizal controls and 18 plants were inoculated with *Glomus* sp. spores. On MH (1K, 10P) and MH (1K, 50P) 18 plants were grown as non mycorrhizal controls and 12 plants were inoculated with *Glomus* sp. spores. On MH (5K, 10P) and MH (5K, 20P) 8 plants were grown as non mycorrhizal controls and 12 plants were inoculated with *Glomus* sp. spores. On MH (5K, 50P) 15 plants were grown as non mycorrhizal controls and 5 plants were inoculated with *Glomus* sp. spores. The plants were grown in a growth cabinet with a photoperiod of 16 h light and 8 h darkness and a constant temperature of 22°C. The light intensity for plants grown on MH media containing 1 mM K was around 350 μmol m^{-2} s^{-1}. The light intensity for plants grown on MH media containing 5 mM K was around 125 μmol m^{-2} s^{-1}. The reason for the different light intensities was that only one growth cabinet fitted with light bulbs with a capacity of 350 μmol m^{-2} s^{-1} was available. One uninoculated plant of each treatment was harvested after six weeks. All survived uninoculated plants and plants inoculated with *Glomus* sp. were harvested after nine weeks. Shoots were separated from roots and the roots were gently pulled out of the solidified medium and washed in sterile

distilled water. Shoots of all replicates and roots of all replicates were combined for each treatment and oven dried at 40–50°C for several days.

3.2.6 Investigation of mycorrhizal colonisation

The presence of extraradical mycelium and fungal spores was determined six, seven, eight and nine weeks after inoculation using a binocular microscope (Olympus UK Ltd., Watford, UK). Because arbuscular mycorrhiza is an obligate symbiosis (Smith and Read, 2008) the fungi can only develop new spores when mycorrhizal colonisation is successful. Plants were determined as mycorrhizal when newly produced spores were present.

3.2.7 Determining of elemental concentrations

Element concentrations were determined in plant roots and shoots using ICP-MS as described in Section 2.2.5. The measurements were undertaken without any tomato leaves standards. Therefore, no drift correction was performed.

3.3 RESULTS

3.3.1 No mycorrhizal infection of *Medicago truncatula* on MSR medium

Medicago truncatula was grown in the presence or absence of *Glomus* sp. on MSR medium. The experiment was performed four times with more than ten replicates in each treatment. After six weeks the plant roots were observed for mycorrhizal colonisation using a binocular microscope. Although plant growth was successful no mycorrhizal infection occurred. No hyphal growth within the medium or development of new spores could be detected. Some spores of *Glomus* sp. germinated but hyphal growth was not directed towards the plant roots.

3.3.2 Mycorrhizal infection of *Medicago truncatula* on MH media

Medicago truncatula was grown in the presence or absence of *Glomus* sp. on MH media. Mycorrhizal infection was investigated six, seven, eight and nine weeks after inoculation. All plants grown on MH (1K, 10P) and MH (1K, 20P) media showed branching hyphae and development of new spores after eight weeks but the amounts of hyphae and spores were not quantified. More spores had been produced after nine weeks. Two plants grown on MH (1K, 50P) medium showed hyphal

branching after nine weeks of growth but no development of new spores. The remaining plants grown on MH (1K, 50P) medium did not shown signs of mycorrhizal infection. None of the plants grown on MH media containing 5 mM K showed mycorrhizal infection irrespective of the P concentration in the media.

3.3.3 Phosphorus concentrations in *Medicago truncatula* on MH media

Phosphorus concentrations in roots and shoots of six-week old uninoculated *M. truncatula* plants grown on MH (1K, 10P), MH (1K, 20P), MH (1K, 50P), MH (5K, 10P), MH (5K, 20P) and MH (5K, 50P) media are shown in Figure 3.5.

The amount of K in the media did not seem to have an effect on P concentrations in shoots of *M. truncatula* plants. Since only one plant was investigated for each treatment no conclusion could be drawn whether external K supply affected P concentrations in roots of plants grown with an external P supply of 50 μM P. Plants grown on media with 50 μM P had higher P concentrations in their shoots than plants grown on media with 10 or 20 μM P.

Phosphorus concentrations in roots and shoots of nine-week old inoculated and uninoculated *M. truncatula* plants grown on MH (1K, 10P), MH (1K, 20P) and MH (1K, 50P) are shown in Figure 3.6. Mycorrhizal infection seemed to increase the P concentrations in plants grown on media with 10 μM P. No increase in P concentrations in shoots seemed to occur due to mycorrhizal colonisation in plants grown on media with 20 μM P. None of the plants grown on media with 50 μM P were colonised by *Glomus* sp.

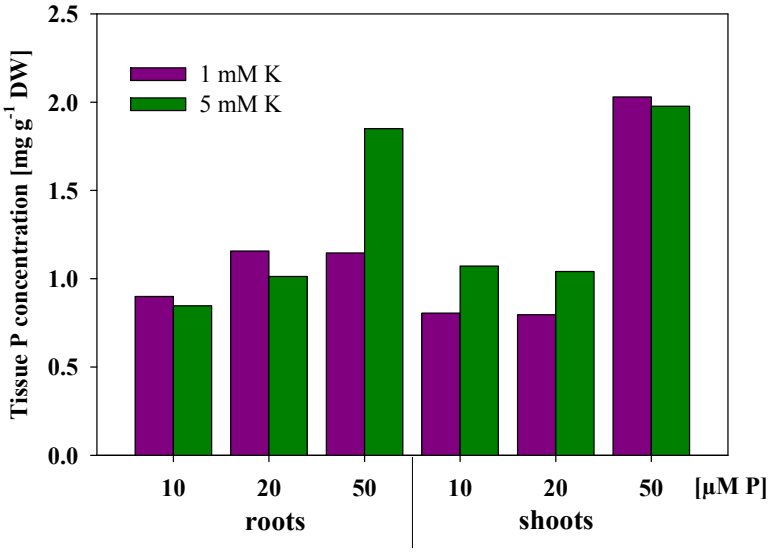

Figure 3.5: Phosphorus concentrations [mg g^{-1} DW] in roots and shoots of six-week old *Medicago truncatula* plants (n = 1) grown on MH (1K, 10P), MH (1K, 20P), MH (1K, 50P), MH (5K, 10P), MH (5K, 20P) and MH (5K, 50P) media.

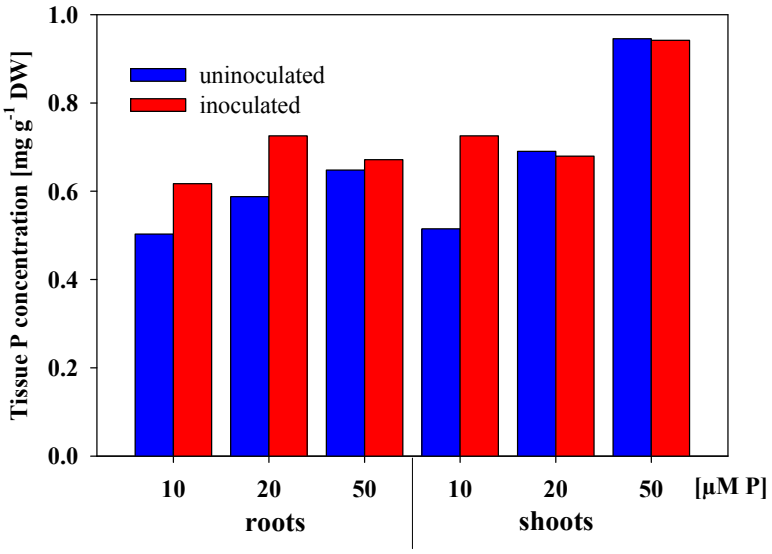

Figure 3.6: Phosphorus concentrations [mg g^{-1} DW] in roots and shoots (n = 1) of nine-week old inoculated and uninoculated *Medicago truncatula* plants grown on MH (1K, 10P), MH (1K, 20P) and MH (1K, 50P). Inoculated plants grown on MH (1K, 10P) and MH (1K, 20P) were mycorrhizal whereas inoculated plants grown on MH (1K, 50P) were not infected by *Glomus* sp.

3.4 DISCUSSION

An Arbuscular Mycorrhizal–Plant (AM-P) *in vitro* culture system to grow mycorrhizal *Medicago truncatula* plants for investigating ion transport by mycorrhizal fungi and accumulation by plants was developed by Dupré de Boulois et al. (2006). The *in vitro* system with MSR medium as growth medium (Declerck et al., 1998) was successfully applied to study transport of radiocaesium by mycorrhizal fungi to their host plant *M. truncatula* (Dupré de Boulois et al., 2006) and to investigate the influence of potassium (K) and phosphorus (P) on radiocaesium transport (Gyuricza et al., 2010a). Nevertheless, when *M. truncatula* was grown on MSR medium in association with *Glomus* sp. in the experiment presented here no mycorrhizal colonisation occurred although the plants grew well. The total P concentration in solidified MSR medium was 68 µM. Furthermore, when *M. truncatula* was grown on MH (1K, 50P) medium with a total concentration of 50 µM P no mycorrhizal infection occurred. Successful mycorrhizal colonisation of plants depends on several factors. One of the key factors is the availability of P. Since improved P uptake is the main benefit of the AM symbiosis (Parniske, 2008; Smith and Read, 2008) high P availability to plants influences colonisation with AM fungi (Thomson et al., 1986; Braunberger et al., 1991). The percentage of root length colonised by AM fungi of maize (*Zea mays*) and *Trifolium subterraneum* plants was reduced when the plants were supplied with high external P concentrations (Thomson et al., 1986; Braunberger et al., 1991). No depletion of P was detected in the hyphal compartment by external hyphae of *G. intraradices* when mycorrhizal roots were supplied with high concentrations of P (Versaw et al., 2002). However, since roots and hyphae had access to the high P supply in the root compartment the hyphae growing in the root compartment could have transported P to their hosts. Furthermore, P is transported bidirectional in external hyphae which could be one of the reasons that no P depletion was detected in the hyphal compartment. Therefore, the experiment conducted by Versaw et al. (2002) does not necessarily lead to the conclusion that mycorrhiza do not transport P to their host plants when P supply is high. When *M. truncatula* was grown on MH (1K, 10P) and MH (1K, 20P) media, with lower P concentrations of 10 µM and 20 µM P respectively, the plants were successfully infected with *Glomus* sp.

By contrast, the AM symbiosis was not developed when *M. truncatula* was grown on MH media containing 5 mM K even when the P concentration was low

(MH (5K, 10P) and MH (5K, 20P)). There could be at least two reasons for this. First, the AM association might be restricted by high K availability. Second, plants on MH media containing 5 mM K were grown under a light intensity of around 125 µmol m^{-2} s^{-1} whereas plants on MH media containing 1 mM K were grown under a light intensity of around 350 µmol m^{-2} s^{-1}. It seemed that increasing the K concentrations in the media did not influence the P concentrations in roots and shoots of non mycorrhizal *M. truncatula* plants (Figure 3.5). Additionally, increasing K supply did not alter mycorrhizal colonisation rate of *M. truncatula* (Gyuricza et al., 2010a). Therefore a negative influence on mycorrhizal colonisation due to increased K supply seems unlikely. In an established symbiosis plants deliver carbohydrates to the fungal partners (Harrison, 1997; Smith and Read, 2008) and thus, functioning of the mycorrhizal symbiosis is dependent on photosynthetic activity of the host plants (Azcón-Aguilar and Bago, 1994). Fungi need energy to take up nutrients from soil. Therefore, the more effective the fungus is in nutrient uptake, the higher becomes the demand for carbohydrates (Azcón-Aguilar and Bago, 1994). Thus, a reduction in root carbohydrate availability leads to reduced mycorrhizal functioning. Bücking and Shachar-Hill (2005) supplied carbohydrates to mycorrhizal modified carrot roots and showed that the carbon supply promoted fungal development and resulted in increased P uptake by the fungus and increased transfer of P to the roots. A few publications exist that report the influence of different light intensities on mycorrhizal infection and functioning (Hayman, 1974; Tester et al., 1986; Son and Smith, 1988; Smith and Gianinazzi-Pearson, 1990). The development of arbuscules in mycorrhizal roots of onion plants (*Allium cepa*) did not differ when plants were grown under light intensities of 190 or 410 µmol m^{-2} s^{-1} irrespective of P supply (Smith and Gianinazzi-Pearson, 1990). By contrast, when grown at low P supply the fraction of root length infected in mycorrhizal onion plants (Son and Smith, 1988) and in mycorrhizal *Trifolium* plants (Tester et al., 1986) was lower at low irradiance of 250 or 100 µmol m^{-2} s^{-1}, respectively, than at high irradiance of 600 or 450 µmol m^{-2} s^{-1}, respectively, due to a lower rate of formation of entry points from the fungus. Although mycorrhizal colonisation was never suppressed due to low irradiance (Hayman, 1974; Tester et al., 1986; Son and Smith, 1988; Smith and Gianinazzi-Pearson, 1990), low light intensities during the growth period of *M. truncatula* in the experiment reported here, in combination with other, unknown factors, might have inhibited mycorrhizal colonisation. Judging mycorrhizal

colonisation by the development of new spores, no conclusion can be drawn about the formation of arbuscules within the roots. Light conditions commonly observed in natural habitats of *M. truncatula* are up to 1500 μmol m^{-2} s^{-1} and, to avoid carbon limitations, *M. truncatula* needs light intensities above 300 μmol m^{-2} s^{-1} (Barker et al., 2006). If the plants grown on MH media containing 5 mM K under a light intensity of around 125 μmol m^{-2} s^{-1} experienced carbon limitation this could have had an influence on mycorrhizal colonisation due to a shortage of photosynthetic products.

Based on these observations it was decided to perform all subsequent experiments involving mycorrhizal *M. truncatula* plants on MH media containing 20 μM P in a growth cabinet with a light intensity of around 350 μmol m^{-2} s^{-1}. The reason to use MH media containing 20 μM P instead of MH media containing 10 μM P was that mycorrhizal colonisation increased P concentrations in plants grown on MH media containing 10 μM P (Figure 3.6). However, mycorrhizal colonisation did not seem to affect P concentrations in shoots of plants grown on MH media containing 20 μM P (Figure 3.6). For subsequent experiments an influence of AM fungi on P concentrations in *M. truncatula* plants was not desired.

Finally, the process of root-colonisation by the fungus involves complex interactions between fungal hyphae and plant root cells (Harrison and Dixon, 1993; Bonfante and Perotto, 1995). Several plant and fungal genes and signal molecules are involved in the process (for review see Parniske, 2008). No general conclusion can be drawn on how long it takes for the symbiosis to develop since many factors influence the process. Therefore, based on the observation that spore production of *Glomus* sp. began after eight weeks of growth under *in vitro* conditions it was decided to grow the plants for nine weeks in subsequent experiments to allow sufficient time for the symbiosis of *M. truncatula* with *Glomus* sp. to develop.

3.5 CONCLUSIONS

- Mycorrhizal colonisation of *Medicago truncatula* roots under *in vitro* conditions requires low external P supply and sufficient light intensities
- Experimental conditions to study mycorrhizal infection of *M. truncatula* plants were adequate with plant growth on MH medium containing 20 μM P in a growth cabinet with a light intensity of around 350 μmol m^{-2} s^{-1} and a growth duration of nine weeks

Chapter 4

Caesium toxicity in *Medicago truncatula*

4.1 INTRODUCTION

4.1.1 Caesium in the environment

Caesium is an alkali metal and is the most electropositive and active of all metals (Avery, 1995). The only stable isotope is ^{133}Cs and its mineral source is pollucite (Davis, 1963; Coughtrey and Thorne, 1983). Caesium occurs in soil as a monovalent cation and is retained in the upper soil layers because it is strongly adsorbed to clay minerals (Davis, 1963; Coughtrey and Thorne, 1983). Soils contain between 0.3 and 25 μg Cs g^{-1} which is non toxic to plants (Davis, 1963; Coughtrey and Thorne, 1983; Avery, 1995; White and Broadley, 2000). However, human activities, such as accidents at nuclear power plants and radioactive fallout from nuclear weapons tests, have led to radiocaesium contaminations of soil worldwide. The two most common radionuclides of Cs are ^{134}Cs and ^{137}Cs (U.S. Environmental Protection Agency). Although their concentrations in soil are six orders of magnitudes lower than of ^{133}Cs, these isotopes are of environmental concern because of their long half-lives (^{134}Cs = 2.06 years, ^{137}Cs = 30.17 years) and the emission of harmful β and γ radiation during decay (White and Broadley, 2000). Radiocaesium enters the food chain through vegetation and therefore has an impact on human health.

4.1.2 Uptake and transport of caesium in plants

Caesium is chemically similar to K. The ionic radii of K and Cs are 133 and 165 pm respectively and are sufficiently similar that K^+ transport proteins are mainly responsible for Cs^+ uptake by plants (Avery, 1995; White and Broadley, 2000). Early studies on uptake and accumulation of Cs by plants showed that the uptake of Cs depends on the availability of K (reviewed by Davis, 1963; Coughtrey and Thorne, 1983). If K is available the uptake of Cs is low but reduced K availability increases Cs uptake.

Based on studies investigating Cs uptake from nutrient solutions, White and Broadley (2000) modelled mechanisms of Cs uptake by plants under K-replete

conditions. They proposed that, independent of the external Cs concentration, most of the influx of Cs^+ into root cells is maintained by voltage insensitive cation channels (VICCs). Furthermore, K^+/H^+ symporters (KUPs) also transport Cs^+ into root cells and outward rectifying K channels (KORCs) are responsible for loading Cs^+ into the xylem (Figure 2.1; White and Broadley, 2000). The expression of genes encoding these transporters is dependent on the K status of plants. Potassium starvation increases the expression of K^+/H^+ symporters in plants (Hampton et al., 2004, 2005). Therefore, under K-deficient conditions, more Cs^+ is transported by K^+/H^+ symporters and the concentration of Cs in plant shoots increases (Hampton et al., 2004, 2005).

Several studies showed close correlations between plant ^{133}Cs and plant ^{137}Cs concentrations (Coughtrey and Thorne, 1983; Tsukada and Hasegawa, 2002; Tsukada et al., 2002; Salt et al., 2004; Soudek et al., 2006). Furthermore, it was demonstrated that the distribution of ^{133}Cs in polished rice was similar to the distribution of ^{137}Cs (Tsukada et al., 2002). Since plants do not appear to discriminate between ^{133}Cs and ^{137}Cs, ^{133}Cs can be used to predict behaviour of ^{137}Cs in the environment (Tsukada et al., 2002; Salt et al., 2004; Uchida et al., 2009). Based on these observations it was decided to use ^{133}Cs to investigate Cs accumulation of *Medicago truncatula*.

4.1.3 Aim

The aim of this chapter was to determine non toxic Cs concentrations for growth of *Medicago truncatula* under K-deficient and K-replete conditions. Therefore, *M. truncatula* was grown under *in vitro* conditions on a complete nutrient medium containing either 1.65 or 21.65 mM K. The plants were supplied with increasing concentrations of CsCl and the effects of Cs on plant growth and mineral composition of plants were investigated.

4.2 MATERIAL AND METHODS

4.2.1 Organisms

Medicago truncatula Gaertn. var. *truncatula* Jemalong A17 was chosen as the experimental plant. The seeds were obtained from the South Australian Research and Development Institute (SARDI), Genetic Resource Centre, Australia.

4.2.2 Growth conditions

The non mycorrhizal plants were grown in the Arbuscular Mycorrhizal–Plant (AM-P) *in vitro* culture system (Sections 3.2.2 and 3.2.5). The plants were grown on MSR medium (Table 3.1) with a concentration of 1.65 mM K. Additionally, plants were grown on MSR medium with an addition of 20 mM KCl (MSR+K medium) resulting in a total K concentration of 21.65 mM.

To investigate Cs toxicity the plants were supplied with increasing concentrations of CsCl. Plants which were grown on MSR medium containing 1.65 mM K were supplied with 0, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.15, 0.3, 1 and 3 mM of CsCl. Plants which were grown on MSR+K medium containing 21.65 mM K were supplied with 0, 0.003, 0.01, 0.03, 0.1, 0.15, 0.3, 1, 1.5 and 3 mM of CsCl. Six plants were grown for each treatment. The plants were grown for six weeks in a growth cabinet with a photoperiod of 16 h light and 8 h darkness and a constant temperature of 22°C. The light intensity was between 125 and 200 μmol m^{-2} s^{-1}. Plant height was measured from the cotyledons to the tip of the highest leaf.

4.2.3 Determining of elemental concentrations

Element concentrations were determined in plant roots and shoots using ICP-MS, as described in Section 2.2.5. The measurements were undertaken without any tomato leaves standards. Therefore, no drift correction was performed.

4.2.4 Statistical analysis

For determination of the influence of increasing external Cs supply on plant growth and mineral composition in plant tissues regression analyses were performed using GenStat (12th Edition, VSN International, Hemel Hempstead, UK). For some analyses the values of the external Cs concentration were log transformed to the base 10 to fit generalised logistic and exponential models. To investigate the effect of external Cs supply on plant growth a generalised logistic curve was fitted with the equation

$$y = \alpha + \gamma / (1 + \tau \times \exp(-\beta \times (x - \mu)))^{1/\tau} + \varepsilon$$

The parameters α and γ define the asymptotes, τ is a power-law parameter, β is the slope parameter and μ is the point of inflexion for the explanatory variable. To

investigate the effect of external Cs supply on the concentrations of Ca, K and Mg in plants exponential curves were fitted with the equation

$$y = \alpha + \beta \times \rho^x + \varepsilon$$

The parameter α defines the asymptote, β gives the range of the curve between the value at x equals zero and the asymptote and ρ shows the rate of exponential decrease. To investigate the effect of external Cs supply on the concentration of Cs in plants linear regression was performed with the equation

$$y = \alpha \times x$$

The parameter α defines the slope of the line. A linear regression was performed because Cs accumulation in plant tissues was measured after six weeks of growth and toxic external Cs concentrations were excluded from the analysis.

4.3 RESULTS

4.3.1 Plant growth

Medicago truncatula was grown in the Arbuscular Mycorrhizal–Plant (AM-P) *in vitro* culture system for six weeks (Figure 4.1). Potassium concentrations in the medium were either 1.65 mM (MSR) or 21.65 mM (MSR+K). The different K concentrations in the media led to differences in K concentration and K content of the plants when Cs supply was low (Figure 4.2). Plants which had been grown on MSR medium with the lower K concentration of 1.65 mM had a nearly constant K content in their leaves independent of leaf mass (Figure 4.2A) but the leaf K concentration decreased with increasing leaf mass (Figure 4.2B). By contrast, plants which had been grown on MSR+K medium with the higher K concentration of 21.65 mM had increasing K contents in their leaves with increasing leaf mass (Figure 4.2A) but the leaf K concentration remained constant independent of leaf mass (Figure 4.2B).

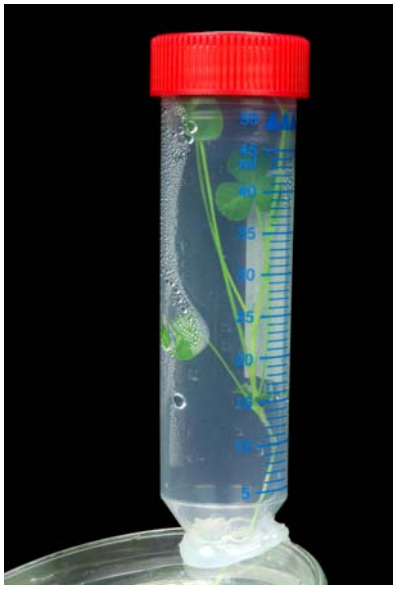

Figure 4.1: Six-week old *Medicago truncatula* plant on MSR medium in an AM-P *in vitro* culture system.

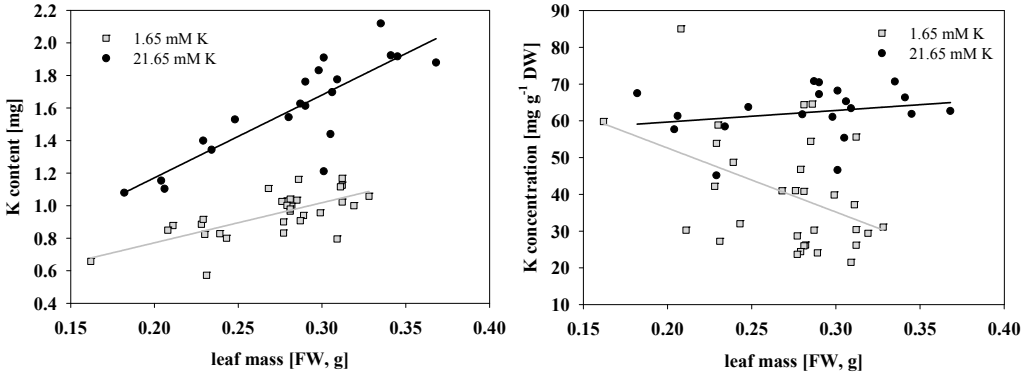

Figure 4.2: Potassium content (A) and K concentration (B) in leaves of single *Medicago truncatula* plants in relation to fresh leaf mass of plants which had been grown on MSR medium (grey) or MSR+K medium (black). Only plants which had been grown on medium with Cs concentrations ≤0.1 mM were included. The relationships between leaf K concentrations or K content *versus* leaf mass were fitted to the equation for straight lines.

For the Cs toxicity experiments the Cs concentrations in the MSR and MSR+K media varied between 0.0001 and 3 mM. High external Cs supply inhibited growth of *M. truncatula* and also affected the health of the plants (Figure 4.3), at both K concentrations of 1.65 and 21.65 mM. Figure 4.4 shows a *M. truncatula* plant under Cs toxicity. Shoot and root growth was suppressed and most of the leaves showed necrosis.

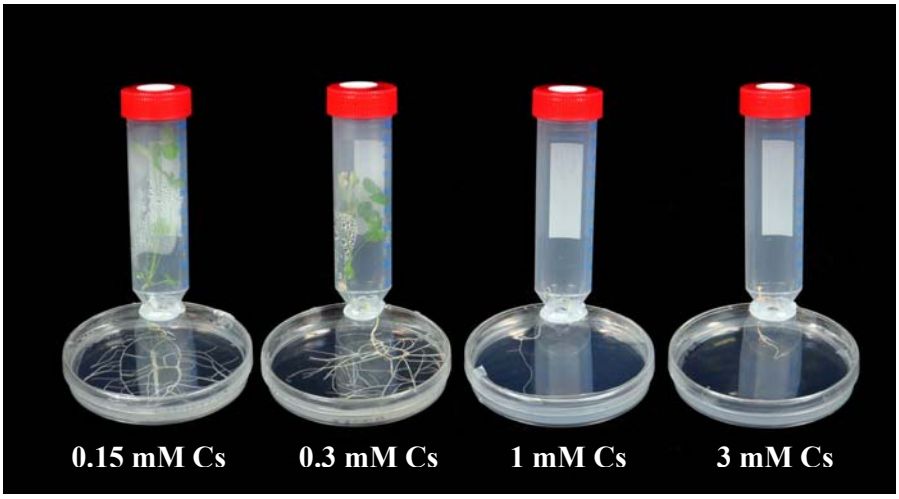

Figure 4.3: *Medicago truncatula* plants on MSR medium containing 0.15 mM, 0.3 mM, 1 mM or 3 mM CsCl. Increasing Cs concentrations inhibited plant growth and led to the death of plants at Cs concentrations of ≥1 mM.

Figure 4.4: *Medicago truncatula* plant on MSR+K medium containing 1 mM CsCl. Shoot and root growth was inhibited and the leaves showed necrosis due to Cs toxicity.

The inhibitory effect of increasing concentrations of external Cs supply on plant growth was dependent on the external K supply. The relationship between plant height and external Cs concentration fitted the equation of generalised logistic curves (Table 4.1). Plant height decreased with increasing Cs supply (Figure 4.5) and plants that were grown on MSR medium were more sensitive to Cs toxicity than plants that were grown on MSR+K medium. Plant height decreased as the external Cs

concentration increased above 0.15 mM in the presence of 1.65 mM K, or above 0.3 mM in the presence of 21.65 mM K. Furthermore, plants died at an external Cs concentration of 1 mM in the presence of 1.65 mM K, or at 3 mM in the presence of 21.65 mM K.

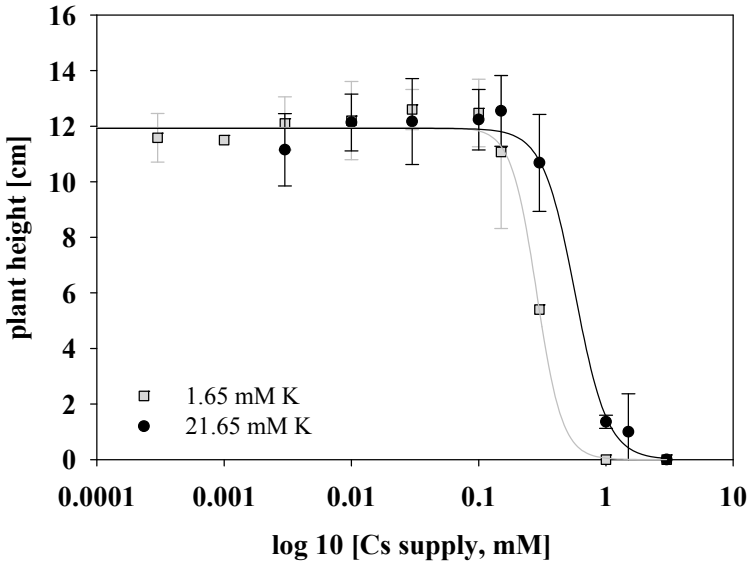

Figure 4.5: Plant height in relation to external Cs concentrations for plants with an external K supply of 1.65 mM (grey) or 21.65 mM (black). Data show mean values and 95% confidence intervals (n = 6). The relationship between plant heights *versus* external Cs supply was fitted to generalised logistic curves. The parameters for these equations are given in Table 4.1.

Table 4.1: Parameters for the regression lines in Figure 4.5. The relationship between plant height [cm] and external Cs supply [mM] was fitted to generalised logistic curves: $y = \alpha + \gamma / (1 + \tau \times \exp(-\beta \times (x - \mu)))^{1/\tau} + \varepsilon$.

Relationship	Parameter	Estimate
Plant height *versus* external [Cs] at 1.65 mM [K]	β	-11.01
	μ	-0.58
	τ	1.91
	γ	12.24
	α	-0.22
Plant height *versus* external [Cs] at 21.65 mM [K]	β	-69.70
	μ	-0.51
	τ	19.00
	γ	12.00
	α	-0.18

4.3.2 Caesium accumulation in *Medicago truncatula* plants dependent on K status

Caesium concentrations in roots and shoots increased with increasing external Cs supply. Generally, Cs concentrations in shoots were higher than in roots. Caesium concentrations in shoots were higher when plants were grown on medium containing 1.65 mM K than when plants were grown on medium containing 21.65 mM K at the same Cs supply and the rate of increase in Cs shoot concentrations with external Cs supply differed significantly between plants on 1.65 mM K medium and plants on 21.65 mM K medium ($p < 0.001$; Figure 4.6). Therefore, higher external K supply reduced the accumulation of Cs in shoots.

In contrast to shoot Cs concentrations, root Cs concentrations were lower when plants were grown at 1.65 mM K than when plants were grown at 21.65 mM K at the same Cs supply and the rate of increase in Cs root concentrations with external Cs supply differed significantly between plants on 1.65 mM K medium and plants on 21.65 mM K medium ($p < 0.001$; Figure 4.6).

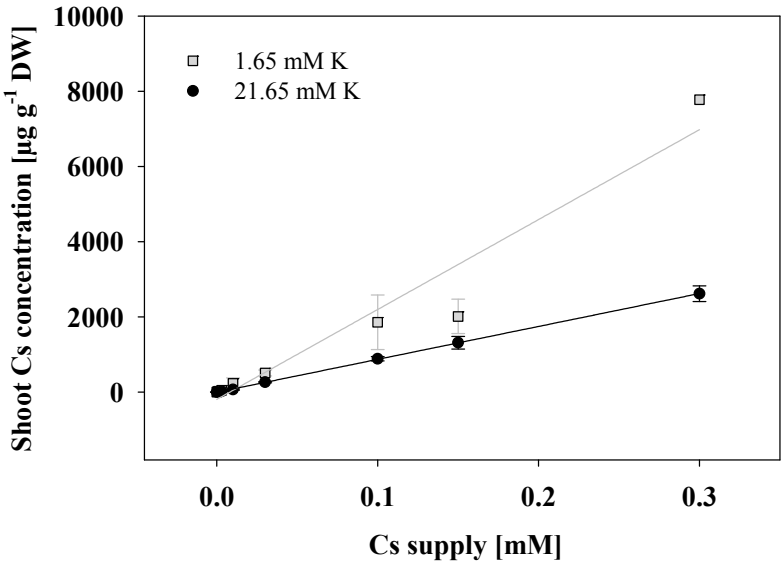

Figure 4.6: Caesium concentrations in shoots in relation to external Cs supply for plants with an external K supply of 1.65 mM (grey) or 21.65 mM (black). Data show mean values and 95% confidence intervals (n = 6). The relationships between shoot Cs concentrations *versus* external Cs supply were fitted to the equation for straight lines.

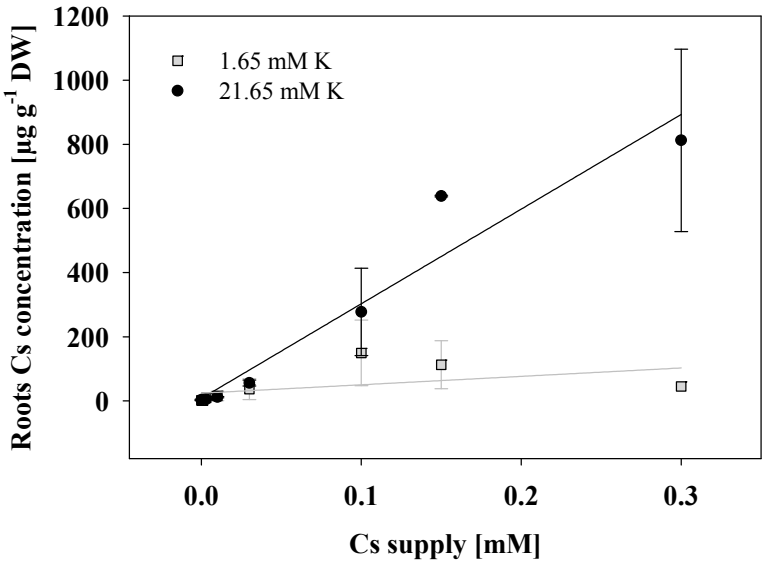

Figure 4.7: Caesium concentrations in roots in relation to external Cs supply for plants with an external K supply of 1.65 mM (grey) or 21.65 mM (black). Data show mean values and 95% confidence intervals (n = 6). The relationships between root Cs concentrations *versus* external Cs supply were fitted to the equation for straight lines.

4.3.3 Mineral accumulation in *Medicago truncatula* plants dependent on K and Cs status

Potassium concentrations in roots and shoots decreased with increasing external Cs supply. The relationships between K concentrations in shoots and external Cs concentrations fitted the equation of negative exponential curves (Table 4.2). Generally, plants which were grown on MSR+K medium with a K concentration of 21.65 mM had higher K concentrations in shoots than plants which were grown on MSR medium with a K concentration of 1.65 mM (Figure 4.8). However, the rate of change of K concentrations in shoots in relation to external Cs supply did not differ significantly in dependence on external K supply (Parameter β in Table 4.2).

Calcium and magnesium concentrations in shoots decreased with increasing external Cs supply. The relationships between Ca or Mg concentrations in shoots and external Cs concentration fitted the equations of negative exponential curves (Table 4.2). Under low external Cs supply plants which were grown on MSR+K medium with a K concentration of 21.65 mM had lower Ca and Mg concentrations in shoots than plants which were grown on MSR medium with a K concentration of 1.65 mM (Figures 4.9 and 4.10). The rates of change of Ca and Mg concentrations in

shoots in relation to external Cs supply differed significantly ($p < 0.001$) in dependence on external K supply (Parameters β in Table 4.2).

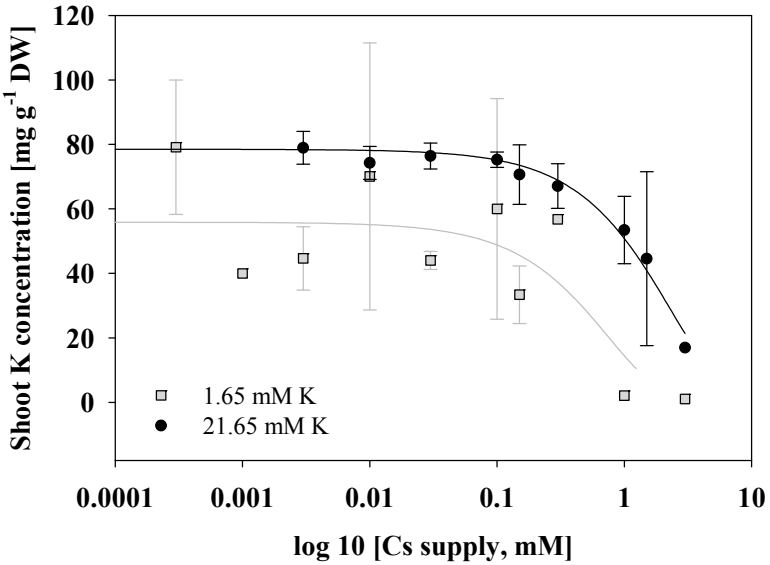

Figure 4.8: Potassium concentrations in shoots in relation to external Cs supply for plants with an external K supply of 1.65 mM (gr3y) or 21.65 mM (black). Data show mean values and 95% confidence intervals (n = 6). The relationships between shoot K concentrations *versus* external Cs supply were fitted to exponential curves. The parameters for these equations are given in Table 4.2.

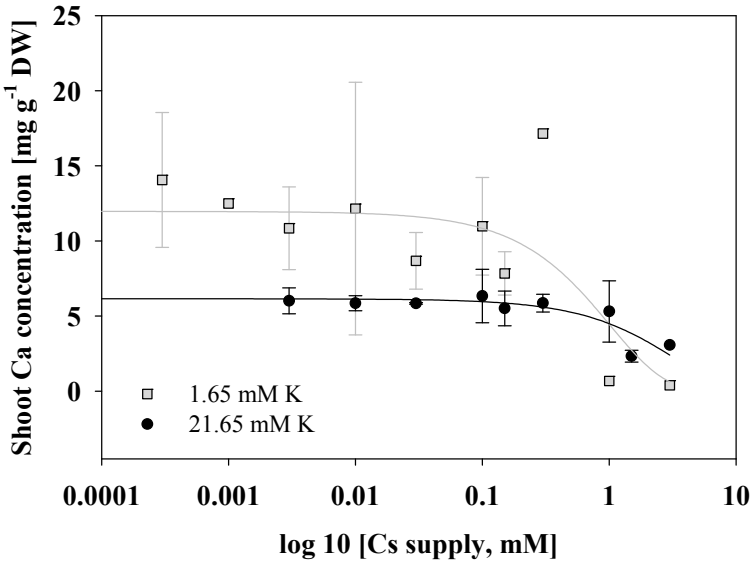

Figure 4.9: Calcium concentrations in shoots in relation to external Cs supply for plants with an external K supply of 1.65 mM (grey) or 21.65 mM (black). Data show mean values and 95% confidence intervals (n = 6). The relationships between shoot Ca concentrations *versus* external Cs supply were fitted to exponential curves. The parameters for these equations are given in Table 4.2.

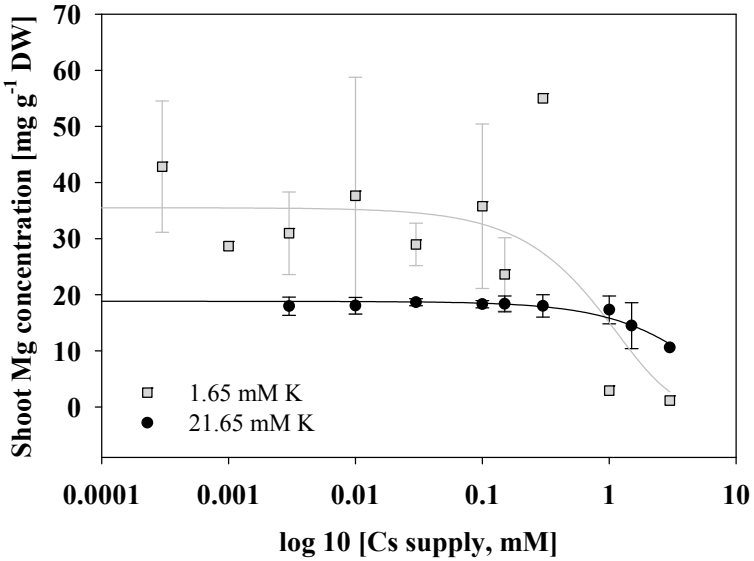

Figure 4.10: Magnesium concentrations in shoots in relation to external Cs supply for plants with an external K supply of 1.65 mM (grey) or 21.65 mM (black). Data show mean values and 95% confidence intervals (n = 6). The relationships between shoot Mg concentrations *versus* external Cs supply were fitted to exponential curves. The parameters for these equations are given in Table 4.2.

Table 4.2: Parameters for the regression lines in Figures 4.8, 4.9 and 4.10. The relationships between $[K]_{shoot}$ [mg g^{-1} DW] (Figure 4.8), $[Ca]_{shoot}$ [mg g^{-1} DW] (Figure 4.9) and $[Mg]_{shoot}$ [mg g^{-1} DW] (Figure 4.10) with external Cs supply [mM] were fitted to exponential curves: $y = \alpha + \beta \times \rho^x + \varepsilon$.

Relationship	Parameter	Estimate
$[K]_{shoot}$ *versus* external [Cs] at 1.65 mM [K]	α	59.65
	β	-38.72
	ρ	3.04
$[K]_{shoot}$ *versus* external [Cs] at 21.65 mM [K]	α	85.65
	β	-38.72
	ρ	3.04
$[Ca]_{shoot}$ *versus* external [Cs] at 1.65 mM [K]	α	13.13
	β	-9.67
	ρ	2.44
$[Ca]_{shoot}$ *versus* external [Cs] at 21.65 mM [K]	α	6.51
	β	-1.99
	ρ	2.44
$[Mg]_{shoot}$ *versus* external [Cs] at 1.65 mM [K]	α	35.98
	β	-22.34
	ρ	4.69
$[Mg]_{shoot}$ *versus* external [Cs] at 21.65 mM [K]	α	19.12
	β	-3.44
	ρ	4.69

There was no relationship between fresh weights (FWs) of shoots and shoot Cs concentrations for plants grown with either 1.65 or 21.65 mM external K supply (Figure 4.11). However, shoot FW was inversely related to Cs_{shoot}:K_{shoot} ratios for both plants grown with 1.65 or 21.65 mM external K supply (Figure 4.12).

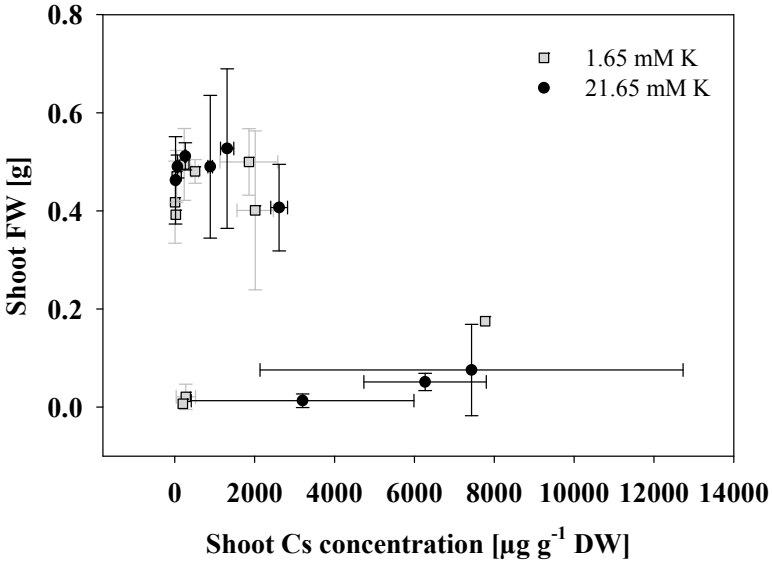

Figure 4.11: Fresh weights of shoots in relation to shoot Cs concentrations for plants with an external K supply of 1.65 mM (grey) or 21.65 mM (black). Data show mean values and 95% confidence intervals (n = 6).

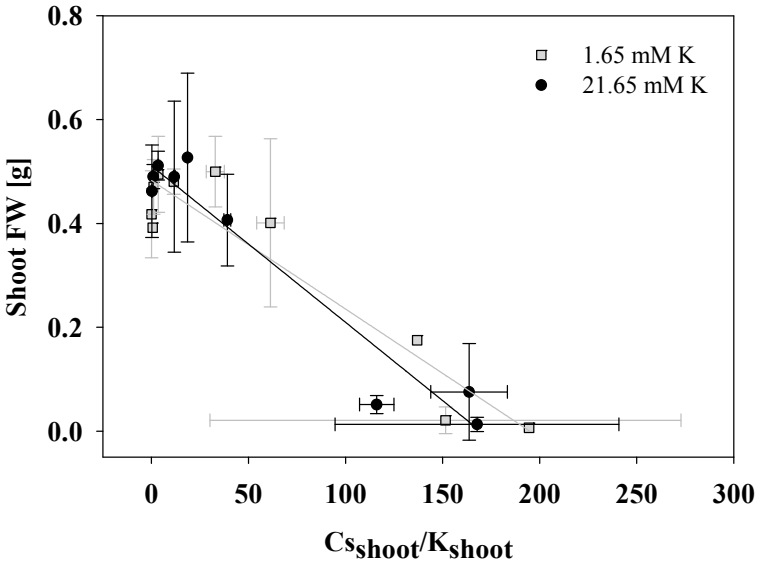

Figure 4.12: Fresh weights of shoots in relation to Cs_{shoot}:K_{shoot} ratios for plants with an external K supply of 1.65 mM (grey) or 21.65 mM (black). Data show mean values and 95% confidence intervals (n = 6). The relationships between FWs *versus* Cs_{shoot}:K_{shoot} ratios were fitted to the equation for straight lines.

4.4 DISCUSSION

High concentrations of Cs are toxic for plants (Hampton et al., 2004; Isaure et al., 2006). Toxicity symptoms include reduced shoot and root growth and necrosis of plant tissues (White and Broadley, 2000). In *Arabidopsis thaliana*, 1 mM Cs stress reduced the concentrations of chlorophyll a and b in cells which had been grown in medium without any K salts (Le Lay et al., 2006). Furthermore, synchrotron-based X-ray fluorescence microscopy suggested that Cs was located in chloroplasts (Le Lay et al., 2006). In this study, caesium toxicity induced necrosis of leaves in *Medicago truncatula* (Figure 4.4). Additionally, plant height decreased at Cs concentrations higher than 0.15 mM Cs in the presence of 1.65 mM K in the medium and at Cs concentrations higher than 0.3 mM Cs in the presence of 21.65 mM K in the medium (Figure 4.5). Caesium concentrations of 1 and 3 mM caused death of plants which had been grown in the presence of 1.65 and 21.65 mM K, respectively. However, soils contain between 0.3 and 25 μg Cs g^{-1} (Davis, 1963; Coughtrey and Thorne, 1983) and the concentration of K in most soil solutions lies between 0.1 and 1 mM (White and Karley, 2010). Therefore, Cs is not toxic to plants in natural environments.

Medicago truncatula accumulated Cs in roots and shoots. Generally, Cs concentrations in shoots were higher than in roots. The accumulation of Cs was dependent on the external Cs and K supply. Concentrations of Cs in plant shoots increased linearly with increasing Cs supply at both external K concentrations (Figure 4.6). Sacchi et al. (1997) measured Cs^+ influx into roots after 30 min of exposure to Cs, resulting in a non linear relationship of Cs^+ influx and external Cs concentrations >0.1 mM. However, Cs concentrations in plant tissues in the experiment reported here were determined after six weeks of growth leading to a linear relationship of Cs concentrations in plants and external Cs supply (Figure 4.6).

Increasing K availability in the medium decreased Cs concentrations in shoots (Figure 4.6). This is in agreement with other studies which showed that increasing K availability decreases Cs accumulation in plant shoots (Nishita et al., 1962; Davis, 1963; Smolders et al., 1996; Zhu et al., 2000, 2002; Tsukada and Hasegawa, 2002; Hampton et al., 2004; Le Lay et al., 2006). By contrast, Cs concentrations in roots of plants which had been grown in the presence of 21.65 mM K were higher than Cs concentrations in roots of plants which had been grown in the presence of 1.65 mM K. Potassium availability influenced Cs concentrations in roots

differently than Cs concentrations in shoots. Hence, Cs_{root}:Cs_{shoot} ratios varied in dependence of K availability with increasing external Cs supply. Increasing external Cs supply increased the Cs_{root}:Cs_{shoot} ratio in the presence of 21.65 mM K but decreased the Cs_{root}:Cs_{shoot} ratio in the presence of 1.65 mM K. The K_{root}:K_{shoot} ratios in the presence of 1.65 or 21.65 mM K changed in the same way in relation to increasing Cs supply as did the Cs_{root}:Cs_{shoot} ratios in the presence of 1.65 or 21.65 mM K (Figure 4.13). Outward-rectifying K channels (KORCs) are permeable to Cs and have been proposed to be responsible for loading Cs into the xylem (White and Broadley, 2000). At high K availability relatively more K and Cs were stored in roots than in shoots with increasing Cs supply (Figure 4.13). A possible explanation could be that the root cells sensed K starvation due to increased competition for uptake between K^+ and Cs^+ with increasing Cs supply. It has been demonstrated that K starvation reduced the expression of *KORCs* and thereby K efflux to shoots (Hampton et al., 2005; Liu et al., 2006). A reduced expression of *KORCs* would lead to reduced transport of K and Cs to shoots and therefore relatively more K and Cs would remain in roots (Figure 4.13). By contrast, at reduced K availability more transport of K and Cs from roots to shoots occurred with increasing Cs supply (Figure 4.13). Due to the low availability of external K the expression of genes encoding KORCs (Hampton et al., 2005; Liu et al., 2006) was possibly already low independent of the external Cs supply. However, it has been demonstrated that most of the Cs that is delivered to shoots is returned to the roots *via* the phloem (Buysse et al., 1996; Hampton, 2005). Therefore, it seems possible that with increasing Cs supply the transport of Cs, and possibly K, *via* the phloem was inhibited or changed leading to relatively higher concentrations of K and Cs in shoots (Figure 4.13). Nevertheless, since Cs concentrations in shoots were higher than Cs concentrations in roots (Figures 4.6 and 4.7) the total Cs accumulation was lower in plants that had been grown under high K supply than in plants that had been grown under low K supply.

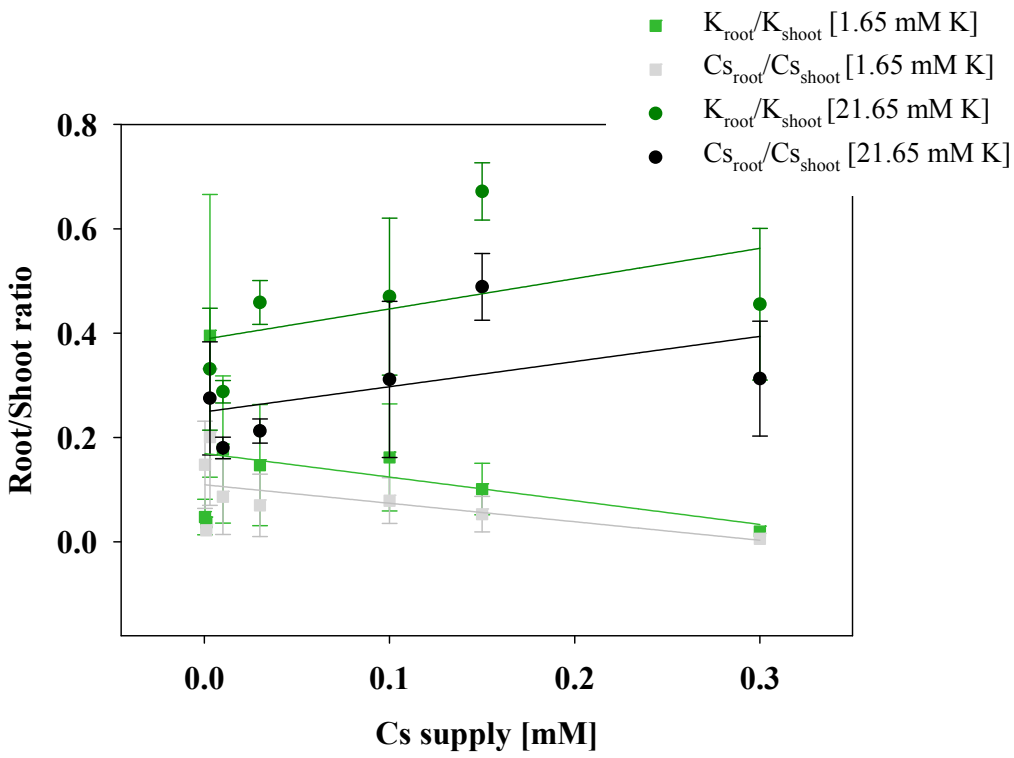

Figure 4.13: Cs_{root}:Cs_{shoot} ratios in relation to external Cs supply for plants with an external K supply of 1.65 mM (grey) or 21.65 mM (black) and K_{root}:K_{shoot} ratios in relation to external Cs supply for plants with an external K supply of 1.65 mM (light green) or 21.65 mM (dark green). The relationships between the root to shoot ratios *versus* external Cs supply were fitted to the equation for straight lines.

Hampton et al. (2004) formulated three hypotheses to explain Cs toxicity in *A. thaliana*. These were [1] caesium inhibits K uptake and causes K starvation, [2] caesium concentrations in shoots are toxic *per se*, and [3] caesium competes with K for essential biochemical functions and, therefore, Cs toxicity is related to the Cs_{shoot}:K_{shoot} ratio. In the experiment presented here, increasing external Cs supply decreased K concentrations in shoots of *M. truncatula* (Figure 4.8) and therefore might have caused K starvation. Decrease in K concentrations in tissues due to increasing external Cs supply was shown in previous studies (Hampton et al., 2004; Le Lay et al., 2006). Nevertheless, it had been concluded that Cs toxicity was unlikely to be caused by K starvation alone because K-starved and Cs-intoxicated plants showed different transcriptional profiles (Hampton et al., 2004). Since there was no relationship between the FW of *M. truncatula* shoots and concentrations of Cs in shoots (Figure 4.11), Cs toxicity was not related to the Cs concentration in shoots *per se* which is in agreement with results obtained by Hampton et al. (2004).

However, shoot FW was inversely related to Cs_{shoot}:K_{shoot} ratios in both plants which had been grown in the presence of 1.65 or 21.65 mM K (Figure 4.12). This suggests that Cs toxicity was related to the Cs_{shoot}:K_{shoot} ratio. Due to the chemical similarity between K and Cs, Cs can replace intracellular K but Cs cannot substitute for K in metabolism (Davis, 1963; Avery, 1995). Isaure et al. (2006) used ion beam micro-chemical imaging to demonstrate that Cs distribution in *A. thaliana* was similar to K distribution and therefore, the cations could compete for binding sites. Thus, Cs toxicity to plants depends on Cs/K ratios in the substrate and the resulting Cs/K ratios in plant tissues rather than on the total external Cs (Davis, 1963; Avery, 1995; Hampton et al., 2004).

Potassium has vital functions in the cytoplasm of cells which include enzyme activation, stabilisation of protein synthesis, and maintenance of cytoplasmic pH homeostasis and is also important for the osmotic potential of cells (Leigh and Wyn Jones, 1984; Amtmann et al., 2006; White and Karley, 2010). If the availability of K is low, other cations such as sodium (Na), Mg and Ca can substitute for K in the vacuole of plants (Leigh and Wyn Jones, 1984). Therefore, plants with low K concentrations in their shoots contain high Na, Mg and Ca concentrations if these cations are available (Popp and Kinzel, 1981; Smith et al., 1982; Leigh and Wyn Jones, 1984). This effect also occurred in *M. truncatula* plants, because plants that had been grown in the presence of 1.65 mM K had higher Ca and Mg concentrations in shoots than plants that had been grown in the presence of 21.65 mM K (Figures 4.9 and 4.10). Nevertheless, increasing external Cs supply decreased Ca and Mg concentrations in shoots of *M. truncatula*. This decrease was most obvious in plants that had been grown in the presence of 1.65 mM K and therefore had high Cs concentrations in their shoots. Root cells contain two types of depolarisation-activated Ca channels, maxi cation channels and voltage dependent cation channels. Both are permeable to a wide variety of monovalent and divalent cations including Mg and Cs (White, 1998). These channels are unlikely to transport Cs under typical ionic conditions in soil (White and Broadley, 2000) but the Cs concentrations in the experiment presented here were much higher than under normal circumstances in soil. Therefore, at high external Cs supply the depolarisation-activated Ca channels could transport Cs into root cells and thereby reduce Ca and Mg uptake due to biochemical competition.

Results obtained in this chapter were used to determine external Cs concentrations for Cs accumulation studies of mycorrhizal and non mycorrhizal *M. truncatula* plants. The external Cs concentrations needed to be high enough to be detectable in plant tissues using ICP-MS but should not be toxic to plants. Caesium toxicity was dependent on external K supply. It was therefore decided to use, in subsequent experiments, an external Cs supply of 0.1 mM for plants grown in the presence of 1.65 mM K and an external Cs supply of 0.15 mM for plants grown in the presence of 21.65 mM K.

4.5 CONCLUSIONS

- Caesium is toxic to *Medicago truncatula* with 1 or 3 mM Cs supply causing death of plants when K supply was low or high, respectively
- High external K supply decreased Cs concentrations in shoots and, to an extent, protects the plants against Cs toxicity
- Increasing external Cs supply decreased K concentrations in shoots and therefore might cause K starvation
- Caesium toxicity was related to the Cs_{shoot}:K_{shoot} ratio and therefore might occur due to biochemical competition between Cs and K
- Experimental conditions to study Cs accumulation by *M. truncatula* plants in subsequent experiments were defined as an external Cs supply of 0.1 mM for plants grown in the presence of 1.65 mM K and an external Cs supply of 0.15 mM for plants grown in the presence of 21.65 mM K

Chapter 5

The influence of arbuscular mycorrhizal fungi on caesium accumulation by *Medicago truncatula*

5.1 INTRODUCTION

5.1.1 Phytoremediation and 'safer' crop

Radiocaesium contamination of the environment has been caused by global fallout from nuclear weapon testing, discharge from nuclear power plants, waste disposal and accidents at nuclear installations. The accident at the nuclear power plant in Chernobyl in 1986 released 0.085×10^{18} becquerel (Bq) of ^{137}Cs into the environment (IAEA, 2006) and large areas (150,000 km^2) in Russia, Belarus and Ukraine had ^{137}Cs contamination densities above 37 kBq m^{-2} (Fesenko et al., 2007; Jacob et al., 2009). Soils around nuclear facilities are also contaminated with ^{137}Cs due to routine release of radionuclides during nuclear energy production (Lasat et al., 1998; Zhu and Shaw, 2000; Willey et al., 2001; Watt et al., 2002).

Conventional engineering-based methods to clean up radionuclide contaminated soils are based on treatments with dispersing and chelating chemicals (Entry et al., 1996, 1997). These methods require the removal and transport of contaminated soil, which is very costly and time consuming and may result in additional dispersal of pollutants (Entry et al., 1996, 1997). Furthermore, the heavy equipment used to remove contaminated soil from the sites leads to soil compaction affecting bulk density, porosity and water holding capacity of the soil. The chemicals used to remove the radionuclides from the soil also alter mineral nutrient availability and harm microorganisms. Therefore, after the soil is replaced, the establishment of plants can be challenging (Entry et al., 1996, 1997).

Phytoremediation, the use of plants to remove pollutants from contaminated soil, provides an alternative to conventional remediation strategies. Dushenkov (2003) defined four methods of phytoremediation: "(a) phytoextraction, in which high biomass radionuclide-accumulating plants and appropriate soil amendments are used to transport and concentrate radionuclides from the soil into the above-ground shoots, which are harvested with conventional agricultural methods, (b) rhizofiltration, in which plant roots are used to precipitate and concentrate

radionuclides from polluted effluents, (c) phytovolatilisation, in which plants extract volatile radionuclides from soil and volatilise them from the foliage and (d) phytostabilisation, in which plants stabilise radionuclides in soils, thus rendering them harmless." Phytoremediation is cheaper than engineering-based methods and potentially more sustainable since it is carried out *in situ* and reduces greatly negative influences on soil properties and microorganisms (Entry et al., 1996, 1997; Pilon-Smits, 2005). However, there are also limitations to phytoremediation. The rooting depths of plants limits successful phytoremediation to upper soil layers and pollutants have to be phytoavailable (Pilon-Smits, 2005). Depending on the pollutant itself, and the extent of contamination, phytoremediation might take several decades and might not be feasible.

Many areas that were affected by the Chernobyl accident contained agricultural land (Fesenko et al., 2007; Jacob et al., 2009). Most regions had sandy or peaty soils where uptake of ^{137}Cs by plants is high (Fesenko et al., 2007). Therefore, countermeasures had to be applied to reduce ^{137}Cs accumulation by plants and transfer of ^{137}Cs to humans *via* the food chain. In the first ten years after the accident in Chernobyl, soil based countermeasures such as deep ploughing and fertilisation with N:P:K fertilisers were undertaken (Fesenko et al., 2007). Fesenko et al. (2007) reported that deep ploughing led to a 8–16 fold decrease and the application of N:P:K fertilisers to a 1.5–3 fold decrease in ^{137}Cs concentrations in plants. Field sites in Russia, Belarus and Ukraine were chosen to test the efficiency of different soil based countermeasures to reduce ^{137}Cs accumulation by plants (Vidal et al., 2001; Camps et al., 2004). Deep ploughing, the application of N:P:K fertilisers and reseeding with three grass species (*Phleum pratense*, *Bromus inermis* and *Festuca pratensis*) reduced the transfer of ^{137}Cs to vegetation (Vidal et al., 2001; Camps et al., 2004). However, it was concluded that a complete characterisation of the soil properties is necessary to decide on the right agricultural countermeasure because, for example, the effect of ploughing depends on the heterogeneity of soils (Vidal et al., 2001; Camps et al., 2004). Nisbet et al. (1993) reviewed data from field experiments in Belarus and Ukraine as well as from small scale experiments conducted under controlled conditions and concluded that the success of K fertilisation in reducing Cs transfer to plants depends on the K availability in soil and the replacement of Cs from soil sorption sites by K.

Plants show significant natural genetic variation for Cs accumulation (see Chapter 2). Differences also occur between varieties of the same species (White et al., 2003; Willey, 2005). This offers the opportunity to select or breed 'safer' crop cultivars that accumulate less Cs (White et al., 2003; Hampton et al., 2005; Willey, 2005). However, the expression of genes encoding Cs transport proteins depends on the K status of plants and due to differences in K fertilisation techniques different crop varieties would be needed for different agricultural areas (Hampton et al., 2005).

5.1.2 The role of arbuscular mycorrhizal fungi in Cs accumulation by plants

Since arbuscular mycorrhizal (AM) fungi deliver nutrients to their host plants it was suggested that the role of AM fungi in radionuclide uptake should be investigated (Entry et al., 1996; Zhu and Shaw, 2000). Based on the low mobility of ^{137}Cs in soil and its low concentration in the soil solution Entry et al. (1996) proposed that mycorrhizal infection of roots should enhance Cs uptake. However, Entry et al. (1996) suggested that not all mycorrhizal fungi will be equally effective in transporting Cs to plants and that specific plant and fungi associations should be tested. Zhu and Shaw (2000) suggested that knowledge about the role of mycorrhiza in radionuclide uptake should be used to improve the design of agricultural countermeasures and phytoremediation strategies.

Studies on the influence of mycorrhiza on Cs accumulation by plants are not consistent (Table 1.2). As outlined in Chapter 1 of this thesis, there are several reasons which could have led to different results. In particular, the plant and fungus species, and concentrations of K and Cs in the growth media can greatly influence Cs accumulation of plants. The expression of genes encoding plant transport proteins can be altered in the AM symbiosis. It is possible for AM fungi to influence Cs uptake, and Cs redistribution within the plant, by altering the expression of plant genes encoding voltage insensitive cation channels (VICCs), K^+/H^+ symporters (KUPs) and outward rectifying K^+ channels (KORKs). It has been hypothesised that if AM fungi contribute to improved plant K status, then the complement of K transporters in roots of mycorrhizal plants would reflect that of K-replete plants (Wiesel et al., 2008; White et al., 2010). This implies that Cs uptake by roots of mycorrhizal plants would occur mainly through VICCs and that associations with AM fungi would reduce the accumulation of Cs by plants in K-limited environments.

Another factor that could influence the effect of AM fungi on Cs accumulation by plants is the rhizosphere Cs concentration. Most of the authors listed in Table 1.2 used radioactive ^{137}Cs for their experiments (Rogers and Williams, 1986; Dighton and Terry, 1996; Entry et al., 1999; Rosén et al., 2005). Joner et al. (2004), Dubchak et al. (2010) and Gyuricza et al. (2010b) used radioactive ^{134}Cs for their experiments. Berreck and Haselwandter (2001) used stable ^{133}Cs for their experiments. In the experiments reported in this thesis stable ^{133}Cs was used. When radioactive Cs isotopes are used total Cs concentrations applied are considerably lower than when stable Cs is used.

This thesis was part of a European project, called MYCOREMED ("Role of arbuscular mycorrhizal fungi on the accumulation of radiocaesium by plants"). Early results obtained for this thesis suggested that AM fungi did not influence Cs accumulation in *Medicago truncatula* when supplied with high concentrations of stable ^{133}Cs. By contrast, early results obtained by V. Gyuricza (Gyuricza et al., 2010b), who was also a PhD student of the MYCOREMED project, suggested that AM fungi decreased Cs accumulation in *M. truncatula* when supplied with low concentrations of carrier-free radioactive ^{134}Cs. In both projects, *M. truncatula* var. *truncatula* Jemalong A17 was grown in the Arbuscular Mycorrhizal–Plant *in vitro* culture system. Whereas in the thesis presented here, *Glomus* sp. MUCL 43195 was used, Gyuricza et al. (2010b) used *Glomus intraradices* MUCL 43194. Different growth media were used as well but the most obvious difference between the experiments of Gyuricza et al. (2010b) and the experiments presented in this thesis was that Gyuricza et al. (2010b) supplied low concentrations of Cs whereas here high concentrations of Cs were used. Based on these observations, it was hypothesised that at low external Cs supply AM fungi could reduce the transport of Cs to plants due to storage of Cs in their hyphae, but that at high external Cs supply AM fungi do not affect the transport of Cs to plants possibly due to the increased uptake of Cs by plant roots themselves.

5.1.3 Aims

The first aim of this chapter was to determine K-deficient conditions for *Medicago truncatula.* The plants were grown under *in vitro* conditions on complete nutrient media containing 0.2, 1, 5 or 25 mM K and K concentrations in roots and shoots were determined.

The second aim of the chapter was to test the hypothesis that AM fungi reduce Cs accumulation by plants under K-deficient conditions. Therefore, non mycorrhizal and mycorrhizal *M. truncatula* plants were grown under *in vitro* conditions on a complete nutrient medium containing 1 mM K without or with the addition of 0.05 mM Cs. Element concentrations in tissues of non mycorrhizal and mycorrhizal plants were investigated.

The third aim of the chapter was to investigate whether the influence of AM fungi on Cs accumulation by plants is determined by external Cs supply. *Medicago truncatula* was grown in pots containing a fertilised sand:clay mixture without or with *Glomus intraradices*. Different concentrations of stable and radioactive Cs were applied to the sand:clay mixture. Concentrations of ^{134}Cs in plant tissues were measured. In addition, the influence of external Cs supply on mycorrhizal colonisation of *M. truncatula* was determined.

5.2 MATERIAL AND METHODS

5.2.1 The effects of potassium supply on growth of *Medicago truncatula* plants (Experiment A)

5.2.1.1 Organisms

Medicago truncatula Gaertn. var. *truncatula* Jemalong A17 was chosen as the experimental plant. The seeds were obtained from the South Australian Research and Development Institute (SARDI), Genetic Resource Centre, Australia.

5.2.1.2 Growth conditions

The non mycorrhizal plants were grown in the Arbuscular Mycorrhizal–Plant (AM-P) *in vitro* culture system as described in Sections 3.2.2 and 3.2.5. The plants were grown on variations of the modified Hoagland's (MH) medium containing 1 mM K (Table 3.2). First, the K concentration was reduced to a concentration of 0.2 mM K by increasing the Ca concentration to 9.8 mM. The accompanying anion was NO_3^-. Second, the K concentration was increased to a concentration of 5 mM K and the Ca concentration was reduced to 5 mM. The accompanying anion was NO_3^-. Third, the K concentration was increased to a concentration of 25 mM K and the Ca concentration was reduced to 5 mM. The accompanying anions were NO_3^- and Cl^-.

The pH of the MH media was adjusted to 5.5 with NaOH and the MH media were solidified using 6 g l^{-1} agar (A1296, Sigma Aldrich, St. Louis, MO, USA).

Eight plants per K treatment were grown for nine weeks in a growth cabinet with a photoperiod of 16 h light and 8 h darkness at a constant temperature of 22°C. The light intensity was approximately 350 μmol m^{-2} s^{-1}. Potassium concentrations in plant roots and shoots were determined using ICP-MS as described in Section 2.2.5.

5.2.1.3 Statistical analysis

To investigate whether increasing external K supply had an influence on plant fresh weights a general analysis of variance was performed using GenStat (12th Edition, VSN International, Hemel Hempstead, UK).

To investigate the effect of external K supply on the concentration of K in plant roots and shoots regression analyses were performed using GenStat. Exponential curves were fitted to the equation

$$y = \alpha + \beta \times \rho^x + \varepsilon.$$

5.2.2 Caesium accumulation by non mycorrhizal and mycorrhizal *Medicago truncatula* plants (Experiment B)

5.2.2.1 Organisms

Medicago truncatula Gaertn. var. *truncatula* Jemalong A17 was chosen as the experimental plant. The seeds were obtained from the South Australian Research and Development Institute (SARDI), Genetic Resource Centre, Australia.

The arbuscular mycorrhizal fungus *Glomus* sp. MUCL 43195 was used. The strain was obtained from the Glomeromycota In Vitro Collection (GINCO), Louvain-la-Neuve, Belgium.

5.2.2.2 Growth conditions

The non mycorrhizal and mycorrhizal plants were grown in the Arbuscular Mycorrhizal–Plant (AM-P) *in vitro* culture system as described in Sections 3.2.2 and 3.2.5. The plants were grown on modified Hoagland's (MH) medium containing 1 mM K (Table 3.2) without or with the addition of 0.05 mM CsCl. The concentration of Cs was based on observations obtained in Chapter 4. The concentration of Cs in modified Strullu-Romand (MSR) medium containing

1.65 mM K (Table 3.1) that was non toxic for *M. truncatula* was 0.1 mM Cs (see Chapter 4). Therefore, it was concluded that a concentration of 0.05 mM Cs in MH medium containing 1 mM K would be non toxic for *M. truncatula*. The plants were grown in a growth cabinet with a photoperiod of 16 h light and 8 h darkness at a constant temperature of 22°C. The light intensity was around 350 µmol m^{-2} s^{-1}. The plants were harvested after nine weeks. The experiment was repeated four times referred to as Experiments 1, 2, 3 and 4. Three plants were combined for each sample to obtain enough plant material for analyses. In Experiment 1, four non mycorrhizal and four mycorrhizal plant samples grown in the absence of Cs and three non mycorrhizal and three mycorrhizal plant samples grown in the presence of Cs were harvested. In Experiment 2, six non mycorrhizal and two mycorrhizal plant samples grown in the absence of Cs and four non mycorrhizal and two mycorrhizal plant samples grown in the presence of Cs were harvested. In Experiment 3, five non mycorrhizal and five mycorrhizal plant samples grown in the absence of Cs and four non mycorrhizal and four mycorrhizal plant samples grown in the presence of Cs were harvested. In Experiment 4, three non mycorrhizal and three mycorrhizal plant samples grown in the absence of Cs and three non mycorrhizal and three mycorrhizal plant samples grown in the presence of Cs were harvested. Half of the harvested plant roots and shoots were frozen in liquid N_2 whilst the other half was oven dried at 40–50°C for several days. The dried roots and shoots were used to determine element concentrations in plant tissues using ICP-MS as described in Section 2.2.5.

5.2.2.3 Determination of mycorrhizal infection

At harvest, all inoculated plants were examined for mycorrhizal colonisation using a binocular microscope (Olympus UK Ltd., Watford, UK). When the AM fungus had produced new spores the plants were classified as mycorrhizal and only those were used for further investigations. From Experiments 2, 3 and 4 some mycorrhizal plants that had been grown without or with Cs in the media were stained using an ink and vinegar technique (Vierheilig et al., 1998) to determine the mycorrhizal colonisation rate as described in Section 2.2.3.1. Roots from Experiment 2 were cleared by boiling in 2.5% KOH for three minutes. The staining failed in Experiment 2. Therefore, roots from Experiments 3 and 4 were cleared in 10% KOH for 24 h at 40ºC. To determine the percentage of mycorrhizal colonisation, 200 intersections per root sample were checked for the presence or absence of arbuscules,

vesicles, hyphae and spores. The percentage of mycorrhizal colonisation (% myc) was calculated from these data (Equation 2.1).

5.2.2.4 Statistical analysis

To determine the influence of AM fungi and of Cs supply on element concentrations in plant tissues an analysis of multiple experiments using the method of residual maximum likelihood (REML) was performed using GenStat. The analysis allows the investigation of treatment effects by excluding between experiment effects.

5.2.3 The effects of caesium supply on caesium accumulation by non mycorrhizal and mycorrhizal *Medicago truncatula* plants (Experiment C)

The experiment was conducted in collaboration with Sergiy Dubchak and Prof. Katarzyna Turnau at the Jagiellonian University, Krakow, Poland.

5.2.3.1 Organisms

Medicago truncatula Gaertn. var. *truncatula* Jemalong A17 was chosen as the experimental plant. The seeds were obtained from the South Australian Research and Development Institute (SARDI), Genetic Resource Centre, Australia.

An inoculum containing root fragments and *Glomus intraradices* spores (strain BIO, obtained from BIORIZE, Dijon, France) from pot cultures maintained at the Jagiellonian University, Krakow, Poland was used.

5.2.3.2 Growth conditions

The plants were grown in pots containing 0.64 kg of a mixture of sterilised sand and clay (3:1 v/v or w/w). For sterilisation the substrate was heated to 100ºC for 1 h. The procedure was repeated three times at 24 h intervals. Before the start of the experiment the substrate was fertilised with 22.5 g rock phosphate and 64 mg KNO_3 per pot.

Caesium was added in the form of Cs_2CO_3 and as a $^{134}CsCl$ water solution with an initial activity of 1 MBq 10 ml^{-1} (Institute of Atomic Energy POLATOM, Otwock-Świerk, Poland). The ^{134}Cs source was diluted in deionised water to achieve a concentration of 1000 Bq l^{-1} and its pH was adjusted to 6.5 with 10% KOH. Five different concentrations of stable ^{133}Cs and of radioactive ^{134}Cs were applied (Table

5.1). Stable ^{133}Cs was used to alter external Cs supply whereas radioactive ^{134}Cs was used as a tracer to measure low concentrations of Cs in plant tissues.

Table 5.1: Activity concentrations of ^{134}Cs and concentrations of ^{133}Cs per pot containing 0.64 kg substrate. Two plants per pot were maintained.

	A	**B**	**C**	**D**	**E**
^{134}Cs [Bq]	3125	6500	12500	25000	50000
^{133}Cs [µg]	0.1	0.4	2	10	50

The total Cs concentrations applied in the different treatments were as followed:

Treatment A: 0.00015625 µg Cs g^{-1} substrate

Treatment B: 0.000625 µg Cs g^{-1} substrate

Treatment C: 0.003125 µg Cs g^{-1} substrate

Treatment D: 0.015625 µg Cs g^{-1} substrate

Treatment E: 0.078125 µg Cs g^{-1} substrate

Three pots containing two non mycorrhizal plants each, and three pots containing two mycorrhizal plants each, were maintained for each of the five Cs treatments. The pots were kept in Sun bags (Sigma Aldrich, St. Louis, MO, USA) and plants were grown for ten weeks in a growth chamber with a photoperiod of 12 h light and 12 h darkness, at 47% humidity and a constant temperature of 21°C. The plants were watered with sterilised distilled water and fertilised once a week with Long Ashton solution (Hewitt, 1966). At harvest, fresh weights of shoots and roots were determined. The shoots of the two plants per pot were combined for γ-spectrometry measurements. The root of one plant per pot was used for γ-spectrometry measurements and the root of the second plant was used to determine mycorrhizal colonisation rate.

5.2.3.3 Gamma-spectrometry measurements

The activity of ^{134}Cs in roots and shoots of *M. truncatula* was determined using a gamma-spectrometer with a semiconductor p-type coaxial HP-Ge detector with a relative efficiency of 15% and a resolution of 2.5 keV at 1.33 MeV, shielded by 10 cm of lead with an inner lining of 2 mm Cd and 18 mm Cu. Roots and shoots were oven dried at 40ºC for 26 h. The plant tissues were cut with scissors and homogenised. Plant materials were transferred to plastic vessels with the same

surface area and covered with 5–10 ml of deionised water and 100% EtOH (1/1, v/v) containing sucrose. The samples were dried completely in an oven at 50ºC.

The activity concentration (A) of ^{134}Cs was derived as averaged value from its two most intensive gamma-lines ($E_{\gamma 1}$ = 604.6 keV and $E_{\gamma 2}$ = 795.8 keV, respectively). The ^{134}Cs activity concentration for each of those gamma-emission lines was evaluated according to the equation (Equation 5.1; Dubchak et al., 2010):

$$A\left[Bq \cdot kg^{-1}\right] = \frac{(N_0 - N_b)_{sample}}{(N_0 - N_b)_{ref}} \cdot \frac{A_{ref}}{m} \qquad \text{[eq. 5.1]},$$

whereby

N_0 – count rate in corresponding peak of radionuclide (counts per second – cps);

N_b – background count rate (cps);

A_{ref} – activity of reference source;

m – mass of sample (kg).

5.2.3.4 Determination of mycorrhizal colonisation

To determine mycorrhizal colonisation rates, roots of *M. truncatula* were prepared according to the modified method of Phillips and Hayman (1970). The roots were washed with tap water and cleared in 10% KOH at room temperature (RT) for 22 h. After washing the roots in tap water to remove KOH, 5% lactic acid was added and the roots were acidified at RT for 1 h. Subsequently, the roots were transferred to a staining solution containing 0.05% aniline blue and concentrated (80%) lactic acid. The roots were stained at RT for 20 h. The roots were cut into 1 cm long pieces and 15 randomly chosen, young root pieces per plant were aligned on a microscope slide. The stained roots were observed for mycorrhizal infection using a Nikon microscope (Nikon eclipse E800, Nikon Instruments Europe B.V., Amstelveen, The Netherlands). The frequency of mycorrhiza in the root system (F%), intensity of mycorrhizal colonisation in the whole root system (M%), intensity of mycorrhizal colonisation in mycorrhizal parts of the root (m%), arbuscule abundance in the whole root system (A%) and arbuscule abundance in mycorrhizal parts of the root (a%) (Trouvelot et al., 1986) were determined using Mikoryza (Version 1.1 Beta 2001, Dariusz Orlowski, Jagiellonian University, Krakow, Poland). Photographs were

taken using a Nikon Digital camera (Nikon Digital Sight DS-L1, Nikon Instruments Europe B.V., Amstelveen, The Netherlands).

5.2.3.5 Statistical analysis

To investigate whether Cs supply and mycorrhizal infection influenced plant fresh weights and concentrations of Cs in plant tissues, general analyses of variance were performed using GenStat. Caesium concentrations in shoots were log transformed to the base 10 to meet the ANOVA premises. To investigate whether increasing Cs concentrations in the substrate had an influence on mycorrhizal colonisation of roots a general analysis of variance was performed using GenStat. Significant differences between average values were determined using Fisher's protected least significant difference test using GenStat.

5.3 RESULTS

5.3.1 The effects of potassium supply on growth of *Medicago truncatula* plants (Experiment A)

Medicago truncatula was grown on MH media containing different concentrations of K. Generally, the fresh weights (FWs) of roots were higher than the FWs of shoots (General analysis of variance, $F_{1,63} = 164.27$, $p < 0.001$; Figure 5.1). The FWs of roots were not affected by increasing concentrations of K in the media (Figure 5.1). However, the FWs of shoots increased over the range of 0.2 to 5 mM external K supply (General analysis of variance, $F_{3,63} = 2.77$, $p = 0.05$; Figure 5.1).

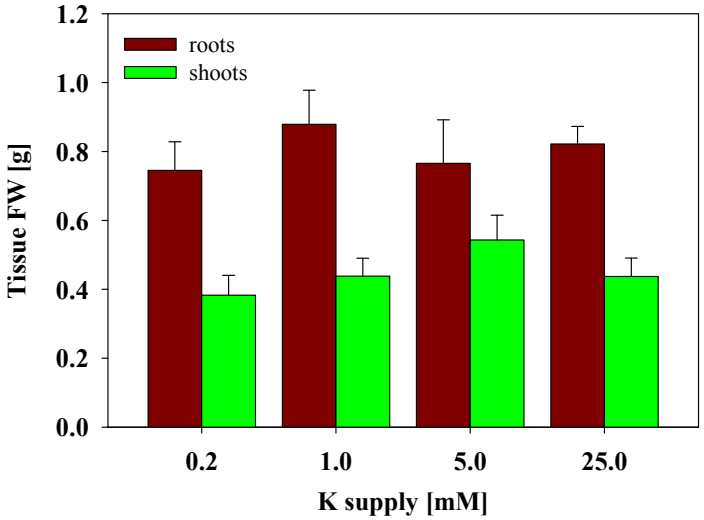

Figure 5.1: Fresh weights of roots and shoots of *Medicago truncatula* grown on MH media containing 0.2, 1, 5 or 25 mM K. Data show mean values and 95% confidence intervals (n = 8).

Increasing K concentrations in the media led to increasing concentrations of K in plant roots and shoots (Figure 5.2). The relationship between K concentrations in plant tissues and external K concentration followed the equation of exponential curves (Table 5.2). The concentration of K in roots was higher than the concentration of K in shoots at high external K supply (Parameter α in Table 5.2). At low external K supply concentrations of K in roots and shoots did not differ (Figure 5.2).

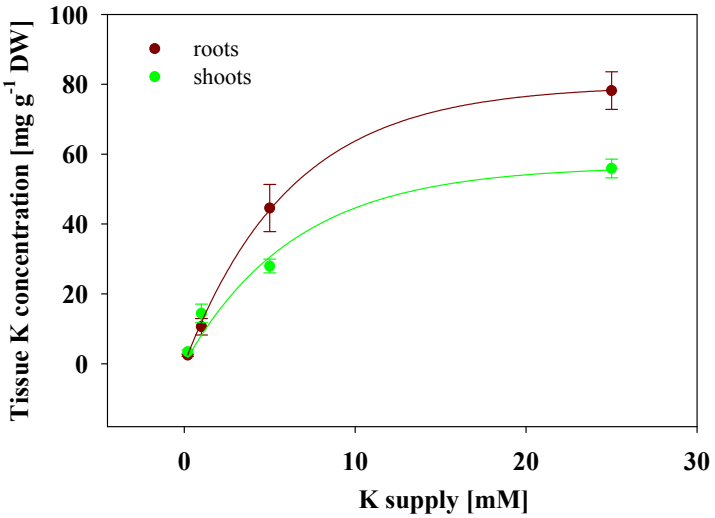

Figure 5.2: The effect of K supply on K concentrations in roots and shoots of *Medicago truncatula*. Data show mean values and 95% confidence intervals (n = 8). The relationships between external K supply and tissue K concentrations were fitted to exponential curves. The parameters for these equations are given in Table 5.2.

Table 5.2: Parameters for the regression lines in Figure 5.2. The relationships between $[K]_{root}$ and $[K]_{shoot}$ with external K supply were fitted to exponential curves: $y = \alpha + \beta \times \rho^x + \varepsilon$

Relationship	Parameter	Estimate
$[K]_{root}$ versus external [K]	α	80.78
	β	-80.71
	ρ	0.86
$[K]_{shoot}$ versus external [K]	α	55.79
	β	-52.41
	ρ	0.86

5.3.2 Caesium accumulation by non mycorrhizal and mycorrhizal *Medicago truncatula* plants (Experiment B)

5.3.2.1 Plant growth

Medicago truncatula was grown in an *in vitro* system on MH medium containing 1 mM K without or with the addition of 0.05 mM Cs. Non mycorrhizal plants and mycorrhizal plants colonised by *Glomus* sp. were grown. The plants were harvested after nine weeks and fresh weights (FWs) of roots and shoots were determined (Figure 5.3).

Although, in the four experiments performed, the plants were grown under identical conditions in a growth cabinet, the FWs of roots differed significantly between experiments (REML variance component analysis, $F_3 = 20.85$, $p < 0.001$; Figure 5.3). However, roots that had been grown in the absence of Cs were significantly heavier than roots that had been grown in the presence of Cs (REML variance component analysis, $F_1 = 18.39$, $p < 0.001$; Table 5.3). Colonisation with *Glomus* sp. did not influence the FWs of roots, regardless if Cs was added to the medium or not. The FWs of shoots also differed significantly between experiments (REML variance component analysis, $F_3 = 31.39$, $p < 0.001$; Figure 5.3). Neither the presence of Cs in the medium nor colonisation with *Glomus* sp. influenced the FWs of shoots.

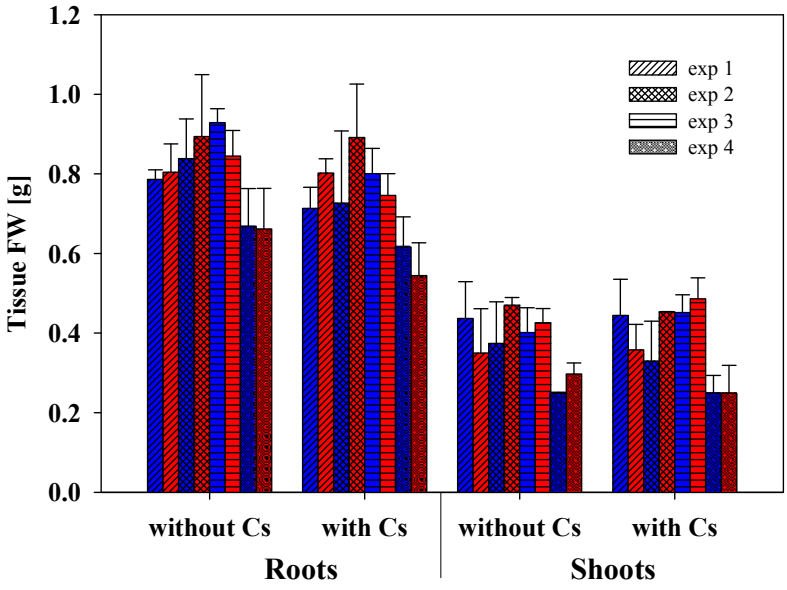

Figure 5.3: Fresh weights of *Medicago truncatula* roots and shoots. Plants had been grown on MH medium without or with the addition of Cs. Fresh weights of non mycorrhizal (blue) and mycorrhizal (red) plants from Experiments (exp) 1, 2, 3 and 4. Data show mean values and 95% confidence intervals (n = 2–6).

Table 5.3: Fresh weights of *Medicago truncatula* roots grown on MH medium without or with the addition of Cs. Predicted means and standard error of differences from an analysis of multiple experiments using the method of residual maximum likelihood.

	Without caesium	**With caesium**
Root FW [g]	0.803	0.730
	± 0.026	

5.3.2.2 Mycorrhizal colonisation

Before harvest, all plants that had been inoculated with *Glomus* sp. were investigated for their mycorrhizal status. Plants were determined as mycorrhizal when newly produced spores were present. Figure 5.4 shows new spores produced by *Glomus* sp. in association with *M. truncatula*. The average colonisation rate of *M. truncatula* plants that had been grown in the absence of Cs in the medium was 12.3%, whereas the average colonisation rate of *M. truncatula* plants that had been grown in the presence of Cs in the medium was 9.8%. Figure 5.5 shows arbuscules in root cells of *M. truncatula* plants. Only plants that could be identified clearly as mycorrhizal were used for investigations of element concentrations in plant tissues.

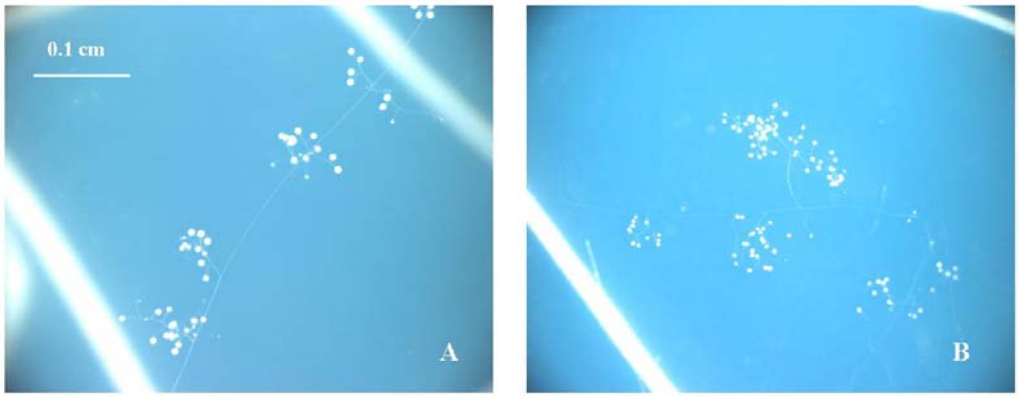

Figure 5.4: *Glomus* sp. spores developed in symbiosis with *Medicago truncatula* grown on MH medium (A) and on MH medium containing 0.05 mM Cs (B).

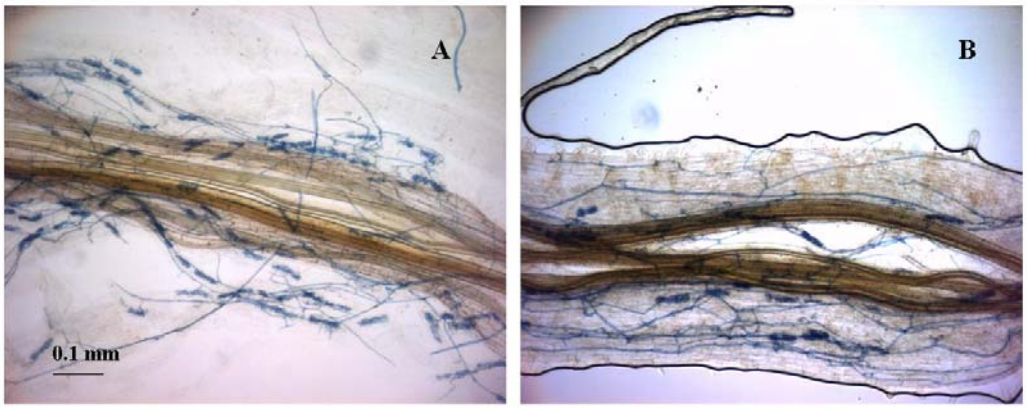

Figure 5.5: Arbuscules and hyphae of *Glomus* sp. in roots of *Medicago truncatula* plants grown on MH medium (A) and on MH medium containing 0.05 mM Cs (B).

5.3.2.3 Element concentrations in Medicago truncatula *plants*

It was hypothesised that mycorrhizal colonisation would increase K concentrations in plant tissues and thereby reduce Cs concentrations in plants. To test this hypothesis, K concentrations (Figure 5.6) and Cs concentrations (Figure 5.7) in roots and shoots of non mycorrhizal and mycorrhizal *M. truncatula* plants were determined. Since AM fungi transport P to their host plants, P concentrations in roots and shoots of non mycorrhizal and mycorrhizal *M. truncatula* plants were also determined (Figure 5.8). To allow comparisons of the influence of AM fungi on mineral accumulation by plants under *in vitro* conditions and when grown in soil, Ca concentrations (Figure 5.9) and Mg concentrations (Figure 5.10) in roots and shoots of non mycorrhizal and mycorrhizal *M. truncatula* plants were also determined.

Potassium concentrations in roots differed significantly between experiments (REML variance component analysis, $F_3 = 25.51$, $p < 0.001$; Figure 5.6). Plants that had been grown in the absence of Cs had lower K concentrations in roots than plants that had been grown in the presence of Cs (REML variance component analysis, $F_1 = 30.18$, $p < 0.001$; Table 5.4). However, mycorrhizal colonisation did not influence K concentrations in roots. Potassium concentrations in shoots also differed significantly between experiments (REML variance component analysis, $F_3 = 10.36$, $p < 0.001$; Figure 5.6). Neither the presence of Cs in the medium nor mycorrhizal colonisation influenced K concentrations in shoots.

Caesium concentrations in roots differed greatly between experiments (REML variance component analysis, $F_3 = 197.23$, $p < 0.001$; Figure 5.7). The influence of mycorrhizal colonisation on Cs concentrations in roots depended on the experiment (REML variance component analysis, $F_3 = 12.32$, $p < 0.001$; Figure 5.7). In Experiment 1, mycorrhizal colonisation reduced Cs concentrations in roots whereas in Experiment 2, mycorrhizal colonisation increased Cs concentrations in roots. In Experiments 3 and 4, mycorrhizal colonisation did not influence Cs concentrations in roots. Caesium concentrations in shoots also differed between experiments (REML variance component analysis, $F_3 = 258.32$, $p < 0.001$; Figure 5.7). Excluding the effect of experiments using a REML analysis, it was observed that mycorrhizal colonisation reduced Cs concentrations in shoots (REML variance component analysis, $F_1 = 7.67$, $p = 0.021$; Table 5.5).

The concentrations of P in roots differed between experiments (REML variance component analysis, $F_3 = 38.44$, $p < 0.001$; Figure 5.8). The presence of Cs in the medium increased P concentrations in roots (REML variance component analysis, $F_1 = 11.59$, $p = 0.002$; Table 5.4) but mycorrhizal colonisation did not influence P concentrations in roots. The concentrations of P in shoots also differed between experiments (REML variance component analysis, $F_3 = 14.19$, $p < 0.001$; Figure 5.8). The presence of Cs in the medium did not influence P concentrations in shoots but mycorrhizal colonisation increased P concentrations in shoots (REML variance component analysis, $F_1 = 4.41$, $p = 0.042$; Table 5.5).

The concentrations of Ca in roots differed between experiments (REML variance component analysis, $F_3 = 12.76$, $p < 0.001$; Figure 5.9). Neither the presence of Cs in the medium nor mycorrhizal colonisation influenced Ca concentrations in roots. The concentrations of Ca in shoots also differed between experiments (REML

variance component analysis, $F_3 = 5.14$, $p = 0.010$; Figure 5.9). The presence of Cs in the medium did not influence Ca concentrations in shoots, but non mycorrhizal plants had higher Ca concentrations in their shoots than mycorrhizal plants (REML variance component analysis, $F_1 = 9.00$, $p = 0.005$; Table 5.5).

The concentrations of Mg in roots differed between experiments (REML variance component analysis, $F_3 = 22.71$, $p < 0.001$; Figure 5.10). Plants that had been grown on medium without Cs had higher concentrations of Mg in their roots than plants that had been grown with Cs in the medium (REML variance component analysis, $F_1 = 94.67$, $p < 0.001$; Table 5.4). Furthermore, non mycorrhizal plants had higher Mg concentrations in their roots than mycorrhizal plants (REML variance component analysis, $F_1 = 6.31$, $p = 0.019$; Table 5.5). The concentrations of Mg in shoots also differed between experiments (REML variance component analysis, $F_3 = 4.21$, $p = 0.021$; Figure 5.10). Plants that had been grown without Cs in the medium had lower Mg concentrations in their shoots than plants that had been grown with Cs in the medium (REML variance component analysis, $F_1 = 22.20$, $p < 0.001$; Table 5.4). In Experiment 1, non mycorrhizal plants had lower Mg concentration in their shoots than mycorrhizal plants (REML variance component analysis, $F_3 = 3.53$, $p = 0.037$; Figure 5.10) whereas in Experiments 2, 3 and 4 mycorrhizal colonisation did not influence Mg concentrations in shoots.

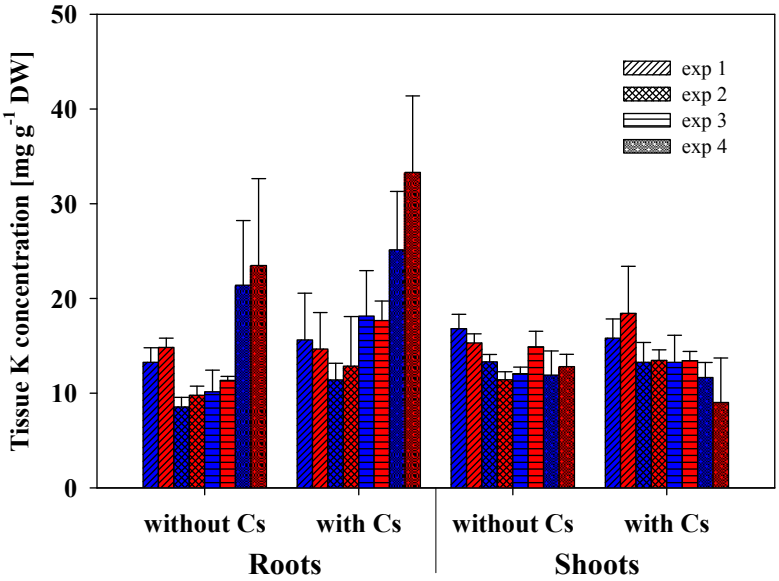

Figure 5.6: Potassium concentrations in *Medicago truncatula* roots and shoots. Plants were grown on MH medium without or with the addition of Cs. Potassium concentrations of non mycorrhizal (blue) and mycorrhizal (red) plants from Experiments (exp) 1, 2, 3 and 4. Data show mean values and 95% confidence intervals (n = 2–6).

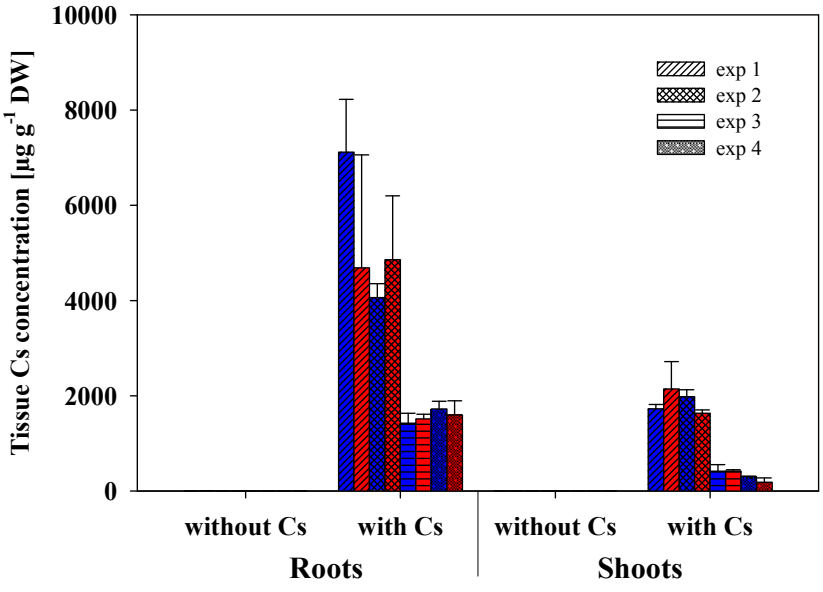

Figure 5.7: Caesium concentrations in *Medicago truncatula* roots and shoots. Plants were grown on MH medium without or with the addition of Cs. Caesium concentrations of non mycorrhizal (blue) and mycorrhizal (red) plants from Experiments (exp) 1, 2, 3 and 4. Data show mean values and 95% confidence intervals (n = 2–6).

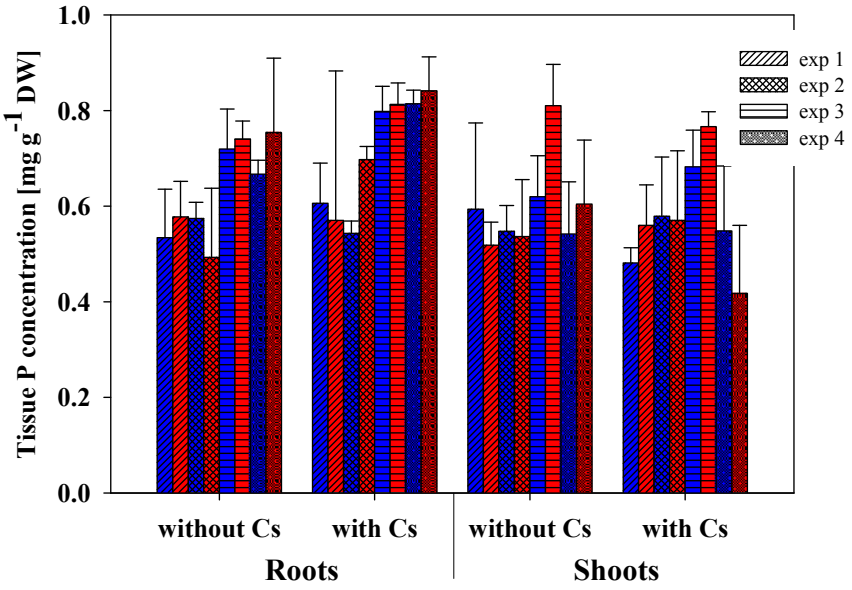

Figure 5.8: Phosphorus concentrations in *Medicago truncatula* roots and shoots. Plants were grown on MH medium without or with the addition of Cs. Phosphorus concentrations of non mycorrhizal (blue) and mycorrhizal (red) plants from Experiments (exp) 1, 2, 3 and 4. Data show mean values and 95% confidence intervals (n = 2–6).

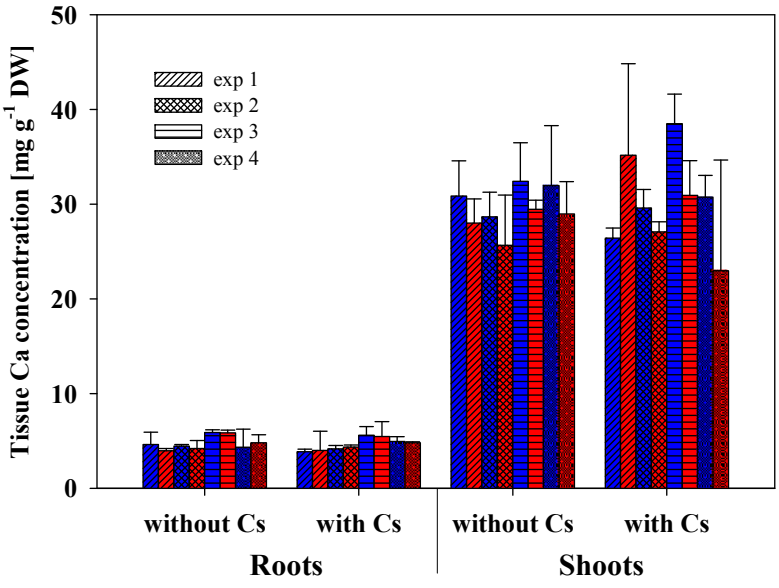

Figure 5.9: Calcium concentrations in *Medicago truncatula* roots and shoots. Plants were grown on MH medium without or with the addition of Cs. Calcium concentrations of non mycorrhizal (blue) and mycorrhizal (red) plants from Experiments (exp) 1, 2, 3 and 4. Data show mean values and 95% confidence intervals (n = 2–6).

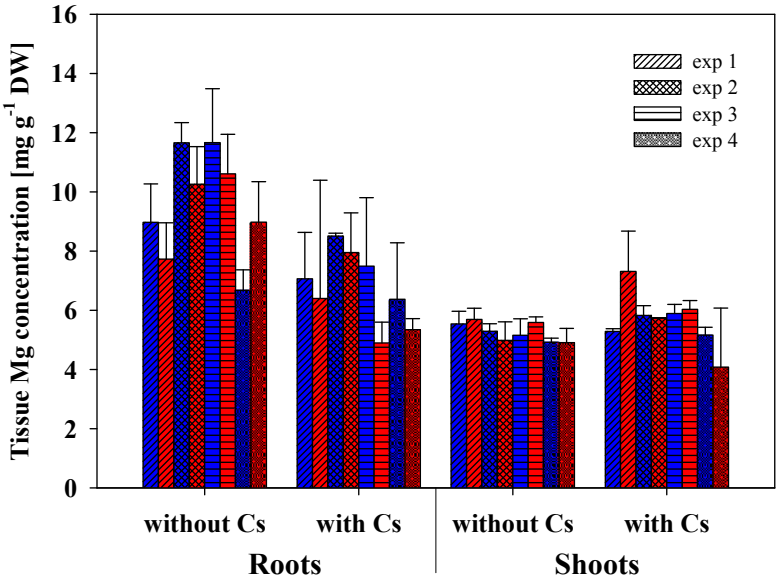

Figure 5.10: Magnesium concentrations in *Medicago truncatula* roots and shoots. Plants were grown on MH medium without or with the addition of Cs. Magnesium concentrations of non mycorrhizal (blue) and mycorrhizal (red) plants from Experiments (exp) 1, 2, 3 and 4. Data show mean values and 95% confidence intervals (n = 2–6).

Table 5.4: Concentrations of K, Mg and P in roots and concentrations of Mg in shoots of *Medicago truncatula* plants grown on MH medium without or with the addition of Cs. Predicted means and standard error of differences from an analysis of multiple experiments using the method of residual maximum likelihood.

Element concentration	Tissue	Without caesium	With caesium
K [mg g^{-1} DW]	roots	14.1	18.6
		± 1.173	
Mg [mg g^{-1} DW]	roots	9.568	6.754
		± 0.3974	
P [mg g^{-1} DW]	roots	0.6324	0.7103
		± 0.02561	
Mg [mg g^{-1} DW]	shoots	5.26	5.665
		± 0.1852	

Table 5.5: Concentrations of Mg in roots and concentrations of Ca, Cs and P in shoots of non mycorrhizal or mycorrhizal *Medicago truncatula* plants. Predicted means and standard error of differences from an analysis of multiple experiments using the method of residual maximum likelihood.

Element concentration	Tissue	Non mycorrhizal	Mycorrhizal
Mg [mg g^{-1} DW]	roots	8.551	7.771
		± 0.3974	
Ca [mg g^{-1} DW]	shoots	31.15	28.53
		± 1.264	
Cs [ug g^{-1} DW]	shoots	1107	1094
		± 78.39	
P [mg g^{-1} DW]	shoots	0.5741	0.5978
		± 0.02891	

5.3.3 The effects of caesium supply on caesium accumulation of non mycorrhizal and mycorrhizal *Medicago truncatula* plants (Experiment C)

5.3.3.1 Plant growth and accumulation of caesium

Medicago truncatula was grown without or with an inoculum containing *Glomus intraradices* in a sand:clay mixture with the addition of 0.1, 0.4, 2, 10 or 50 µg Cs per pot (Figure 5.11).

The FWs of roots were not influenced by the different concentrations of Cs in the substrate but non mycorrhizal roots were significantly heavier than mycorrhizal roots (General analysis of variance, $F_{1,29} = 17.78$, $p < 0.001$; Figure 5.12). The FWs of shoots were not influenced by supply of Cs or by mycorrhizal colonisation (Figure 5.13).

Concentrations of Cs in roots increased with higher supply of Cs to the substrate (General analysis of variance, $F_{4,29} = 211.70$, $p < 0.001$; Figure 5.14) but mycorrhizal colonisation did not influence Cs concentrations in roots. Concentrations of Cs in shoots also increased with higher supply of Cs to the substrate (General analysis of variance, $F_{4,29} = 84.98$, $p < 0.001$; Figure 5.15). The concentrations of ^{133}Cs and ^{134}Cs in the substrate were not increased in the same order of magnitude. Proportionally higher concentrations of ^{133}Cs in comparison to ^{134}Cs at high total Cs supply lead to a dilution of ^{134}Cs. Due to the dilution effect differences in ^{134}Cs concentrations in tissues of *M. truncatula* at different external Cs supply might be underestimated. The influence of mycorrhizal colonisation on Cs concentrations in shoots was dependent on Cs supply. At a Cs supply of 0.1 µg per pot mycorrhizal

colonisation increased Cs concentrations in shoots whereas at a Cs supply of 0.4 µg per pot mycorrhizal colonisation decreased Cs concentrations in shoots. At higher concentrations of external Cs supply of 2–50 µg per pot mycorrhizal colonisation did not influence Cs concentrations in shoots (General analysis of variance, $F_{4,29} = 4.85$, $p = 0.007$; Figure 5.15).

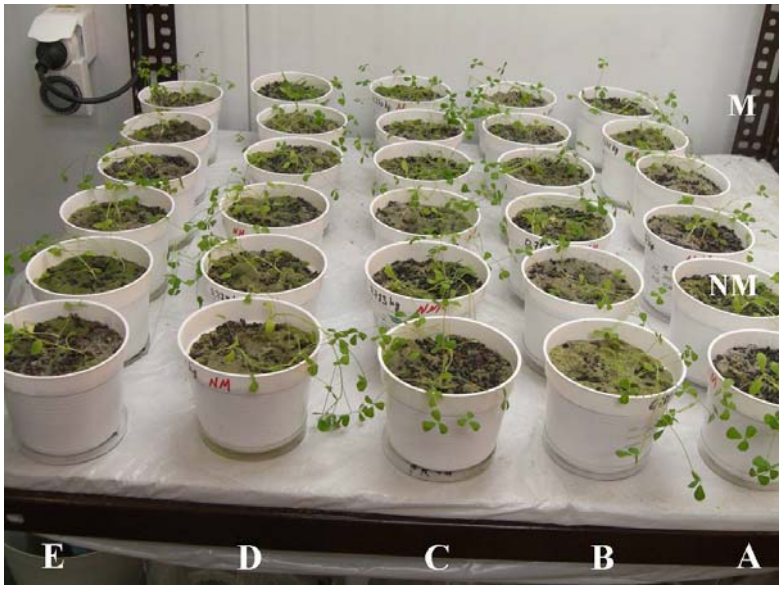

Figure 5.11: Ten-week old *Medicago truncatula* plants grown on a sand:clay mixture with different concentrations of Cs. Plants were grown with the addition of 0.1 µg (A), 0.4 µg (B), 2 µg (C), 10 µg (D) and 50 µg (E) Cs per pot. The three rows in the front of the picture are non mycorrhizal plants (NM) and the three rows in the back of the picture are mycorrhizal plants (M).

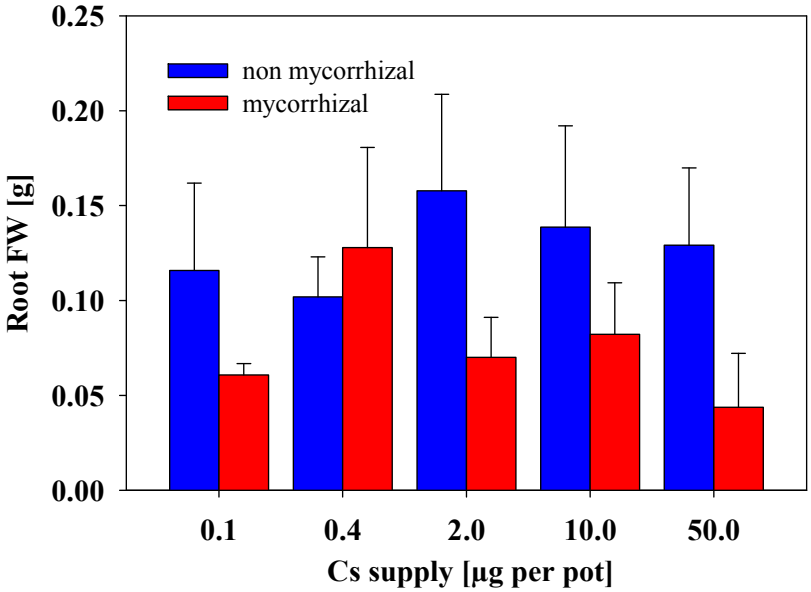

Figure 5.12: The effect of Cs supply on fresh weights of non mycorrhizal (blue) and mycorrhizal (red) *Medicago truncatula* roots grown on a sand:clay mixture. Data show mean values and 95% confidence intervals (n = 3).

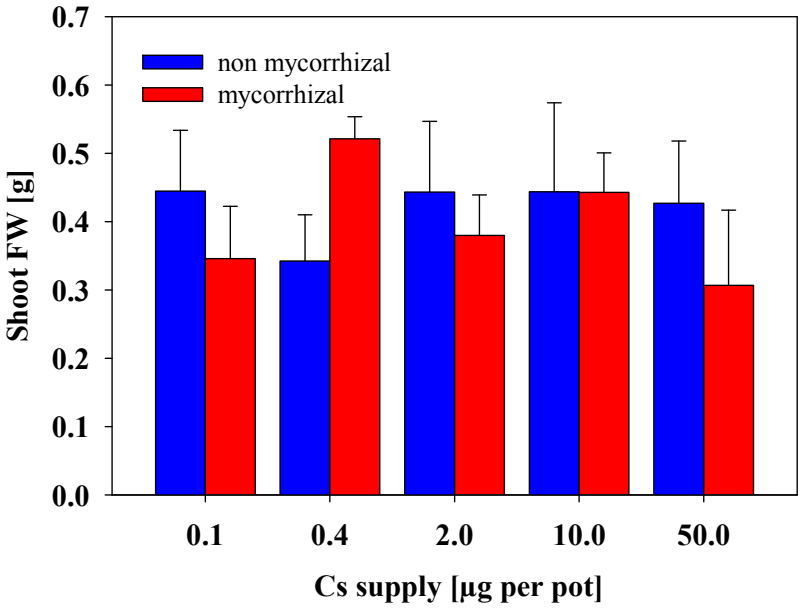

Figure 5.13: The effect of Cs supply on fresh weights of non mycorrhizal (blue) and mycorrhizal (red) *Medicago truncatula* shoots grown on a sand:clay mixture. Data show mean values and 95% confidence intervals (n = 3).

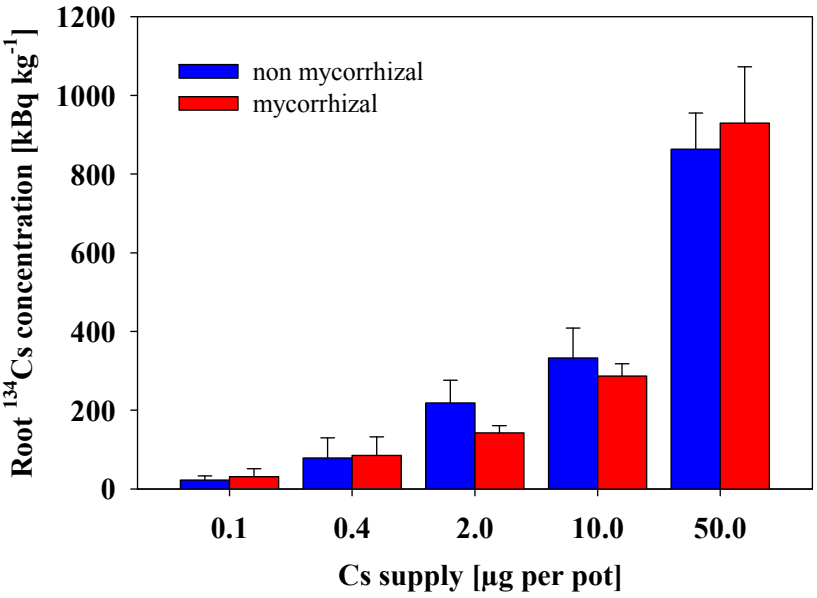

Figure 5.14: The effect of Cs supply on ^{134}Cs activity concentrations in non mycorrhizal (blue) and mycorrhizal (red) *Medicago truncatula* roots grown on a sand:clay mixture. Data show mean values and 95% confidence intervals (n = 3).

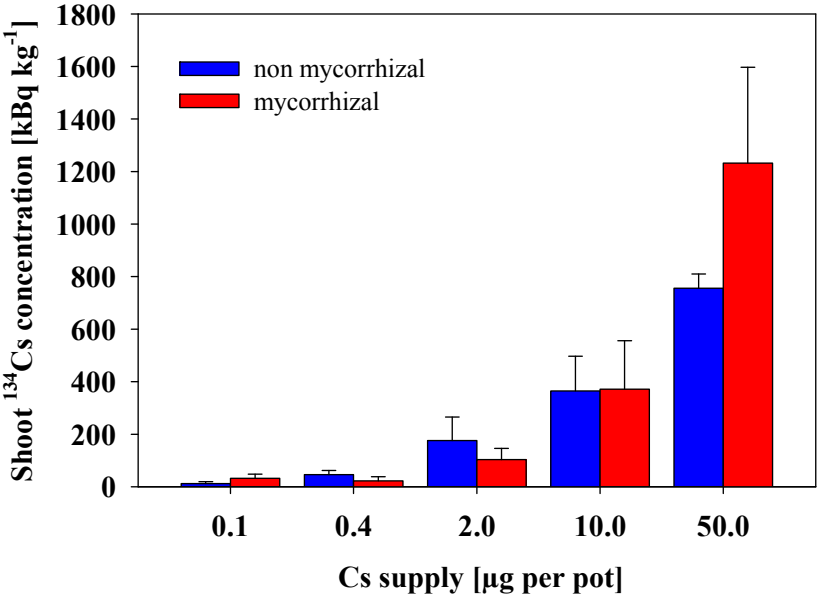

Figure 5.15: The effect of Cs supply on ^{134}Cs activity concentrations in non mycorrhizal (blue) and mycorrhizal (red) *Medicago truncatula* shoots grown on a sand:clay mixture. Data show mean values and 95% confidence intervals (n = 3).

5.3.3.2 Mycorrhizal colonisation influenced by external caesium supply

The frequency of mycorrhiza in the root system (F%), intensity of mycorrhizal colonisation in the whole root system (M%), intensity of mycorrhizal colonisation in mycorrhizal parts of the root (m%), arbuscule abundance in the whole root system (A%) and arbuscule abundance in mycorrhizal parts of the root (a%) were determined for *M. truncatula* that had been grown in a sand:clay mixture with different concentrations of Cs (Table 5.6).

With higher external Cs supply all mycorrhizal parameters in *M. truncatula* roots decreased. The frequency of mycorrhiza in the root system (F%) was between 60% and 78% when external Cs supply was low but decreased significantly when external Cs supply was high (General analysis of variance, $F_{4,14} = 11.76$, $p < 0.001$; Table 5.6). The intensity of mycorrhizal colonisation in the whole root system (M%) decreased significantly with higher Cs supply (General analysis of variance, $F_{4,14} = 7.04$, $p = 0.006$; Table 5.6) and the intensity of mycorrhizal colonisation in mycorrhizal parts of the root (m%) decreased as well (General analysis of variance, $F_{4,14} = 9.79$, $p = 0.002$; Table 5.6). The arbuscule abundance of the whole root system (A%) decreased from 22% at an external Cs supply of 0.1 µg per pot to less than 1% at an external Cs supply of 50 µg per pot (General analysis of variance, $F_{4,14} = 3.59$, $p = 0.046$; Table 5.6). The arbuscule abundance in mycorrhizal parts of the root decreased from 78% at an external Cs supply of 0.1 µg per pot to 26% at an external Cs supply of 50 µg per pot (General analysis of variance, $F_{4,14} = 4.20$, $p = 0.030$; Table 5.6).

Figure 5.16 shows arbuscules in roots of *M. truncatula*. It seemed that, at high external Cs supply of 10 and 50 µg per pot, the abundance of hyphae growing on the root surface and the formation of vesicles within the root increased. However, the entry points of the fungus into root cells and the abundance of arbuscules seemed to decrease throughout the root when external Cs supply was high.

Table 5.6: Mycorrhizal parameters for roots of *Medicago truncatula* inoculated with *Glomus intraradices* grown in a sand:clay mixture containing 0.1, 0.4, 2, 10 or 50 µg Cs per pot. Frequency of mycorrhiza in the root system (F%), intensity of mycorrhizal colonisation in the whole root system (M%), intensity of mycorrhizal colonisation in mycorrhizal parts of the root (m%), arbuscule abundance in the whole root system (A%) and arbuscule abundance in mycorrhizal parts of the root (a%). Data show mean values and 95% confidence intervals. Letters beside the values show significant differences (Fisher's protected least significant difference test).

Cs supply	**F%**	**M%**	**m%**	**A%**	**a%**
0.1 µg per pot	59.21 **bc** ± 19.51	27.46 **c** ± 12.43	45.53 **c** ± 11.22	22.96 **c** ± 16.81	78.46 **b** ± 22.83
0.4 µg per pot	77.78 **c** ± 4.36	26.55 **c** ± 5.01	34.10 **bc** ± 6.50	20.00 **bc** ± 7.71	74.09 **b** ± 14.60
2 µg per pot	68.89 **bc** ± 15.71	20.11 **bc** ± 5.39	29.33 **b** ± 6.05	14.27 **abc** ± 9.66	67.20 **b** ± 24.40
10 µg per pot	51.11 **b** ± 4.35	12.33 **ab** ± 3.64	24.02 **ab** ± 6.92	6.92 **ab** ± 2.59	56.11 **ab** ± 11.83
50 µg per pot	22.22 **a** ± 8.72	3.16 **a** ± 1.17	14.42 **a** ± 3.15	0.98 **a** ± 1.18	26.04 **a** ± 23.28

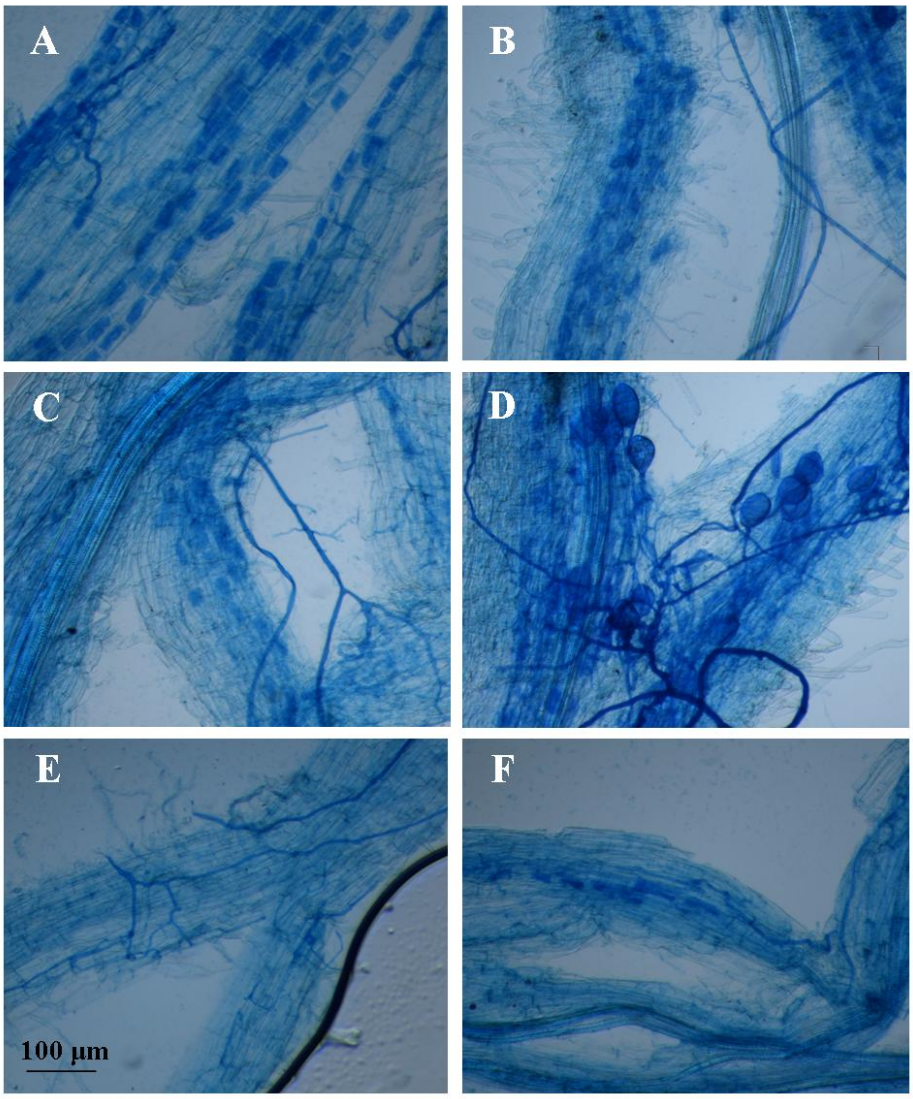

Figure 5.16: Mycorrhizal structures in root cells of *Medicago truncatula* plants grown in the presence of 0.1 µg Cs (A), 0.4 µg Cs (B), 2 µg Cs (C), 10 µg Cs (D) or 50 µg Cs (E, F) per pot. Mycorrhizal colonisation and abundance of arbuscules was high when the supply of Cs was low. However, at high supply of Cs the abundance of arbuscules decreased whereas the abundance of hyphae and vesicles seemed to increase.

5.4. DISCUSSION

5.4.1 Increasing external potassium supply led to increasing concentrations of K in plant roots and shoots (Experiment A)

The critical value of K for plants is around 5–20 mg g^{-1} DW (Leigh and Wyn Jones, 1984; White and Karley, 2010). Potassium concentrations in roots and shoots of *M. truncatula* plants that had been grown in the presence of 0.2 and 1 mM K in the medium had tissue K concentrations lower than 20 mg g^{-1} DW (Figure 5.1). Additionally, older leaves of *M. truncatula* plants died after a few weeks when plants had been grown in the presence of 0.2 and 1 mM K. Since K is readily redistributed within the plant *via* the phloem from mature to developing tissues, leaves of K-deficient plants have a shorter lifetime than of K-replete plants (White and Karley, 2010). Furthermore, K-deficient plants grow slower than K-replete plants and shoots are more susceptible to K-deficiency than roots (White and Karley, 2010). The FWs of shoots increased over the range of 0.2 to 5 mM external K supply (Figure 5.1).

The aim of this experiment was to determine K-deficient conditions for *M. truncatula*. Since shoot K concentrations were lower than 20 mg g^{-1} DW when plants had been grown on medium containing 1 mM K the plants were K-deficient.

5.4.2 Arbuscular mycorrhizal fungi and Cs accumulation by *Medicago truncatula* plants

5.4.2.1 The influence of arbuscular mycorrhizal fungi on Cs concentrations in K-deficient Medicago truncatula *plants (Experiment B)*

To test the hypothesis that AM fungi reduce Cs accumulation by plants under K-deficient conditions, *M. truncatula* was grown in the absence or presence of *Glomus* sp. on media containing 1 mM K without or with the addition of 0.05 mM Cs. Four experiment replicates (Experiments 1, 2, 3 and 4) were performed under identical conditions in a growth cabinet. However, the FWs of both roots and shoots and the element concentrations in plant tissues varied significantly between experiments. There was no obvious reason for these differences.

Plants that had been grown without Cs in the medium had higher FWs of roots than plants that had been grown with the addition of 0.05 mM Cs (Table 5.3). It was previously observed that a Cs:K ratio in the medium of 1:20 is non toxic for *M.*

truncatula (see Chapter 4). However, different types of media with different solidifying agents were used in the experiments of Chapters 4 and 5 and a slightly toxic effect of Cs might have occurred. Furthermore, the average colonisation rate of *M. truncatula* plants that had been grown in the absence of Cs was 12.3%, whereas the average colonisation rate of *M. truncatula* plants that had been grown in the presence of Cs was 9.8%. However, these rates are based on a small number of plants and are not necessarily representative for all plants used in the experiments, especially since none of the plants investigated for mycorrhizal colonisation were sampled from Experiments 1 or 2. Colonisation rates of around 10% are low because roots of *M. truncatula* are frequently colonised to more than 50% by *Glomus* species after a growth period of more than four weeks (Burleigh and Harrison, 1998; Joner et al., 2004; Gyuricza et al., 2010b). However, a low colonisation rate does not automatically mean that the nutrient transfer *via* the fungus is low as well. Several authors did not find any relationship between the percentage of AM colonisation with shoot P concentrations or plant growth (Jensen, 1982; Sanders and Fitter, 1992; Klironomos, 2000; Jakobsen et al., 2001; Smith et al., 2004). Therefore, element transfer including K and Cs by *Glomus* sp. to *M. truncatula* might still have occurred although the colonisation rates were around 10%.

The concentrations of K and P in roots were higher when plants were grown with Cs than when plants were grown without Cs (Table 5.7). Since all plants were K-deficient, genes encoding K^+/H^+ symporters (KUPs) were probably expressed independent of the presence of Cs (Hampton et al., 2004; 2005; Amtmann et al., 2006). Since the expression of genes encoding outward rectifying K^+ channels (KORCs) is low when plants are K-starved (Hampton et al., 2005; Liu et al., 2006) the transport of K from roots to shoots is limited leading to higher concentrations of K in roots. Caesium availability might have enhanced the K-deficiency of plants due to biochemical competition resulting in higher K concentrations in roots in the presence of Cs than in the absence of Cs. So far, no processes are known that link P and Cs transport. Therefore, the increase of P concentrations in roots in the presence of Cs cannot be explained. When Cs was present Mg concentrations decreased in roots but increased in shoots (Table 5.7) which means that the transfer of Mg from roots to shoots seemed to be increased due to the presence of Cs. This is in contrast to results obtained in Chapter 4 where increasing supply of Cs led to decreasing concentrations of Mg in shoots (Figure 4.10).

Colonisation of roots with *Glomus* sp. led to an increase in P concentrations of shoots (Table 5.7). Since transport of P is one of the major functions of the AM symbiosis even when P is not limiting for plants (Smith and Read, 2008) the increase in P concentrations in shoots is not surprising. Concentrations of Ca in shoots and of Mg in roots were lower in mycorrhizal than in non mycorrhizal plants (Table 5.7). Schultz et al. (2010) showed that Mg concentrations in roots of mycorrhizal *M. truncatula* cv. F83005 were lower than in non mycorrhizal roots but Mg concentrations in roots of mycorrhizal and non mycorrhizal *M. truncatula* cv. A17 did not differ. Lower concentrations of Ca in shoots and Mg in roots of *M. truncatula* (Table 5.7) are in contrast to results obtained in Chapter 2 where it was shown that mycorrhizal colonisation increased concentrations of Ca and Mg in plant tissues (Table 2.11 and 2.12). Although the influence of AM fungi on Ca and Mg is not clear, it has been reported previously that AM fungi can enhance Ca and Mg concentrations in plants mainly on acidic soils (Clark and Zeto, 2000). Berreck and Haselwandter (2001) showed increased concentrations of Ca in mycorrhizal roots in comparison to non mycorrhizal roots when K was supplied at 66.3 µg K g^{-1} substrate but Mg concentrations in roots were not affected by AM colonisation. It is possible that AM fungi and plants competed for Ca and Mg uptake and therefore colonisation with AM fungi reduced Ca and Mg concentrations in shoots and roots, respectively (Table 5.7). Concentrations of K in plant tissues were not influenced by AM fungi (Table 5.7).

Table 5.7: Effects of Cs supply and of mycorrhizal colonisation by *Glomus* sp. on concentrations of Ca, K, Mg and P in roots and shoots of *Medicago truncatula*.

Element	**Roots**		**Shoots**	
	Caesium	**Mycorrhiza**	**Caesium**	**Mycorrhiza**
Ca	no effect	no effect	no effect	decrease
K	increase	no effect	no effect	no effect
Mg	decrease	decrease	increase	no effect
P	increase	no effect	no effect	increase

Generally, Cs concentrations in roots were higher than in shoots (Figure 5.7). This is in agreement with Gyuricza et al. (2010b) who showed higher concentrations of ^{134}Cs in roots of *M. truncatula* than in shoots. However, all other publications investigating the influence of AM fungi on Cs accumulation by plants have shown

higher concentrations of Cs in shoots than in roots (Dighton and Terry, 1996; Entry et al., 1999; Berreck and Haselwandter, 2001; Joner et al., 2004).

Concentrations of Cs in tissues of *M. truncatula* plants differed greatly between experiments (Figure 5.7). Tissue Cs concentrations in Experiments 1 and 2 were around three times higher than in Experiments 3 and 4, although all plants had been supplied with 0.05 mM Cs. The accumulation of Cs differs between plant species and is dependent on K and Cs availability in the growth medium (Andersen, 1967; Buysse et al., 1996; Broadley et al., 1999a; Berreck and Haselwandter, 2001). Neither K or Cs concentrations in the growth medium, nor plant species differed between experiments. Hence, there is no obvious explanation for the differences in tissue Cs concentrations.

The effect of mycorrhizal colonisation on Cs concentrations in roots of *M. truncatula* depended on the experiment (Figure 5.7). In Experiment 1, mycorrhizal colonisation reduced Cs concentrations in roots. Berreck and Haselwandter (2001) showed a decrease in Cs concentrations in mycorrhizal roots of *Agrostis tenuis* (colonial bentgrass) after four and six weeks of growth when K was supplied at 66.3 µg K g^{-1} substrate but no influence of AM fungi on Cs concentrations in roots was detected after eight and ten weeks of growth. When *A. tenuis* was additionally supplied with 196 µg K g^{-1} substrate, AM fungi did not influence Cs concentrations in roots at any harvest time (Berreck and Haselwandter, 2001). In Experiment 2, mycorrhizal colonisation increased Cs concentrations in roots (Figures 5.7). Mycorrhizal colonisation also increased P concentrations in roots in the presence of Cs in Experiment 2 (Figure 5.8). It has been suggested that K^+ is a balancing cation for P anions in AM hyphae (Ryan et al., 2003, 2007) and that K might be transported by AM fungi in association with polyphosphates (Smith and Read, 2008). Gyuricza et al. (2010a) studied the influence of K and P availability on uptake and transport of ^{134}Cs by AM fungi. *Medicago truncatula* was grown in symbiosis with *Glomus intraradices* in a split *in vitro* system where the extraradical hyphae grew in a separate compartment than the mycorrhizal roots (Gyuricza et al., 2010a). The authors supplied ^{134}Cs and varying concentrations of K and P to the extraradical hyphae of *G. intraradices* but the plant roots did not have access to ^{134}Cs. Gyuricza et al. (2010a) demonstrated that increasing P availability led to increased uptake of ^{134}Cs by AM fungi and subsequent transport to *M. truncatula*. Gyuricza et al. (2010a) speculated that the increased P availability to AM fungi led to an increase in P

transport and accompanied an increase in K and Cs transport to plants. No effect of AM fungi on Cs concentrations in roots was detected in Experiments 3 and 4 (Figure 5.7) which is in agreement with other publications (Entry et al., 1999; Joner et al., 2004; Gyuricza et al., 2010b). An analysis of multiple experiments using REML methods showed that Cs concentrations in shoots of mycorrhizal plants were generally slightly lower than those of non mycorrhizal plants (Table 5.5). The biological relevance of the statistically significant decrease of Cs concentrations in shoots of mycorrhizal plants has to be treated with caution since the average concentrations of Cs in non mycorrhizal and mycorrhizal plants were very similar (Table 5.5). Gyuricza et al. (2010b) observed a decrease in shoot Cs concentrations in *M. truncatula* due to mycorrhizal colonisation independent of external K supply. Furthermore, a decrease in shoot Cs concentration was also observed in mycorrhizal *Agrostis tenuis* at 66.3 μg K g^{-1} substrate during the first eight weeks of growth (Berreck and Haselwandter, 2001). Conversely, other studies have reported that AM fungi do not influence shoot Cs concentrations in the grass species *Sorghum sudanense* and *Lolium perenne* or in *Medicago truncatula* (Rogers and Williams, 1986; Joner et al., 2004; Rosén et al., 2005) or even increase shoot Cs concentrations (Entry et al., 1999; Rosén et al., 2005; Dubchak et al., 2010). However, the differences in shoot Cs concentrations between non mycorrhizal and mycorrhizal *M. truncatula* plants were small (Figure 5.7). Furthermore, the addition of AM fungal inocula to soil might not lead to higher colonisation rates of host plants (Entry et al., 1999). Therefore, the use of arbuscular mycorrhiza to grow 'safer' crop might be ineffective. A similar conclusion had also been drawn by Entry et al. (1999) and Rosén et al. (2005) who concluded that the use of AM fungi in phytoremediation might be ineffective.

5.4.2.2 Arbuscular mycorrhizal fungi did not influence Cs concentrations in Medicago truncatula *plants supplied with different concentrations of Cs (Experiment C)*

To test the hypothesis that AM fungi reduce Cs concentrations in plants at low external Cs supply but that AM fungi do not affect Cs concentrations in plants at high external Cs supply *M. truncatula* was grown in association with *Glomus intraradices* in fertilised sand:clay mixtures containing different concentrations of stable and radioactive Cs.

The FWs of roots and shoots were not influenced by different supplies of Cs. Since the highest concentration of total Cs applied was 0.078 μg g^{-1} substrate and the plants were K-replete, Cs was not toxic to *M. truncatula* (see Chapter 4; White and Broadley, 2000; Hampton et al., 2004). The FWs of shoots were not influenced by mycorrhizal colonisation but non mycorrhizal roots were heavier than mycorrhizal roots (Figure 5.12). Some plants allocate a lower proportion of total plant weight to roots when colonised by AM fungi (Smith and Read, 2008). Non mycorrhizal *Agrostis tenuis* roots had higher DWs than mycorrhizal roots when grown at high K supply (Berreck and Haselwandter, 2001). Mycorrhizal colonisation increased the DWs of *M. truncatula* plants (Joner et al., 2004) or had no influence on root or shoot DWs of *M. truncatula* (Gyuricza et al., 2010b).

Concentrations of ^{134}Cs increased in roots (Figure 5.14) and shoots (Figure 5.15) with increasing Cs supply. This is in agreement with results obtained in Chapter 4. The colonisation with *Glomus intraradices* did not influence ^{134}Cs concentrations in roots at any external Cs supply (Figure 5.14). However, the effect of mycorrhizal colonisation on ^{134}Cs concentrations in shoots was dependent on external Cs supply (Figure 5.15). Mycorrhizal colonisation increased ^{134}Cs concentrations in shoots at an external Cs supply of 0.0002 μg g^{-1} but decreased ^{134}Cs concentrations in shoots at an external Cs supply of 0.0006 μg g^{-1}. These results do not lead to a definite conclusion about the influence of AM fungi on Cs accumulation in plants at low external Cs supply. At higher external Cs supply, AM fungi did not influence ^{134}Cs concentrations in shoots of *M. truncatula* (Figure 5.15). The higher Cs concentrations applied in the experiment of 0.003 μg g^{-1} to 0.078 μg g^{-1} were still lower than natural occurring Cs concentrations in soil of 0.3 to 25 μg g^{-1} (Davis, 1963; Coughtrey and Thorne, 1983; White and Broadley, 2000). It is therefore concluded that AM fungi probably do not influence Cs accumulation in plants under natural conditions in soil. It had been proposed that AM fungi should be considered in agricultural countermeasures and phytoremediation strategies for radiocaesium contaminated areas (Entry et al., 1996; Zhu and Shaw, 2000). Another strategy proposed for contaminated agricultural land is the cultivation of 'safer' crop plants that do not accumulate Cs (White et al., 2003; Hampton et al., 2005; Willey, 2005) and many agricultural plants are colonised by AM fungi (Smith and Read, 2008). However, results obtained in this thesis suggest that the influence of AM fungi in relation to phytoremediation or 'safer' crop plants is negligible.

5.4.3 High external Cs supply reduced mycorrhizal colonisation in *Medicago truncatula* (Experiment C)

The application of high concentrations of Cs led to a decrease in mycorrhizal colonisation of *M. truncatula* (Table 5.6). When external Cs supply was low more than 60% of the root system was colonised after ten weeks of growth. This is in agreement with colonisation rates in *M. truncatula* obtained by Joner et al. (2004) and Gyuricza et al. (2010b). However, at an external Cs supply of 50 µg per pot only 22% of the root system was colonised (Table 5.6). More importantly, the abundance of arbuscules in the whole root system decreased significantly with increasing Cs supply. In *M. truncatula*, arbuscules are initially formed in the cortical cell layers closest to the vascular tissue (Gianinazzi-Pearson et al., 2006). This also seemed to be the case for the few arbuscules that were formed in roots exposed to high concentrations of Cs (Figure 5.16). Furthermore, it seemed that the abundance of hyphae and vesicles was increased but the entry points of the fungus into root cells were decreased when Cs supply was high (Figure 5.16). In the root parts where arbuscule development occurred, several neighbouring cells also contained arbuscules (Figure 5.16). These observations might suggest that the initiation of the symbiosis was affected by Cs. Seven plant genes that are required for the development of AM and rhizobial symbioses have been identified in legumes (Bécard et al., 2004; Parniske, 2008; Smith and Read, 2008). Two of these genes, *CASTOR* and *POLLUX* characterised in *Lotus japonicus*, are involved in Nod-factor induced calcium spiking (Imaizumi-Anraku et al., 2005; Miwa et al., 2006; Charpentier et al., 2008). In *M. truncatula*, the POLLUX ortholog DMI1 (does not make infections) is also involved in calcium spiking (Ané et al., 2004; Parniske et al., 2008) and mutants lacking CASTOR, POLLUX or DMI1 do not develop AM and rhizobial symbioses (Ané et al., 2004; Imaizumi-Anraku et al., 2005). CASTOR and POLLUX are potassium-permeable cation channels (Charpentier et al., 2008). It has been proposed that these proteins act as counter-ion channels that compensate for the rapid charge imbalance produced during the calcium spiking response in root cells (Imaizumi-Anraku et al., 2005; Charpentier et al., 2008; Parniske, 2008). So far, no information exists about the influence of Cs on the development of the AM or rhizobial symbioses. It can only be speculated that Cs might interfere with the transport of K^+ by CASTOR and POLLUX leading to disruptions in the calcium spiking response. However, since many molecules involved in the development of

the AM symbiosis are still unknown many possibilities exist how Cs could interfere with AM development.

5.5 CONCLUSIONS

- *Medicago truncatula* plants were K-deficient when grown on media containing 1 mM K under *in vitro* conditions
- Roots of *M. truncatula* were colonised by *Glomus* sp. to 12% in the absence of Cs and to 10% in the presence of Cs after nine weeks of growth under *in vitro* conditions
- Plant fresh weights and element concentrations in plant tissues of *M. truncatula* varied significantly between identical experiments under *in vitro* conditions
- Concentrations of K in tissues of K-deficient *M. truncatula* plants were not influenced by AM fungi under *in vitro* conditions
- Arbuscular mycorrhizal fungi did not influence Cs concentrations in roots but decreased Cs concentrations in shoots of K-deficient *M. truncatula* plants under *in vitro* conditions
- Arbuscular mycorrhizal fungi did not influence Cs accumulation in K-replete *M. truncatula* plants supplied with different concentrations of Cs in sand:clay mixtures
- High concentrations of Cs led to a decrease in mycorrhizal colonisation of *M. truncatula* grown in a sand:clay mixture

Chapter 6

Gene expression in *Medicago truncatula* in response to caesium and arbuscular mycorrhiza

6.1 INTRODUCTION

6.1.1 Genome of *Medicago truncatula*

Medicago truncatula has been chosen as a model plant for legumes because of its small diploid genome and its susceptibility to transformation and regeneration (Barker et al., 1990; Rose, 2008). Furthermore, *M. truncatula* is efficiently nodulated by *Sinorhizobium meliloti*, whose genome has been sequenced (Galibert et al., 2001), and forms arbuscular mycorrhiza. This makes *M. truncatula* a good model to study these symbioses (Frugoli and Harris, 2001; Rose, 2008). The *M. truncatula* genome shows high levels of macro- and microsynteny to other legumes such as *Medicago sativa*, pea (*Pisum sativum*) and *Lotus japonicus* (Bell et al., 2001; Frugoli and Harris, 2001; Oldroyd and Geurts, 2001; Young et al., 2005; Cannon et al., 2006). The genomes of *M. truncatula* (http://www.medicago.org/genome/) and *L. japonicus* (http://www.kazusa.or.jp/lotus/) are being sequenced and it has been predicted that more than 75% of the *M. truncatula* and *L. japonicus* genespaces are conserved (Young et al., 2005). For sequencing the genome of *M. truncatula* a map-anchored bacterial artificial chromosome (BAC) sequencing strategy was selected because the genome is organised into distinct gene-rich euchromatin separate from repeat-rich pericentromeric regions (http://www.medicago.org/genome/; Cannon et al., 2005). The sequencing process started in 2002 and in March 2009 the *M. truncatula* genome assembly version 3.0 (Mt3.0) was released (http://www.medicago.org/genome/). So far, 3143 BACs have been sequenced (http://www.medicago.org/genome/; accessed 28 July 2010) and 268712 expressed sequence tags (ESTs) are available on the Gene Index Project database (http://compbio.dfci.harvard.edu/tgi/plant.html; accessed 28 July 2010). The annotation of the *M. truncatula* genes is coordinated by the International Medicago Genome Annotation Group (IMGAG; http://www.medicago.org/genome/IMGAG/). In 2005, Affymetrix GeneChip® Medicago genome arrays became available to monitor gene expression in *Medicago truncatula*, *Medicago sativa*, and their symbiont *Sinorhizobium meliloti*

(http://www.affymetrix.com/estore/). The array contains 32,167 *M. truncatula* EST based probe-sets (sequence information from The Institute for Genomic Research (TIGR)), 18,733 *M. truncatula* gene prediction based probe-sets (gene predictions from IMGAG), 1,896 *M. sativa* EST based probe-sets, and 8,305 *S. meliloti* gene prediction based probe-sets. To analyse the *Medicago* transcriptome the *Medicago truncatula* Gene Expression Atlas (MtGEA) web server was developed (Benedito et al., 2008; He et al., 2009). The current version of the web server (MtGEA v2) holds information from 156 Affymetrix GeneChips from 64 different experiments (http://mtgea.noble.org/v2/). The availability of genomic tools for the model legume *M. truncatula* offers the transfer of information to important crop species (Ané et al. 2008; Young and Udvardi, 2009).

6.1.2 Caesium induced changes in plant gene expression

Caesium is chemically similar to K and hence, Cs in the rhizosphere can change the K status of plants (White and Broadley, 2000; Hampton et al., 2004). Furthermore, high concentrations of Cs are toxic to plants (see Chapter 4; Hampton et al., 2004). These effects have implications for plant gene expression. Sahr et al. (2005a) investigated gene expression in *Arabidopsis thaliana* exposed to non toxic concentrations of ^{133}Cs. The gene expression in roots of five-week old plants that had been grown in the presence of 2 mM K and up to 150 µM Cs was investigated using suppression subtractive hybridization and reverse transcription–polymerase chain reaction (Sahr et al., 2005a). Genes involved in defence, stress response and detoxification were up regulated in roots in the presence of Cs as well as genes involved in transport and cellular metabolism. The authors concluded that Cs can be classified as an abiotic oxidative stress factor and that changes in gene expression could also be related to decreased internal K concentrations (Sahr et al., 2005a). A proteomic study of sucrose-fed photosynthetic *A. thaliana* suspension cells grown on medium without any K salts and submitted to a 1 mM Cs stress for 24 h also showed up regulation of antioxidant proteins and proteins involved in transport (Le Lay et al., 2006). Proteins involved in amino-acid, nitrogen, sulphur and glutathione metabolism, and glycolysis and gluconeogenesis were also up regulated but the strongest effect of Cs stress was the up regulation of proteases (Le Lay et al., 2006). To differentiate between changes in gene expression due to K starvation or Cs intoxication, Hampton et al. (2004) investigated the gene expression of 21 day old *A.*

thaliana grown on K-replete medium with 2 mM K, on K-deficient medium with 0.5 mM K and on medium containing 2 mM K and 2 mM Cs. Potassium starvation resulted in changes in gene expression of defence response genes and transcription factors in roots and shoots but also in up regulation of transport proteins, including the K^+/H^+ symporter *AtHAK5* (Hampton et al., 2004). Several genes that showed differential expression due to K starvation were also affected by Cs intoxication, showing that the transcriptional responses to K starvation and Cs intoxication are not independent of each other (Hampton et al., 2004). Nevertheless, the gene expression profile of Cs intoxicated plants showed distinct differences to the profile of K-starved plants. Several K transport proteins in roots and shoots were up regulated in Cs intoxicated plants (Hampton et al., 2004). In *Escherichia coli*, the expression of the *kdp* operon that encodes for a high affinity K^+ uptake system was induced due to exposure to Cs (Jung et al., 2001). The response in gene expression to radioactive ^{134}Cs differs from the response to stable ^{133}Cs (Sahr et al., 2005b). Low concentrations of ^{134}Cs (30 Bq cm^{-3}) in the presence of 0.7 mM K up regulated the expression of 36 genes mainly involved in defence, stress response and detoxification, and in cell growth, division and development in five-week old *A. thaliana* roots (Sahr et al., 2005b). Only five of the 36 genes were also up regulated in *A. thaliana* exposed to ^{133}Cs (Sahr et al., 2005a,b).

6.1.3 Arbuscular mycorrhiza induced changes in plant gene expression

Several plant genes are required for the development of the AM symbiosis and many of these genes are also needed for the development of the rhizobial symbiosis (Parniske, 2008). In legumes, seven genes involved in the development of both symbioses have been identified so far (Kistner et al., 2005; Parniske, 2008). The gene *DMI2* (does not make infections) from *Medicago truncatula* encodes a putative leucine-rich-repeat receptor kinase that seems to recognise extracellular signals from the symbiotic partners (Parniske, 2004, 2008). The *M. truncatula* gene *DMI3* encodes a putative calcium or calmodulin-dependent protein kinase (Parniske, 2004). The proteins DMI2 and DMI3 are essential for the induction of the pre-penetration apparatus (PPA). Recently, Siciliano et al. (2007) created a PPA-targeted suppressive-subtractive cDNA library using transformed root cultures of *M. truncatula*. Eleven genes were identified that showed increased transcription levels in inoculated roots at the time of appressorium development or 48 h later, including the

genes *Expansin-related protein 1 precursor* (*Exp-like*) and *Nodulin-like protein* (*Nod-like*) (Siciliano et al., 2007). It has been suggested that DMI3 up regulates the expression of plant genes such as *Exp-like* and *Nod-like* but suppresses the expression of basal defence related genes like *Avr9/Cf-9 rapidly elicited protein 264* (Siciliano et al., 2007). Other genes required for AM development in legumes are *IPD3*, coding an unknown protein whose mutants are impaired in the infection process, and two cation channels involved in Nod factor induced calcium spiking, *CASTOR* and *POLLUX* from *Lotus japonicus* (Kistner et al., 2005; Parniske, 2008). The ortholog of *CASTOR* is unknown in *M. truncatula* and the ortholog of *POLLUX* is called *DMI1*. In *L. japonicus*, two genes encoding putative nuclear pore components (*NUP85* and *NUP133*) have been identified and it has been speculated that they might be involved in transporting CASTOR and POLLUX to the inner nuclear envelope (Parniske, 2008). Zhang et al. (2010) identified two genes that are necessary for the development of the AM symbiosis but not for the rhizobial symbiosis in *M. truncatula*. The genes *stunted arbuscule* (*STR*) and *STR2* seem to encode half-size ATP-binding cassette (ABC) transporters of a subfamily (ABCG; Zhang et al., 2010). Both proteins are located in the periarbuscular membrane and since half-transporters are predicted to act as dimers, STR and STR2 might act as heterodimers. Potential orthologs of STR and STR2 have been found in many angiosperms but not in *Arabidopsis* which is a non mycorrhizal plant (Zhang et al., 2010).

Several changes in plant gene expression also occur during the establishment of AM symbioses and in mature mycorrhiza in comparison to non mycorrhizal plants. Many researchers have used the model plant *M. truncatula* in symbiosis with different Glomeromycota species to investigate changes in gene expression induced by mycorrhiza. Generally, genes involved in signal transduction, transport, cell structure, primary and secondary metabolism, protein binding, and plant defence show differences in expression in mycorrhizal plants (Frenzel et al., 2005; Hohnjec et al., 2005; Liu et al., 2007; Gomez et al., 2009). Furthermore, Liu et al. (2007) showed that genes implicated in hydrolytic activity, enzyme activity and structural molecular activity were over represented in mycorrhizal roots whereas genes implicated in transcriptional regulation were over represented in shoots of mycorrhizal *M. truncatula* plants.

Some of the mycorrhiza related genes have been characterised in more detail. For example, a blue copper binding protein (MtBcp1) expressed in cells containing arbuscules and in neighbouring cortical cells is probably involved in electron transfer (Hohnjec et al., 2005). A cellulase (MtCel1) probably responsible for cell wall modifications (Liu et al., 2003) and a class III chitinase (MtChit3-3; Elfstrand et al., 2005) are expressed in cells containing arbuscules. A glutathione-S-transferase (MtGst1) probably involved in stress responses is expressed in mycorrhizal root tissue (Wulf et al., 2003). Two lectins (MtLec5 and MtLec7) exclusively expressed in cells containing arbuscules have been proposed to bind to carbohydrates exposed to fungal cell wall surfaces or to act as storage of organic nitrogen (Frenzel et al., 2005). Vapyrin, a cytosolic protein in root cells of *M. truncatula* has been suggested to be involved in cellular remodeling processes necessary for colonisation by AM fungi (Pumplin et al., 2010) and phosphate uptake from the periarbuscular space is mediated by an arbuscule specific phosphate transporter (MtPt4; Harrison et al., 2002). A transcriptional analysis of *M. truncatula* transporters revealed that in addition to MtPT4, at least three peptide transporters, two putative ATP/ADP carriers, an ATPase (Mtha1; Krajinski et al., 2002), four ABC transporters, four plant defensins and three aquaporins were up regulated in four-week old roots colonised by *Glomus intraradices* (Benedito et al., 2010).

Mycorrhiza activated genes show either cell autonomous expression, because they are only expressed in cells containing arbuscules, or cell non autonomous expression, because they are expressed in cells of the colonised region of the root (Harrison, 2005). Two distinct temporal expression patterns were defined by Liu et al. (2003): (1) genes that show increased expression during the initial contact between the symbionts but decreasing expression during the establishment of the symbiosis, which seemed to be mainly involved in stress and defence response and (2) genes that show sustained increase in expression in mycorrhizal roots, many of which are involved in signal transduction.

Some of the mycorrhiza related genes are activated in response to different fungal species of the Glomeromycota whereas other genes are targeted by specific fungal species (Liu et al., 2007; Grunwald et al., 2009). Massoumou et al. (2007) compared the influence of seven different fungal species on the expression of 14 AM specific *M. truncatula* genes and showed that ten of the genes were activated by all fungal species and the remainder of the genes were activated by at least two fungi.

Feddermann et al. (2008) investigated the distinct expression patterns of ten *M. truncatula* genes in response to *G. mosseae*, *G. intraradices* and *Scutellospora castanea*. The *Glomus* species belong to the *Arum*-type of mycorrhiza whereas *S. castanea* belongs to the Gigasporaceae and forms *Paris*-type mycorrhiza. Four of the genes were activated by all three fungi but six genes only responded to *S. castanea* (Feddermann et al., 2008). The authors suggested that the differences in gene expression might be related to the distinctive colonisation pattern (Feddermann et al., 2008). Not all of the genes whose gene expression is influenced by AM fungi respond exclusively to mycorrhiza. Seventy five genes corresponding to a range of functional classes were differentially expressed in *M. truncatula* roots in symbiosis with *G. intraradices* and with *Sinorhizobium meliloti* (Manthey et al., 2004). The beneficial rhizobacterium *Pseudomonas fluorescens* also influenced genes in *M. truncatula* that responded to *G. mosseae* and to *S. meliloti* (Sanchez et al., 2004). Gene expression analysis of twelve mycorrhiza upregulated *M. truncatula* genes showed that root colonisation with *P. fluorescens* activated seven of the twelve plant genes and nodulation with *S. meliloti* activated three of the twelve plant genes (Sanchez et al., 2004). For example, the expression of a gene encoding a nodulin 26-like aquaporin was up regulated by all three microorganisms (Sanchez et al., 2004). Cell wall related genes and transcription factors that were up regulated in mycorrhizal roots were down regulated in *M. truncatula* leaves infected with a pathogen *Pseudomonas syringae* (Bozsó et al., 2009).

6.1.4 Aim

The aim of this chapter was to investigate changes in plant gene expression in relation to Cs supply and to arbuscular mycorrhiza. Non mycorrhizal and mycorrhizal plants were grown under *in vitro* conditions on a complete nutrient medium containing 1 mM K without or with the addition of 0.05 mM Cs and Affymetrix GeneChip® Medicago genome arrays were used to examine gene expression in *M. truncatula* roots and shoots under these conditions.

6.2 MATERIAL AND METHODS

6.2.1 Organisms and growth conditions

Medicago truncatula Gaertn. var. *truncatula* Jemalong A17 and the arbuscular mycorrhizal fungus *Glomus* sp. MUCL 43195 were used and the growth conditions are described in Section 5.2.2. The same plant material that was used in Section 5.2.2 to determine element concentrations in plant tissues was used in the Chapter presented here. Root and shoot material of non mycorrhizal and mycorrhizal plants that had been grown without or with Cs in the medium had been frozen in liquid N_2 and stored at -80°C. Root and shoot material from Experiments 1 and 2 were used for gene expression analysis. Plant materials from each treatment in Experiments 1 and 2, respectively, were combined for the extraction of total RNA to achieve high concentrations of RNA necessary for gene expression analysis. For each treatment (non mycorrhizal and mycorrhizal, absence and presence of Cs), one root and one shoot RNA sample were obtained for Experiment 1 and 2, respectively, which lead to a total of eight root and eight shoot samples.

6.2.2 Ribonucleic acid (RNA) extraction

Total RNA of plant roots was extracted using TRIzol Reagent (Invitrogen Ltd., Paisley, UK). The tissues were pulverised in liquid nitrogen and 1 ml of TRIzol Reagent was added to 100 mg of tissue. Following incubation of the homogenised sample for 5 min at RT, 0.2 ml of chloroform was added. After mixing thoroughly and incubating for 5 min at RT, the samples were centrifuged at 9000 g for 15 min at 4°C (1-15K, Sigma Aldrich, St. Louis, MO, USA). The aqueous phase was mixed with 0.2 ml of chloroform and again centrifuged at 9000 g for 15 min at 4°C. The RNA was precipitated from the aqueous phase by mixing with 0.25 ml isopropanol and 0.25 ml 0.8 M sodium citrate, 1.2 M NaCl. After mixing and incubating for 30 min at RT the samples were centrifuged at 13000 g for 20 min at 4°C. The supernatant was removed and the RNA pellet was washed with 1 ml of 75% ethanol. At the end, the RNA was air-dried and dissolved in RNase-free water by mixing and incubating at 55°C.

Following extraction the RNA from root samples was further purified using a phenol-chloroform step. The sample volume was adjusted to 100 µl with RNase-free water and 100 µl of a phenol/chloroform mixture (1:1, pH 4.3) was added. The

samples were incubated on ice for 5 min and subsequently centrifuged at 9000 g for 5 min at 4°C. The RNA was precipitated from the aqueous phase by mixing with 10 µl 3 M sodium acetate (pH 5.5) and 0.25 ml 100% ethanol. After mixing and incubating for 1 h at -20°C the samples were centrifuged at 18000 g for 20 min at 4°C. The supernatant was removed and the RNA pellet was washed with 1 ml of 80% ethanol. At the end, the RNA was air-dried and dissolved in RNase-free water by mixing and incubating at 55°C.

The RNA from shoot samples was extracted using the RNeasy Mini Kit (Qiagen Ltd., Crawley, UK) according to the manufacturer's instructions. The tissues were pulverised in liquid nitrogen and added to 450 µl of a buffer containing 4.5 µl β-mercaptoethanol. The samples were vortexed and incubated at 56°C for 1 min. The lysate was homogenised with a QIAshredder spin column. The supernatant of the flow through was mixed with 220 µl 100% ethanol, transferred to an RNeasy Mini spin column and centrifuged at 9000 g for 15 s. This was followed by an on-column DNase I digestion. After two wash steps the RNA was eluted with RNase-free water. Extraction of RNA from roots using the RNeasy Mini Kit failed and therefore two different methods were used for the extraction of total RNA from roots and shoots.

The quality and quantity of the RNA was tested using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

6.2.3 Affymetrix GeneChip® Medicago genome array hybridisation

Quality control of the RNA, cDNA synthesis and hybridisation of the Affymetrix GeneChip® Medicago genome array was carried out by NASC's International Affymetrix Service (The European Arabidopsis Stock Centre, University of Nottingham, Loughborough, UK).

The quality of the RNA was assessed using the Agilent 2100 bioanalyzer (Agilent Technologies UK Limited, Stockport, UK) which is an automated bio-analytical device using microfluidics technology that provides electrophoretic separations (Schroeder et al., 2006). Profiles generated on the Agilent 2100 bioanalyzer give information about RNA concentration and RNA integrity (RNA Integrity Number (RIN) – Standardization of RNA Quality Control, Agilent Technologies). In addition, quality and quantity of the RNA was tested using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Labelling and hybridisation steps were performed as recommended by Affymetrix, Inc. The labelling was performed with the GeneChip® IVT Labeling Kit (GeneChip® Expression 3'-Amplification Reagents for IVT Labeling, Affymetrix, Inc., Santa Clara, CA, USA) and the array hydridisation was performed as described in the Affymetrix Genechip® Expression Analysis Technical Manual (Affymetrix Genechip® Expression Analysis Technical Manual, Affymetrix, Inc., Santa Clara, CA, USA). Two microlitres of 50 µM T7-Oligo(dT) Primer was added to 1 µg of total RNA, along with 2 µl of Poly-A RNA controls, during reverse transcription, generating cDNA containing the T7 promoter sequence. After cleanup of the cDNA the GeneChip® IVT Labeling Kit was used to produce amplified and biotinylated targets to hybridise on the Affymetrix GeneChip® Medicago genome array. The biotinylated cRNA was mixed with 10x IVT Labeling Buffer, IVT Labeling NTP Mix and IVT Labeling Enzyme mix and incubated at 37°C for 16 h. The labeled cRNA was cleaned up using IVT cRNA Cleanup Spin Columns and subsequently fragmented to 35–200 nt fragments by metal-induced hydrolysis. Fifteen micrograms of fragmented cRNA were mixed with 5 µl of 3 nM Control Oligonucleotide B2, 15 µl of 20X Eukaryotic Hybridization Controls (*bioB*, *bioC*, *bioD*, *cre*), 3 µl of 10 mg ml^{-1} Herring Sperm DNA, 3 µl of 50 mg ml^{-1} BSA, 150 µl of 2X Hybridization Buffer, 30 µl of DMSO and made up to a final volume of 300 µl with H_2O. The hybridisation cocktail was added to the Affymetrix GeneChip® Medicago genome array and hybridised for 16 h in an Affymetrix GeneChip® Hybridization oven 640 (Affymetrix, Inc., Santa Clara, CA, USA) at 45°C. After hybridisation the array was washed and subsequently stained in an automated station with Streptavidin Phycoerythrin Stain Solution and Antibody Solution containing 0.5 mg ml^{-1} biotinylated antibody. Finally, the array was scanned using an Affymetrix GeneChip® Scanner 3000 (Affymetrix, Inc., Santa Clara, CA, USA) using standard settings.

6.2.4 Annotation of *Medicago* genes

The annotations of the genes on the Affymetrix GeneChip® Medicago genome array were obtained from the *Medicago truncatula* Gene Expression Atlas (MtGEA) (http://mtgea.noble.org/v2/). Furthermore, an updated annotation was added to the *Medicago* genome model in GeneSpring, based on *Arabidopsis thaliana* annotation. The annotation was based on basic local alignment search tool (BLAST)

alignment (Altschul et al., 1990). For the local translated BLAST alignment, a database was created of all peptide sequences from the *Arabidopsis* TAIR 6 genome release (www.arabidopsis.org), using the 'formatdb' command. Target sequences used to design the Affymetrix GeneChip® Medicago probe sets were downloaded from Affymetrix (www.affymetrix.com/estore/). A translated BLAST alignment (v2.2.16; Altschul et al., 1990) identified the most similar *Arabidopsis* coding sequence to individual *Medicago* target sequences, using the 'blastall' command. A threshold of e-value <0.0001 was defined for reliable similarities between *Medicago* and *Arabidopsis* coding sequences.

6.2.5 Analysis of *Medicago* transcriptome data

Non-scaled RNA CEL files containing the raw signal intensity values for each probe on the array were generated from the scanned image of the GeneChip® array. Each RNA CEL file contained signal intensity values for 11 perfect match (PM) probes and 11 mismatch (MM) probes within each probe-set for each of the 61,101 probe-sets on the array. RNA CEL files (roots and shoots) were imported using the RMA pre-processor in GeneSpring GX and globally normalised before further analysis. The analysis of the microarray data using GeneSpring GX (Version 7.3, Agilent Technologies UK Ltd., South Queensferry, UK) was performed in collaboration with Dr Pete Hedley, SCRI, UK. Data were viewed initially using the 'Condition Tree' clustering program in GeneSpring GX. Shoot and root data separated at the first division and were subsequently analysed separately. Per-gene normalisations were applied whereby the signal intensity of each probe-set was normalised to the median value of the probe-set across all samples. The raw signal intensity values were filtered to an intensity value of at least 50 in at least two of the eight samples (to match the number of biological replicates per sample). The normalised data were filtered on expression levels removing all genes with fold-changes between 0.7 and 1.3, compared to the median measurements. For statistical analysis the two replicates for each treatment were combined. Probe-sets with differential hybridisation intensities between treatment type (i.e. without or with Cs) and growth conditions (i.e. non mycorrhizal or mycorrhizal), were identified subsequently using a two-way ANOVA, with a model of treatment type (i.e. without or with Cs), growth conditions (i.e. non mycorrhizal or mycorrhizal) and an

interaction term. The cutoff for the two-way ANOVA was p-value <0.05 with no multiple testing correction.

6.2.6 AMIGO

The Gene Ontology (GO) website (http://www.geneontology.org; The Gene Ontology Consortium, 2000) provides ontologies to describe predicted gene characteristics in a species-independent manner. Information about biological processes, molecular function and cellular component for genes are provided. To investigate if any groups of genes were overrepresented in the lists of differentially expressed genes in response to Cs or mycorrhizal symbiosis in comparison to the whole genome, the GO Term Enrichment tool of AmiGO (version 1.7; http://amigo.geneontology.org/cgi-bin/amigo/term_enrichment; Carbon et al., 2009) was used. The *Arabidopsis* homologs of the *M. truncatula* genes that were influenced by Cs or AM (threshold of e-value <0.0001) were compared against the whole genome of *Arabidopsis* (database filter TAIR) with a maximum p-value of 0.01 and a minimum number of one gene product.

6.3 RESULTS

6.3.1 Quality control of extracted RNA

Non mycorrhizal and mycorrhizal *Medicago truncatula* plants were grown under K-deficient conditions without or with Cs in the medium. Total RNA was extracted from roots and shoots. The quality of the extracted RNA was assessed using the Agilent 2100 bioanalyzer. Clear peaks of 18S and 28S ribosomal RNA are visible in non degraded, high quality RNA. Figure 6.1 shows an electropherogram of RNA that was extracted from mycorrhizal *M. truncatula* roots that had been grown with Cs in the medium.

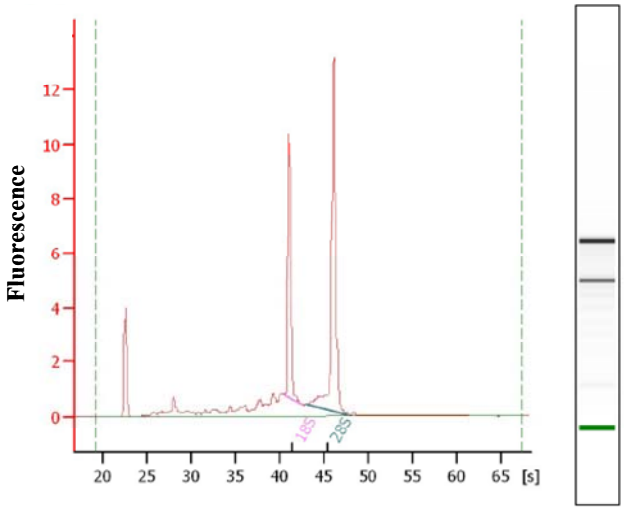

Figure 6.1: Electropherogram of total RNA extracted from mycorrhizal *Medicago truncatula* roots that had been grown with Cs in the medium. Clear peaks of ribosomal 18S and 28S RNA indicate intact RNA.

6.3.2 Quality control analysis of Affymetrix GeneChip® Medicago genome array hybridisation

The RNA was labeled and for each treatment two biological replicates (one replicate from Experiment 1 and the other replicate from Experiment 2) for roots and shoots were hybridised to Affymetrix GeneChip® Medicago genome arrays. The arrays contain 61,101 probe sets, 50,900 of these originate from *M. truncatula*. Observation of the Condition Tree in GeneSpring revealed that root and shoot data separated at the first division (Figure 6.2). Consequently, root and shoot samples were analysed separately.

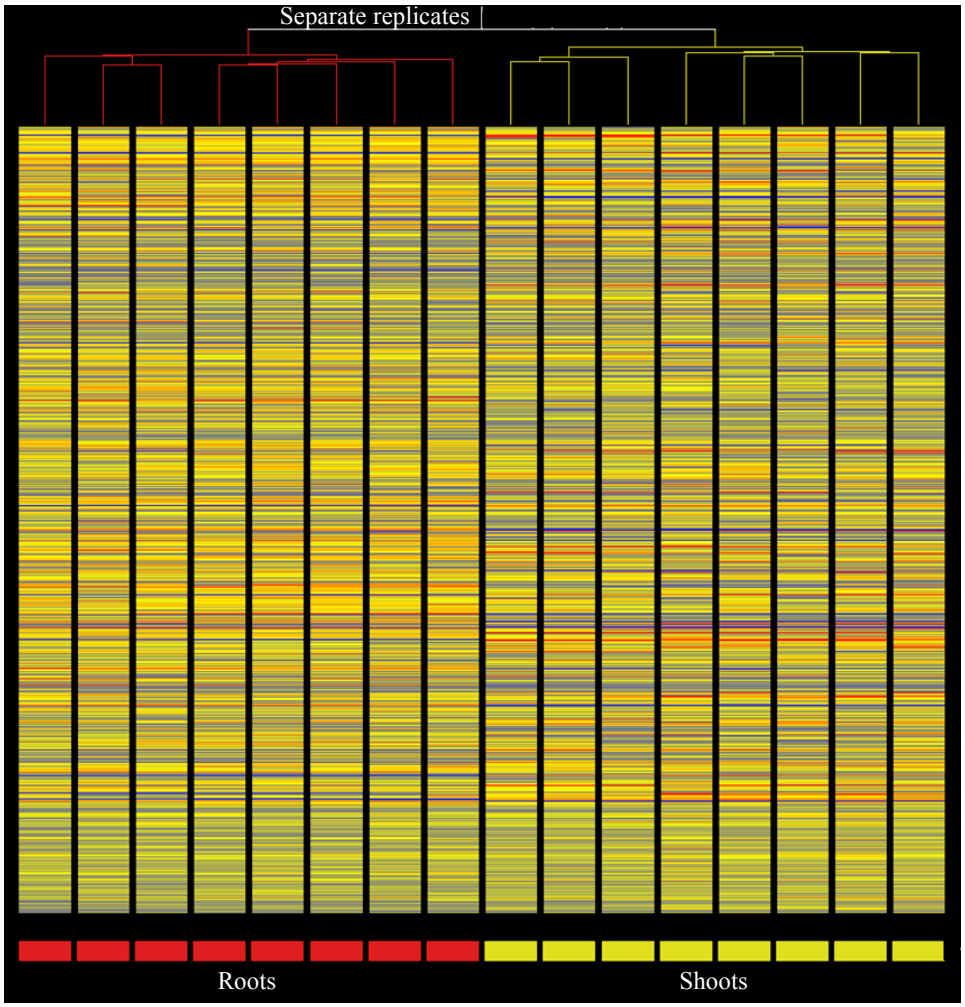

Figure 6.2: Separation of root (red) and shoot (yellow) data in the first division of a 'Condition Tree' clustering program in GeneSpring.

The raw data were filtered to an intensity value of at least 50 in at least two of the eight samples. In roots, 22,250 probe sets passed the filtering step and in shoots 22,324 probe sets were left after the filtering step. The overlap between the gene lists from the two tissues, as defined by a Venn diagram, was 19,730 probe sets. Subsequently, the filtered and normalised data were filtered on expression levels removing all genes with constitutive expression levels between 0.7 and 1.3. In roots, 12,106 probe sets showed differential expression levels in at least one of the samples and in shoots, 15,255 probe sets were left after the filtering step. These gene lists had an overlap of 7,755 probe sets. Subsequent discussion of data will refer to probe sets as 'genes'.

6.3.3 Differentially expressed genes in roots and shoots of *Medicago truncatula* due to Cs or arbuscular mycorrhiza

A two-way ANOVA, with a model of treatment type (i.e. without or with Cs), of growth conditions (i.e. non mycorrhizal or mycorrhizal) and an interaction term, without any multiple testing correction, was used to identify genes in roots and shoots of *M. truncatula* which were differentially expressed due to Cs or arbuscular mycorrhiza. In roots, the expression of 201 *M. truncatula* and 9 *M. sativa* genes was influenced by the presence of Cs in the medium (Table S1, Appendix). The expression of 275 *M. truncatula* and 19 *M. sativa* genes differed between non mycorrhizal and mycorrhizal roots (Table S2, Appendix). The expression of 85 *M. truncatula* and 7 *M. sativa* genes in roots was influenced by both the presence of Cs in the medium and the presence of the mycorrhizal symbiosis (Table S3, Appendix). AmiGO was used to find biological processes, molecular functions and cellular components of genes that were significantly overrepresented in the lists of *M. truncatula* genes that were differentially expressed in response to Cs or arbuscular mycorrhiza. For the analysis the *A. thaliana* orthologs of the *M. truncatula* genes were compared against the whole genome of *A. thaliana*. The whole genome of *A. thaliana* was used because the Affymetrix GeneChip® Medicago genome array contains probes for the major part of the *Medicago* genome. Based on the analysis using the *A. thaliana* genome, genes involved in secondary metabolic processes (11 genes, 7.6%) and cellular lipid metabolic processes (11 genes, 7.6%), especially isoprenoid metabolic processes (9 genes, 6.2%) including isoprenoid biosynthetic processes (7 genes, 4.8%) and terpenoid metabolic processes (7 genes, 4.8%; Table S7, Appendix), were overrepresented in *M. truncatula* roots that had been grown with Cs in the medium. Furthermore, genes involved in oxygen binding (related to cytochrome P450 activity; 10 genes, 6.9%), oxidoreductase activity (25 genes, 17.2%), glucuronosyltransferase activity (3 genes, 2.1%), electron carrier activity (11 genes, 7.6%) and iron ion binding (10 genes, 6.9%; Table S7, Appendix) were overrepresented. By contrast, no biological processes or molecular functions were overrepresented in mycorrhizal roots.

In shoots, the expression of 83 *M. truncatula* and 5 *M. sativa* genes was influenced by the presence of Cs in the medium (Table S4, Appendix). The expression of 371 *M. truncatula* and 25 *M. sativa* genes differed in shoots of non mycorrhizal and mycorrhizal plants (Table S5, Appendix). The expression of 189 *M.*

truncatula and 15 *M. sativa* genes in shoots was influenced by both the presence of Cs in the medium and the presence of the mycorrhizal symbiosis (Table S6, Appendix). According to AmiGO, in shoots of plants that had been grown with Cs in the medium, genes involved in catalytic activity (34 genes, 54%), especially oxidoreductase activity (16 genes, 25.4%; Table S8, Appendix) were overrepresented. Shoots of mycorrhizal plants showed overrepresentation of genes related to catalytic activity (108 genes, 40.3%), especially peroxiredoxin activity (3 genes, 1.1%) and of genes located in the chloroplast (74 genes, 27.6%; Table S9, Appendix).

6.3.4 Expression of genes in roots and shoots of *Medicago truncatula* related to Cs transport

Caesium is transported by cation channels and proton-coupled cation transporters (see Chapter 1; White and Broadley, 2000). To investigate if Cs in the medium affected the expression of genes encoding cation transport proteins in *M. truncatula*, a list of genes encoding cation transport proteins was created using The Arabidopsis Information Resource (TAIR; www.arabidopsis.org, accessed 23 June 2010). The list included all members of the *Arabidopsis* gene families of K^+ channels, K^+/H^+ symporters, cyclic-nucleotide gated channels, glutamate receptors and cation/H^+ antiporters (see Chapter 1; White and Karley, 2010). Using the updated annotation list for the *M. truncatula* genes on the Affymetrix GeneChip® Medicago genome array based on *Arabidopsis* annotations, all *M. truncatula* orthologs to the *Arabidopsis* cation transport proteins were listed. These genes were checked for their annotations in the *Medicago truncatula* Gene Expression Atlas (MtGEA, http://mtgea.noble.org/v2/). The expression levels of all genes whose MtGEA annotations were related to cation transport were identified (Figures 6.3, 6.4, 6.5, 6.6; Table 6.1, 6.2). It has to be noted that the membrane locations and the mechanisms of the cation transport proteins in *M. truncatula* are not known.

Four of the genes related to cation transport were significantly differentially expressed in response to the presence of Cs in the medium or mycorrhizal symbiosis. A putative Na^+ transporter orthologous to *AtHKT1* (probe set Mtr.34734.1.S1_at; Figure 6.3; Munns and Tester, 2008) was down regulated in roots of *M. truncatula* when Cs was present and also in mycorrhizal roots ($p < 0.05$). A putative glutamate receptor cation channel orthologous to *AtGLR2.1* (probe set Mtr.6324.1.S1_at;

Figure 6.4; Davenport, 2002) and a putative K^+ channel orthologous to *AtAKT1* (probe set Mtr.51989.1.S1_s_at; Figure 6.5; Lagarde et al., 1996) were down regulated in shoots of *M. truncatula* in the presence of Cs ($p < 0.05$). A putative Na^+/H^+ exchanger orthologous to *AtCHX19* (probe set Mtr.17230.1.S1_at; Figure 6.6; Sze et al., 2004) was down regulated in shoots in the presence of Cs and was also influenced by an interaction of Cs and mycorrhiza ($p < 0.05$).

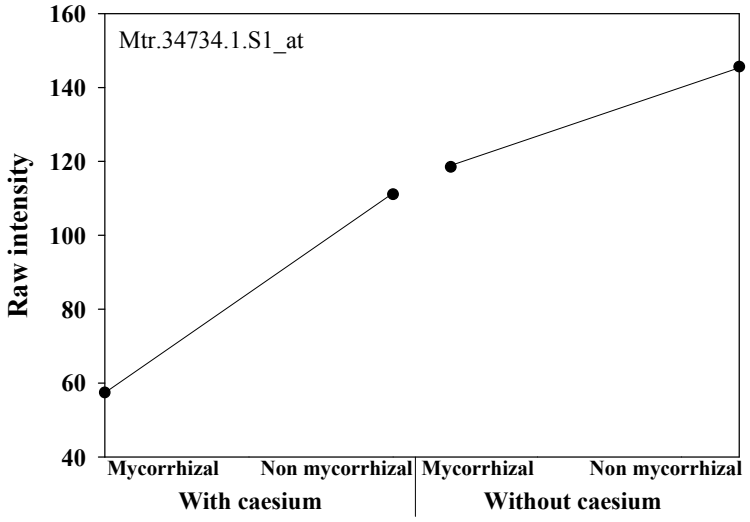

Figure 6.3: Raw intensity values of a putative Na^+ transporter orthologous to *AtHKT1* (probe set Mtr.34734.1.S1_at) in mycorrhizal and non mycorrhizal *Medicago truncatula* roots that had been grown with or without Cs in the medium. The expression of the gene was down regulated in mycorrhizal roots and in roots of plants that had been grown with Cs in the medium.

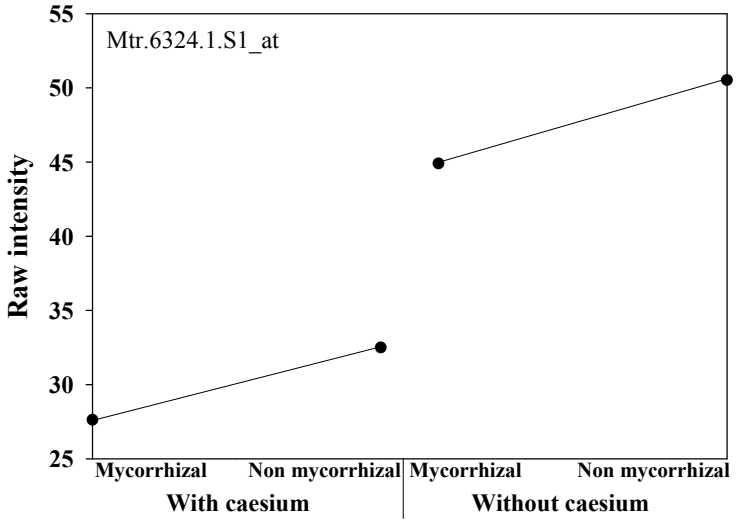

Figure 6.4: Raw intensity values of a putative glutamate receptor cation channel orthologous to *AtGLR2.1* (probe set Mtr.6324.1.S1_at) in mycorrhizal and non mycorrhizal *Medicago truncatula* shoots that had been grown with or without Cs in the medium. The expression of the gene was down regulated in shoots of plants that had been grown with Cs in the medium.

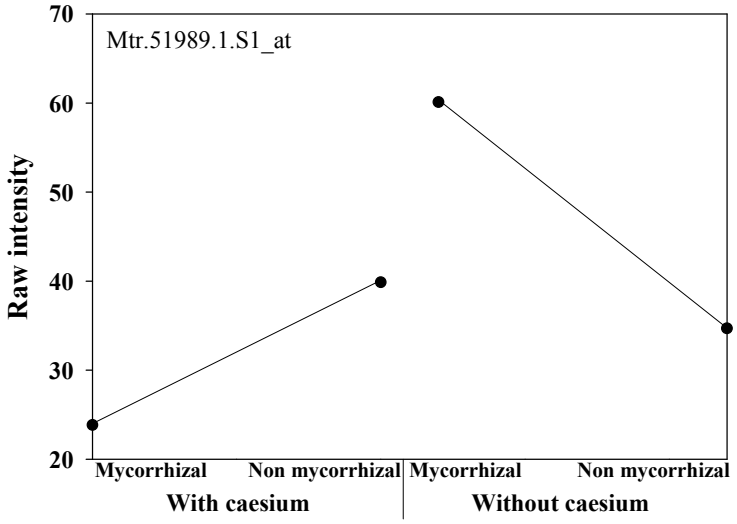

Figure 6.5: Raw intensity values of a putative K^+ channel orthologous to *AtAKT1* (probe set Mtr.51989.1.S1_at) in mycorrhizal and non mycorrhizal *Medicago truncatula* shoots that had been grown with or without Cs in the medium. The expression of the gene was down regulated in shoots of plants that had been grown with Cs in the medium.

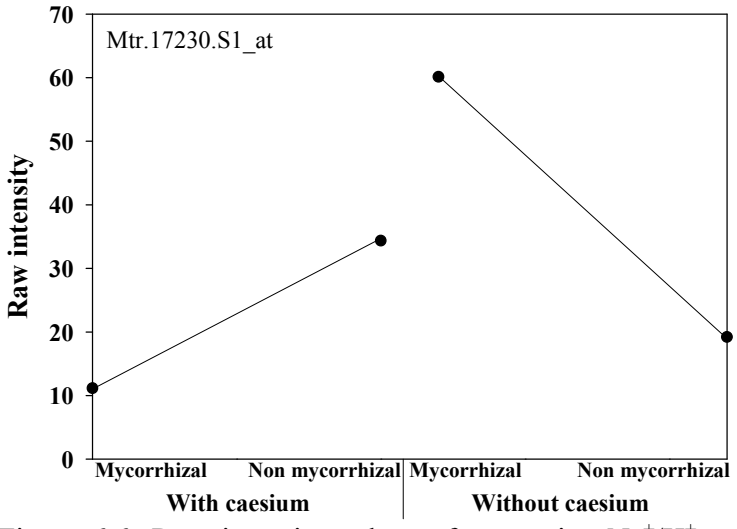

Figure 6.6: Raw intensity values of a putative Na^+/H^+ exchanger orthologous to *AtCHX19* (probe set Mtr.17230.1.S1_at) in mycorrhizal and non mycorrhizal *Medicago truncatula* shoots that had been grown with or without Cs in the medium. The expression of the gene was down regulated in shoots of plants that had been grown with Cs in the medium.

Generally, the intensity values for most probe sets for these selected genes were low and several target genes did not pass the quality control analysis (Section 6.3.2). Furthermore, only two replicates per treatment were processed meaning that an exhaustive statistical analysis was limited. Therefore, normalised raw expression values of all the selected target genes were compared between the different treatments. It is noted that without clear statistical evidence, the following observations are speculative. Some genes encoding putative Cs transport proteins appeared to be influenced by the presence of Cs in the medium or by the arbuscular mycorrhizal symbiosis (Table 6.1). In roots, none of the target genes appeared to be up regulated in the presence of Cs, but six genes showed lower raw expression values in roots that had been grown with Cs than in roots that had been grown without Cs. Four of these genes were putative K^+/H^+ symporters (KUPs; an ortholog to *AtKUP10*, probe set Mtr.41449.1.S1_ at; an ortholog to *AtKUP11*, probe set Mtr.3419.1.S1_at; two orthologs to *AtHAK5*, probe sets Mtr.1577.1.S1_s_at and Mtr.40509.1.S1_at). In mycorrhizal roots, three genes appeared to be up regulated, two of these genes were putative voltage insensitive cation channels (VICCs; an ortholog to cyclic-nucleotide gated channel 1 (*AtCNGC1*), probe set Mtr.13824.1.S1_at; an ortholog to *AtCNGC20*, probe set Mtr.7630.1.S1_at). Six genes seemed to be down regulated in mycorrhizal roots. Three of these genes were putative VICCs (an ortholog to *AtCNGC6*, probe set Mtr.17109.1.S1_s_at; an ortholog to *AtCNGC17*, probe set Mtr.39382.1.S1_at; an ortholog to glutamate receptor 3 (*AtGLR3.3*), probe set Mtr.25331.1.S1_at) and two of these genes were putative KUPs (an ortholog to *AtKUP3*, probe set Mtr.33886.1.S1_at; an ortholog to *AtKUP4*, probe set Mtr.39674.1.S1_at). In shoots, two genes appeared to be up regulated in the presence of Cs (an ortholog to *AtKUP10*, probe set Mtr.41449.1.S1_at; an ortholog to *AtGLR3.4*, probe set Mtr.4374.1.S1_at). Six genes appeared to be down regulated in the presence of Cs. Four of the genes that appeared to be down regulated were involved in calcium transport. Five genes appeared to be up regulated in shoots of mycorrhizal plants. Three of these genes were putative VICCs (an ortholog to *AtCNGC1*, probe set Mtr.33833.1.S1_at; an ortholog to *AtCNGC6*, probe set Mtr.17109.1.S1_s_at; an ortholog to *AtCNGC19*, probe set Mtr.31814.1.S1_at). Four genes appeared to be down regulated in shoots of mycorrhizal plants. Three of these genes were putative KUPs (an ortholog to *AtKUP2*, probe set Mtr.10999.1.S1_at; an ortholog to *AtKUP6*, probe set

Mtr.9837.1.S1_at; an ortholog to *AtKUP11*, probe set Mtr.3419.1.S1_at) and one was a putative VICC (an ortholog to *AtCNGC2*, probe set Mtr.38460.1.S1_at).

All other genes related to cation transport did not seem to be influenced by the presence of Cs in the medium or by the mycorrhizal symbiosis (Table 6.2).

Table 6.1: Raw microarray probe-set expression values of genes encoding cation transport proteins in roots and shoots of non mycorrhizal (non myc) and mycorrhizal (myc) *Medicago truncatula* plants that had been grown without or with caesium in the medium. The *Medicago* probes refer to the probe names for the genes on the Affymetrix GeneChip® Medicago genome array and the *Arabidopsis thaliana* orthologs are given. The genes seemed to be influenced in their expression by the presence of Cs in the medium or by the arbuscular mycorrhizal symbiosis.

			Raw expression values in roots			
			Without caesium		**With caesium**	
Influence	***Medicago* probes**	***Arabidopsis* orthologs**	**Non myc**	**Myc**	**Non myc**	**Myc**
Down regulated (Cs)	Mtr.4890.1.S1_at	*AtGLR3.7*	**180**	**170**	142	155
	Mtr.41449.1.S1_at	*AtKUP10*	**616**	**622**	470	444
	Mtr.3419.1.S1_at	*AtKUP11*	**434**	**465**	355	351
	Mtr.1577.1.S1_s_at	*AtHAK5*	**365**	**522**	353	369
	Mtr.40509.1.S1_at	*AtHAK5*	**4622**	**5667**	4210	4448
	Mtr.32759.1.S1_at	*AtNHX2*	**1017**	**1009**	907	955
Up regulated (Myc)	Mtr.13824.1.S1_at	*AtCNGC1*	170	**240**	177	**225**
	Mtr.7630.1.S1_at	*AtCNGC20*	281	**309**	290	**349**
	Mtr.20198.1.S1_at	*AtCHX20*	85	**105**	74	**102**
Down regulated (Myc)	Mtr.17109.1.S1_s_at	*AtCNGC6*	**1069**	962	**1029**	975
	Mtr.39382.1.S1_at	*AtCNGC17*	**573**	512	**563**	485
	Mtr.25331.1.S1_at	*AtGLR3.3*	**412**	328	**370**	330
	Mtr.33886.1.S1_at	*AtKUP3*	**190**	174	**185**	138
	Mtr.39674.1.S1_at	*AtKUP4*	**433**	371	**368**	335
	Mtr.38308.1.S1_at	*AtCAX5*	**1440**	1367	**1477**	1294

Table 6.1 (continued)

			Raw expression values in shoots			
			Without caesium		With caesium	
	Medicago probes	***Arabidopsis* orthologs**	**Non myc**	**Myc**	**Non myc**	**Myc**
Up regulated (Cs)	Mtr.4374.1.S1_at	*AtGLR3.4*	381	392	**491**	**494**
	Mtr.41449.1.S1_at	*AtKUP10*	482	491	**556**	**577**
Down regulated (Cs)	Mtr.7630.1.S1_at	*AtCNGC20*	**436**	**474**	358	377
	Mtr.27435.1.S1_at	*AtKEA2*	**922**	**831**	807	721
	Msa.1203.1.S1_at	*AtCAX1*	**766**	**625**	492	460
	Mtr.37432.1.S1_at	*AtCAX1*	**1835**	**1440**	1149	1113
	Mtr.34470.1.S1_s_at	*AtCAX3*	**2443**	**1855**	1695	1582
	Mtr.41148.1.S1_at	*AtCAX3*	**8492**	**8599**	6736	6712
Up regulated (Myc)	Mtr.33833.1.S1_at	*AtCNGC1*	302	**409**	313	**358**
	Mtr.17109.1.S1_s_at	*AtCNGC6*	604	**710**	582	**623**
	Mtr.31814.1.S1_at	*AtCNGC19*	1361	**1552**	1167	**1310**
	Mtr.45369.1.S1_at	*AtNHX1*	122	**167**	136	**167**
	Mtr.38308.1.S1_at	*AtCAX5*	718	**815**	748	**928**
Down regulated (Myc)	Mtr.38460.1.S1_at	*AtCNGC2*	**815**	610	**785**	689
	Mtr.10999.1.S1_at	*AtKUP2*	**906**	656	**734**	681
	Mtr.9837.1.S1_at	*AtKUP6*	**265**	234	**264**	177
	Mtr.3419.1.S1_at	*AtKUP11*	**556**	474	**514**	378

Table 6.2: Raw microarray probe-set expression values of genes encoding cation transport proteins in roots and shoots of non mycorrhizal (non myc) and mycorrhizal (myc) *Medicago truncatula* plants that had been grown without or with caesium in the medium. The *Medicago* probes refer to the probe name for the gene on the Affymetrix GeneChip® Medicago genome array and the *Arabidopsis thaliana* orthologs are given. Cyclic-nucleotide gated channels (CNGCs); glutamate receptors (glutamate recept.); Na^+/H^+ antiporters (Na^+/H^+ antip.)

			Raw expression values in roots				**Raw expression values in shoots**			
			Without caesium		**With caesium**		**Without caesium**		**With caesium**	
Gene families	***Medicago* probes**	***Arabidopsis* orthologs**	**Non myc**	**Myc**	**Non myc**	**Myc**	**Non myc**	**Myc**	**Non myc**	**Myc**
K^+ channels	Mtr.2.1.S1_at	*AtAKT1*	9	9	8	8	10	12	10	11
	Mtr.31572.1.S1_s_at	*AtAKT1*	8	9	8	9	9	9	8	8
	Mtr.2571.1.S1_at	*AtAKT2*	11	11	13	18	120	166	179	145
	Mtr.49962.1.S1_at	*AtAKT6*	11	11	10	12	13	15	28	17
	Mtr.21846.1.S1_at	*AtKCO1*	180	199	154	172	102	119	93	107
	Mtr.13869.1.S1_at	*AtKCO5*	237	177	136	187	191	139	141	136
	Mtr.27566.1.S1_at	*AtSKOR*	24	30	33	28	18	27	18	17
K^+/H^+ symporters	Mtr.33087.1.S1_at	*AtKUP2*	63	56	42	64	25	24	28	23
	Mtr.42438.1.S1_at	*AtKUP3*	58	48	58	35	29	26	31	26
	Mtr.45408.1.S1_at	*AtKUP4*	8	8	8	9	9	9	8	8
	Mtr.11919.1.S1_at	*AtKUP6*	10	11	10	10	8	7	7	7
	Mtr.1970.1.S1_at	*AtKUP6*	12	12	10	11	16	14	14	12
	Mtr.32208.1.S1_at	*AtKUP6*	77	104	88	68	19	20	19	15
	Mtr.34174.1.S1_at	*AtKUP6*	246	371	246	196	81	70	75	53
	Mtr.18323.1.S1_at	*AtKUP7*	120	116	109	103	94	107	98	109
	Mtr.9321.1.S1_at	*AtKUP10*	42	37	29	31	20	22	24	23

Table 6.2 (continued)

Gene families	*Medicago* probes	*Arabidopsis* orthologs	Raw expression values in roots				Raw expression values in shoots			
			Without caesium		With caesium		Without caesium		With caesium	
			Non myc	Myc	Non myc	Myc	Non myc	Myc	Non myc	Myc
K^+/H^+ symporters	Mtr.17301.1.S1_s_at	*AtHAK5*	6	6	6	7	6	7	8	6
	Mtr.24768.1.S1_s_at	*AtHAK5*	7	6	6	6	8	7	7	8
	Mtr.46517.1.S1_at	*AtHAK5*	6	6	6	5	6	7	6	6
	Mtr.46518.1.S1_s_at	*AtHAK5*	6	7	6	5	9	9	7	7
CNGCs	Mtr.41996.1.S1_at	*AtCNGC1*	26	29	26	42	13	22	14	14
	Mtr.6340.1.S1_at	*AtCNGC2*	10	9	9	9	16	16	13	14
	Mtr.15718.1.S1_at	*AtCNGC4*	16	16	18	13	27	19	58	28
	Mtr.5994.1.S1_at	*AtCNGC4*	7	7	6	7	246	330	253	213
	Mtr.7912.1.S1_at	*AtCNGC4*	6	6	7	7	79	113	82	70
	Mtr.38571.1.S1_s_at	*AtCNGC10*	65	59	63	72	34	38	30	36
	Mtr.32037.1.S1_at	*AtCNGC13*	82	72	77	215	39	47	25	63
	Mtr.32850.1.S1_at	*AtCNGC14*	11	10	11	13	10	11	10	8
	Mtr.32319.1.S1_at	*AtCNGC15*	10	8	9	8	9	9	7	9
	Mtr.50460.1.S1_at	*AtCNGC15*	6	6	6	6	86	114	105	73
	Mtr.44061.1.S1_at	*AtCNGC19*	38	33	28	33	44	94	55	66
	Mtr.5453.1.S1_at	*AtCNGC19*	36	42	37	40	24	30	31	30
	Mtr.13294.1.S1_at	*AtCNGC20*	138	104	119	103	54	68	67	65
	Mtr.27045.1.S1_at	*AtCNGC20*	64	73	74	79	40	53	49	48
	Mtr.33468.1.S1_at	*AtCNGC20*	7	6	7	7	7	7	6	7
	Mtr.8209.1.S1_at	*AtCNGC20*	5	5	5	5	6	6	5	5

Table 6.2 (continued)

Gene families	*Medicago* probes	*Arabidopsis* orthologs	Raw expression values in roots				Raw expression values in shoots			
			Without caesium		With caesium		Without caesium		With caesium	
			Non myc	Myc	Non myc	Myc	Non myc	Myc	Non myc	Myc
Glutamate recept.	Mtr.28008.1.S1_at	*AtGLR2.4*	5	6	5	5	6	6	5	6
	Mtr.33665.1.S1_at	*AtGLR2.7*	5	6	5	5	6	7	7	6
	Mtr.5819.1.S1_at	*AtGLR2.8*	8	8	8	7	24	33	26	29
	Mtr.10181.1.S1_at	*AtGLR3.3*	60	62	53	66	27	42	37	41
	Mtr.33327.1.S1_at	*AtGLR3.3*	5	6	6	5	7	6	6	6
	Mtr.35041.1.S1_at	*AtGLR3.3*	5	5	5	5	5	5	5	5
	Mtr.6703.1.S1_at	*AtGLR3.3*	6	7	7	6	18	25	43	17
	Mtr.33099.1.S1_at	*AtGLR3.4*	5	5	6	6	6	6	6	6
	Mtr.10073.1.S1_at	*AtGLR3.6*	7	8	8	7	7	7	7	7
	Mtr.6869.1.S1_s_at	*AtGLR3.6*	9	6	7	7	25	15	14	18
	Mtr.28410.1.S1_at	*AtGLR3.7*	18	18	16	18	15	17	16	16
	Mtr.30507.1.S1_at	*AtGLR3.7*	535	414	443	459	329	318	326	347
K^+/H^+ antiporters	Mtr.1727.1.S1_at	*AtKEA1*	10	10	9	10	15	30	23	14
	Mtr.52047.1.S1_at	*AtKEA1*	39	41	27	34	39	55	46	39
	Mtr.14720.1.S1_at	*AtKEA2*	49	60	47	47	58	82	78	64
	Mtr.33232.1.S1_s_at	*AtKEA2*	756	718	817	625	735	800	847	604
	Mtr.13261.1.S1_s_at	*AtKEA3*	9	9	9	11	18	14	16	16
	Mtr.14755.1.S1_at	*AtKEA3*	34	36	35	41	138	113	234	141
	Mtr.11482.1.S1_at	*AtNHD1*	270	254	265	234	561	604	621	482

Table 6.2 (continued)

Gene families	*Medicago* probes	*Arabidopsis* orthologs	Raw expression values in roots				Raw expression values in shoots			
			Without caesium		With caesium		Without caesium		With caesium	
			Non myc	Myc	Non myc	Myc	Non myc	Myc	Non myc	Myc
Na^+/H^+ antip.	Mtr.23441.1.S1_s_at	*AtCHX3*	6	5	5	5	5	5	5	5
	Mtr.25109.1.S1_at	*AtCHX3*	5	4	5	4	5	4	5	5
	Mtr.23442.1.S1_s_at	*AtCHX9*	6	6	7	6	6	6	6	6
	Mtr.1116.1.S1_at	*AtCHX10*	4	4	4	5	4	5	4	4
	Mtr.25106.1.S1_x_at	*AtCHX12*	5	5	5	5	5	5	6	5
	Mtr.26503.1.S1_s_at	*AtCHX12*	4	5	5	5	5	5	5	5
	Mtr.21260.1.S1_at	*AtCHX13*	6	7	7	6	7	9	7	7
	Mtr.21271.1.S1_at	*AtCHX13*	37	31	42	56	10	11	10	12
	Mtr.48853.1.S1_at	*AtCHX13*	5	5	6	5	5	5	6	6
	Mtr.25228.1.S1_at	*AtCHX14*	5	5	5	4	5	5	5	4
	Mtr.25230.1.S1_at	*AtCHX14*	5	5	4	5	5	5	5	5
	Mtr.25232.1.S1_at	*AtCHX14*	5	5	5	4	5	4	5	5
	Mtr.20563.1.S1_at	*AtCHX15*	5	4	4	5	5	5	4	5
	Mtr.20563.1.S1_x_at	*AtCHX15*	6	5	5	5	5	5	5	5
	Mtr.45782.1.S1_at	*AtCHX15*	7	6	7	7	7	8	7	8
	Mtr.50019.1.S1_at	*AtCHX15*	94	86	86	77	47	61	56	44
	Mtr.17229.1.S1_at	*AtCHX18*	344	289	291	298	153	254	179	124
	Mtr.17232.1.S1_at	*AtCHX18*	5	5	5	4	5	5	5	5
	Mtr.1183.1.S1_at	*AtCHX20*	8	8	9	8	12	9	9	10
	Mtr.1185.1.S1_at	*AtCHX20*	7	6	6	5	5	5	6	5
	Mtr.20201.1.S1_at	*AtCHX20*	366	329	387	364	116	166	154	148
	Mtr.41896.1.S1_s_at	*AtCHX20*	12	14	11	12	9	10	12	9

Table 6.2 (continued)

Gene families	*Medicago* probes	*Arabidopsis* orthologs	Raw expression values in roots				Raw expression values in shoots			
			Without caesium		With caesium		Without caesium		With caesium	
			Non myc	Myc	Non myc	Myc	Non myc	Myc	Non myc	Myc
Na^+/H^+ antip.	Mtr.14484.1.S1_at	*AtCHX21*	7	5	6	6	7	7	7	8
	Mtr.22566.1.S1_at	*AtCHX23*	9	8	8	8	10	10	12	11
	Mtr.13232.1.S1_at	*ATHKT1*	14	13	12	9	20	26	39	19
	Mtr.32796.1.S1_at	*ATHKT1*	25	23	22	12	52	57	95	37
	Mtr.39481.1.S1_at	*ATHKT1*	11	11	9	7	8	11	11	8
	Msa.1680.1.S1_at	*AtNHX1*	1680	1706	1725	1564	987	1016	1002	1089
	Mtr.44334.1.S1_at	*AtNHX1*	2575	2538	2577	2426	1347	1479	1417	1521
	Mtr.21664.1.S1_at	*AtNHX2*	51	38	41	42	14	28	24	28
	Mtr.51100.1.S1_at	*AtNHX2*	6	6	6	8	6	7	7	6
	Mtr.28828.1.S1_at	*AtNHX3*	6	6	5	5	6	5	5	5
	Mtr.7095.1.S1_at	*AtNHX4*	9	8	8	9	12	9	11	10
	Mtr.13430.1.S1_at	*AtNHX5*	643	677	735	681	579	711	708	534
	Mtr.35033.1.S1_at	*AtNHX5*	12	12	12	13	10	12	12	13
	Mtr.8400.1.S1_at	*AtNHX6*	36	43	39	38	24	37	28	29
	Mtr.10951.1.S1_at	*AtNHX7*	821	845	850	773	376	411	410	497
	Mtr.2857.1.S1_s_at	*AtNHX7*	207	230	212	195	101	132	126	150
	Mtr.5284.1.S1_at	*AtNHX7*	65	76	72	68	31	42	45	41

Table 6.2 (continued)

Gene families	*Medicago* probes	*Arabidopsis* orthologs	Raw expression values in roots				Raw expression values in shoots			
			Without caesium		With caesium		Without caesium		With caesium	
			Non myc	Myc	Non myc	Myc	Non myc	Myc	Non myc	Myc
cation exchanger	Mtr.33700.1.S1_at	*AtCAX3*	11	7	10	7	222	119	199	207
	Mtr.34470.1.S1_at	*AtCAX3*	6	6	5	5	7	9	6	7
	Mtr.45411.1.S1_at	*AtCAX3*	27	21	29	21	32	27	27	33
	Mtr.5594.1.S1_at	*AtCAX3*	8	8	8	7	1140	743	1000	1021
	Mtr.10011.1.S1_at	*AtCAX7*	7	9	7	7	114	67	83	93
	Mtr.45494.1.S1_s_at	*AtCAX8*	22	25	29	23	17	31	23	23
	Mtr.47770.1.S1_at	*AtCAX8*	247	252	262	226	417	426	359	411
	Mtr.6694.1.S1_at	*AtCAX8*	8	12	11	10	546	283	403	321
	Mtr.38860.1.S1_at	*AtCAX9*	15	14	20	13	6	8	7	7
	Mtr.48663.1.S1_s_at	*AtCAX11*	19	14	16	18	17	21	21	21

6.3.5 Expression of genes in roots of *Medicago truncatula* related to arbuscular mycorrhiza

Some plant genes are exclusively expressed in arbuscular mycorrhizal roots (Wulf et al., 2003; Hohnjec et al., 2006; Krajinski and Frenzel, 2007; Liu et al., 2007; Massoumou et al., 2007; Benedito et al., 2010). Therefore, a list of mycorrhizal *M. truncatula* genes was created. All genes on the list show mycorrhizal specific or at least a much stronger expression in mycorrhizal than in non mycorrhizal roots in accordance to the *Medicago truncatula* Gene Expression Atlas (MtGEA, http://mtgea.noble.org/v2/). Out of the 38 genes of interest, only two genes showed statistically significant mycorrhiza induced expression ($p < 0.05$) in the experiments described here. These were a protease inhibitor (probe set Mtr.35511.1.S1_at; Figure 6.7; Hohnjec et al., 2005) and a serine carboxypeptidase *MtScp1* (probe set Mtr.40285.1.S1_at; Figure 6.8; Liu et al., 2003).

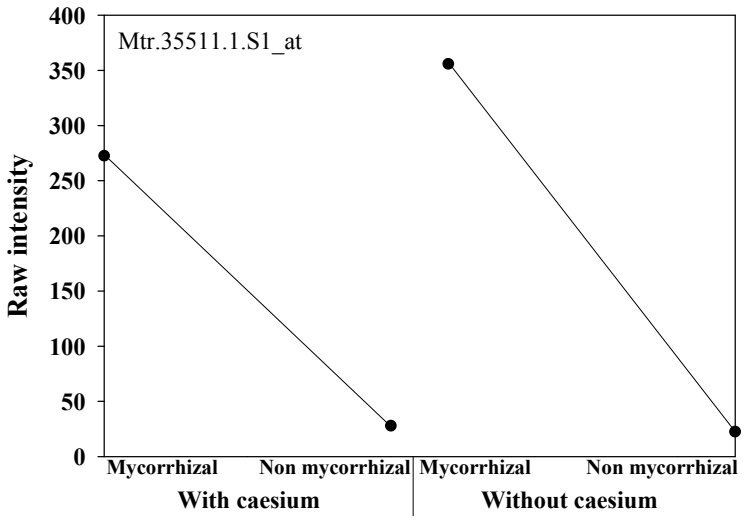

Figure 6.7: Raw intensity values of a protease inhibitor (probe set Mtr.35511.1.S1_at) in mycorrhizal and non mycorrhizal *Medicago truncatula* roots that had been grown with or without Cs in the medium. The expression of the gene was significantly up regulated in mycorrhizal roots.

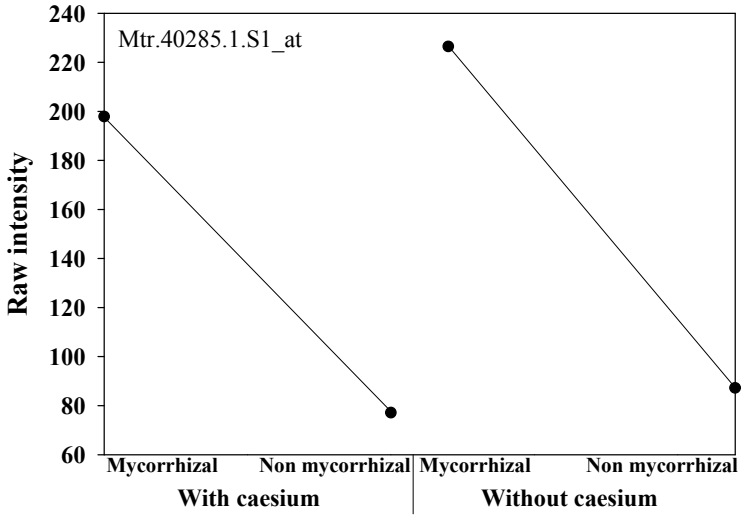

Figure 6.8: Raw intensity values of a serine carboxypeptidase *MtScp1* (probe set Mtr.40285.1.S1_at) in mycorrhizal and non mycorrhizal *Medicago truncatula* roots that had been grown with or without Cs in the medium. The expression of the gene was significantly up regulated in mycorrhizal roots.

However, as explained above the intensity values were generally low and the low replication limited exhaustive statistical analysis. It appeared that an additional seven genes showed higher expression in mycorrhizal roots of *M. truncatula* than in non mycorrhizal ones (Table 6.3). These genes include some of the arbuscule-related AM marker genes in the model legume *M. truncatula* as described by Hohnjec et al. (2006). A germin-like protein *MtGlp1* (probe set Mtr.12500.1.S1_at), a blue copper binding protein *MtBcp1* (probe set Mtr.15627.1.S1_s_at), a glutathione-S-transferase *MtGst1* (probe set Mtr.15957.1.S1_at), a β-tubulin *MtTubb1* (probe set Mtr.17272.1.S1_at) and a phosphate transporter *MtPt4* (probe set Mtr.43062.1.S1_at) appeared to have greater expression in mycorrhizal roots (Table 6.3). The other mycorrhizal *M. truncatula* genes did not seem to be induced in mycorrhizal roots studied in this thesis (Table 6.4).

Table 6.3: Raw microarray probe-set expression values of genes encoding mycorrhizal *Medicago truncatula* genes in roots of non mycorrhizal (non myc) and mycorrhizal (myc) *Medicago truncatula* plants that had been grown without or with caesium in the medium. The *Medicago* probes refer to the probe name for the gene on the Affymetrix GeneChip® Medicago genome array and the gene annotation is based on the *Medicago truncatula* Gene Expression Atlas (MtGEA). The genes seemed to be up regulated in mycorrhizal roots in comparison to non mycorrhizal ones.

		Raw expression values in roots				
		Without caesium		**With caesium**		
***Medicago* probes**	**Annotation MtGEA**	**Non myc**	**Myc**	**Non myc**	**Myc**	**Reference**
Mtr.10562.1.S1_at	Specific tissue protein 2	6	**51**	6	**112**	Liu et al. (2007)
Mtr.12500.1.S1_at	*MtGlp1* (Germin-like protein)	34	**89**	30	**116**	Hohnjec et al. (2006)
Mtr.15627.1.S1_s_at	*MtBcp1* (Blue copper binding protein)	7	**39**	7	**64**	Hohnjec et al. (2006)
Mtr.15957.1.S1_at	*MtGst1* (Glutathione-S-transferase)	18	**34**	14	**68**	Hohnjec et al. (2006)
Mtr.17272.1.S1_at	*MtTubb1* (β-tubulin)	47	**104**	46	**42**	Hohnjec et al. (2006)
Mtr.43062.1.S1_at	*MtPt4* (phosphate transporter)	9	**27**	8	**84**	Hohnjec et al. (2006)
Mtr.46524.1.S1_at	ATP binding cassette transporter	49	**117**	40	**60**	Benedito et al. (2010)

Table 6.4: Raw microarray probe-set expression values of genes encoding mycorrhizal *Medicago truncatula* genes in roots of non mycorrhizal (non myc) and mycorrhizal (myc) *Medicago truncatula* plants that had been grown without or with caesium in the medium. The *Medicago* probes refer to the probe name for the gene on the Affymetrix GeneChip® Medicago genome array and the gene annotation is based on the *Medicago truncatula* Gene Expression Atlas (MtGEA).

		Raw expression values in roots				
		Without caesium		**With caesium**		
***Medicago* probes**	**Annotation MtGEA**	**Non myc**	**Myc**	**Non myc**	**Myc**	**Reference**
Mtr.10089.1.S1_at	Putative cytochrome P450	5	6	5	6	Liu et al. (2007)
Mtr.1103.1.S1_at	ATP binding cassette transporter	9	12	9	11	Benedito et al. (2010)
Mtr.15653.1.S1_at	*MtLec5* (Lectin)	12	24	13	34	Hohnjec et al. (2006)
Mtr.16454.1.S1_at	*MtTi1* (Trypsin inhibitor)	8	11	8	25	Hohnjec et al. (2006)
Mtr.24340.1.S1_at	*MtCel1* (cellulase)	6	7	5	7	Hohnjec et al. (2006)
Mtr.31214.1.S1_s_at	Plant defensin	5	8	5	6	Benedito et al. (2010)
Mtr.31910.1.S1_at	ER retrotranslocon	5	5	4	5	Benedito et al. (2010)
Mtr.3284.1.S1_at	YABBY protein transcription factor	5	9	5	9	Hohnjec et al. (2005)
Mtr.35424.1.S1_at	Kunitz-type trypsin inhibitor 3	7	7	7	16	Massoumou et al. (2007)
Mtr.35484.1.S1_at	Plant defensin	9	7	9	11	Benedito et al. (2010)
Mtr.35854.1.S1_at	Cystine rich anti-fungal protein 2	9	7	8	10	Liu et al. (2007)
Mtr.36985.1.S1_at	H^+-dependent oligopeptide transporter	5	5	5	7	Benedito et al. (2010)
Mtr.36985.1.S1_s_at	H^+-dependent oligopeptide transporter	6	6	6	8	Benedito et al. (2010)
Mtr.37021.1.S1_at	Blue copper binding protein like	6	17	7	34	Liu et al. (2007)
Mtr.37110.1.S1_at	Copper transporter	10	12	8	13	Benedito et al. (2010)

Table 6.4 (continued)

Medicago probes	Annotation MtGEA	Raw expression values in roots				Reference
		Without caesium		With caesium		
		Non myc	Myc	Non myc	Myc	
Mtr.38791.1.S1_at	Ser/Thr protein kinase	589	690	446	453	Hohnjec et al. (2005)
Mtr.38814.1.S1_at	Cytochrome P450	7	6	7	8	Hohnjec et al. (2005)
Mtr.39705.1.S1_at	Mitochondrial carrier	8	8	6	12	Benedito et al. (2010)
Mtr.40286.1.S1_at	Ser carboxypeptidase	8	22	7	28	Hohnjec et al. (2005)
Mtr.42086.1.S1_at	Lipase precursor	8	9	8	6	Massoumou et al. (2007)
Mtr.43470.1.S1_at	*MtHa1* (plasma-membrane H^+-ATPase)	14	14	14	23	Hohnjec et al. (2006)
Mtr.44070.1.S1_at	ATP binding cassette transporter	24	44	10	11	Benedito et al. (2010)
Mtr.45648.1.S1_at	*MtLec7* (lectin)	6	14	9	11	Hohnjec et al. (2006)
Mtr.4771.1.S1_at	Iron/lead transporter superfamily	6	8	7	10	Benedito et al. (2010)
Mtr.50194.1.S1_at	H^+-dependent oligopeptide transporter	7	6	6	6	Benedito et al. (2010)
Mtr.51195.1.S1_at	ATP binding cassette transporter	9	10	14	15	Benedito et al. (2010)
Mtr.7210.1.S1_at	Cysteine rich anti-fungal protein	6	12	7	20	Massoumou et al. (2007)
Mtr.7596.1.S1_at	Major intrinsic protein	7	13	7	13	Benedito et al. (2010)
Mtr.9007.1.S1_at	Isoflavonoid glucosyltransferase	6	9	7	17	Liu et al. (2007)

6.4 DISCUSSION

6.4.1 Gene expression in *Medicago truncatula* is influenced by the presence of caesium and by arbuscular mycorrhizal fungi

Gene expression in roots and shoots of *M. truncatula* was investigated using Affymetrix GeneChip® Medicago genome arrays. Non mycorrhizal and mycorrhizal plants were grown with or without Cs in the medium. Genes expressed in roots and shoots differed greatly and consequently, root and shoot data were analysed separately (Figure 6.2). Out of the 50,900 *M. truncatula* and 1,896 *M. sativa* genes represented on the Affymetrix GeneChip® Medicago genome array, 201 *M. truncatula* and 9 *M. sativa* genes in roots were significantly influenced by Cs (Table S1, Appendix). Genes involved in secondary and lipid metabolism were overrepresented in these lists in comparison to the whole genome (Table S7, Appendix). In particular, genes of the isoprenoid metabolism including terpenoid metabolism were overrepresented. Isoprenoids are compounds built up of simple or multiple C5 units and the isoprenoid pathway has been highly conserved throughout evolution of plants (Bouvier et al., 2005). Many isoprenoids are synthesised in plastids and mitochondria, and they are involved in many aspects of the plant life cycle, i.e. photosynthesis, regulation of gene expression, constituents of membranes, vitamins and antioxidants (Holstein and Hohl, 2004; Bouvier et al., 2005). Terpenoids function as phytoalexins in plant–insect, plant–pathogen and plant–plant interactions (Cheng et al., 2007). Genes involved in glucuronosyltransferase activity and oxidoreductase activity were also overrepresented in the genes affected by Cs (Table S7, Appendix). UDP glycosyltransferases glycosylate a broad spectrum of substrates, including plant hormones and all major classes of plant secondary metabolites (Ross et al., 2001). Reactive oxygen species act as signaling molecules to control processes such as programmed cell death, abiotic stress responses and pathogen defence (Mittler, 2002; Apel and Hirt, 2004). The production of reactive oxygen species can be caused by several stresses including radiation, heavy metals and nutrient deprivation (Mittler, 2002). In shoots, 83 *M. truncatula* and 5 *M. sativa* genes were significantly influenced by Cs (Table S4, Appendix). As in roots, genes involved in oxidoreductase activity were overrepresented (Table S8, Appendix). Caesium might have caused oxidative stress in *M. truncatula* with consequent effects

on secondary metabolism, which is in agreement with the induction of oxidative stress by Cs in *A. thaliana* (Sahr et al., 2005a).

Arbuscular mycorrhiza significantly influenced the expression of 275 *M. truncatula* and 19 *M. sativa* genes in roots but these did not group into any processes (Table S2, Appendix). In shoots, 371 *M. truncatula* and 25 *M. sativa* genes were significantly influenced by arbuscular mycorrhiza (Table S5, Appendix). Many of these gene products were located in chloroplasts (Table S9, Appendix). It has been shown that AM plants can have improved photosynthetic rates in comparison to non mycorrhizal plants (Augé, 2001; Smith and Read, 2008). One of the genes down regulated in shoots of mycorrhizal *M. truncatula* plants was the small subunit of ribulose bisphosphate carboxylase/oxygenase (Rubisco; probe set Mtr.19517.1.S1_at; Table S5, Appendix). The down regulation of the small subunit of Rubisco due to AM fungi had also been observed by Liu et al. (2007). Except for genes related to peroxiredoxin activity in shoots the genes influenced by AM fungi could not be grouped to molecular functions. It suggests that AM fungi influence a wide range of genes with different functions. This effect might be explained by the fact that AM fungi not only influence plant nutrition but can have a variety of effects on plants including improved resistance against plant pathogens or heavy metals (see Chapter 1; van der Heijden and Sanders, 2002).

6.4.2 Gene expression of caesium transport proteins in *Medicago truncatula*

A list of cation transport proteins of *M. truncatula* that might be involved in Cs^+ transport was created. A putative Na^+ transporter orthologous to *AtHKT1* (probe set Mtr.34734.1.S1_at) was significantly down regulated in roots of *M. truncatula* when Cs was present and also in mycorrhizal roots (Figure 6.3). In roots of *Arabidopsis thaliana*, AtHKT1 is thought to be involved in the recovery of Na^+ from the xylem before it reaches the shoot (Munns and Tester, 2008). The Na^+ transporter AtHKT1 is not involved in K^+ transport (Davenport et al., 2007) and it is not known if Cs^+ is transported by AtHKT1. Caesium might have interfered with Na^+ transport by the *M. truncatula* ortholog of AtHKT1 and influenced its expression. Since AM fungi play a major role in nutrient transport (Smith and Read, 2008) and influence the expression of various transport proteins (Benedito et al., 2010) they could also have an effect on the expression of *MtHKT1*. The presence of Cs in the medium down regulated the expression of a putative glutamate receptor cation channel

orthologous to *AtGLR2.1* (probe set Mtr.6324.1.S1_at; Figure 6.4), a putative K^+ channel orthologous to *AtAKT1* (probe set Mtr.51989.1.S1_s_at; Figure 6.5) and a putative Na^+/H^+ exchanger orthologous to *AtCHX19* (probe set Mtr.17230.1.S1_at; Figure 6.6) in shoots of *M. truncatula*. Glutamate receptors in plants function as non-selective ion channels but are also involved in responses to abiotic stresses, and *GLR2.1* is expressed throughout the plant (Davenport, 2002; Dietrich et al., 2010). Caesium might have influenced the expression of *GLR2.1* either as an abiotic stress or by affecting transport processes. The *A. thaliana* voltage gated K^+ channel *AtAKT1* is mainly, but not exclusively, expressed in root cells (Lagarde et al., 1996; Ward et al., 2009) and is probably the dominant K^+ channel involved in K nutrition (Broadley et al., 2001). According to the MtGEA, the *M. truncatula* ortholog (probe set Mtr.51989.1.S1_s_at) is also mainly expressed in roots. Furthermore, AtAKT1 is probably not involved in Cs^+ transport (Broadley et al., 2001) and the expression of *AtAKT1* is not influenced by K^+ (Lagarde et al., 1996; White et al., 2010). The expression values of the ortholog to *AKT1* in *M. truncatula* shoots were very low (Figure 6.5) and the down regulation due to Cs might be an artefact due to the low replication. The putative Na^+/H^+ exchanger *AtCHX19* is mainly expressed in pollen and has been suggested to be involved in the regulation of potassium homeostasis in the course of pollen development (Sze et al., 2004; Gierth and Mäser, 2007). Other members of the *Arabidopsis* CHX family have been shown to be expressed in root cells (Sze et al., 2004) and they have been suggested to be involved in Cs^+ transport into vacuoles (White et al., 2010).

The expression of 15 additional cation transport proteins in roots and 17 additional cation transport proteins in shoots of *M. truncatula* seemed to be influenced by the presence of Cs or colonisation with *Glomus* sp. although the differences in expression levels were not significant (Table 6.1). None of these genes showed higher expression levels in roots when Cs was present. However, Hampton et al. (2004) observed up regulation of two *AtGLR*s in roots of Cs-intoxicated *A. thaliana* plants. In *M. truncatula*, Cs seemed to down regulate the expression of four putative KUPs, including two orthologs of *AtHAK5* (Table 6.1). Sahr et al. (2005a) also showed that *AtHAK5* was down regulated in *A. thaliana* roots exposed to Cs using suppression subtractive hybridisation although the result was not verified with quantitative PCR. By contrast, Hampton et al. (2004) did not see any effect of Cs on the expression of *AtHAK5* in roots of *A. thaliana*. In shoots, orthologs to *AtKUP10*

and *AtGLR3.4* seemed to be up regulated by Cs in *M. truncatula* (Table 6.1). An up regulation of *AtKUP6* and *AtGLR1.3* as well as five *AtCNGC*s was observed in response to Cs intoxication in shoots of *A. thaliana* (Hampton et al., 2004).

Arbuscular mycorrhiza seemed to up regulate the expression of two putative VICCs and to down regulate the expression of three putative VICCs and two putative KUPs in roots. Furthermore, arbuscular mycorrhizal seemed to up regulate the expression of three putative VICCs and to down regulate the expression of one putative VICC and three putative KUPs in shoots of *M. truncatula* (Table 6.1). Up regulation of putative VICCs in *M. truncatula* (an ortholog to *AtCNGC1*; Liu et al., 2007 and an ortholog to *AtCNGC20*; Siciliano et al., 2007) due to AM fungi has been reported previously. In addition, Hohnjec et al. (2005) showed that arbuscular mycorrhiza influenced the expression of two putative KUPs in *M. truncatula*. The analysis of changes in the expression of transport proteins in *M. truncatula* due to AM fungi showed up and down regulation of several putative KUPs in roots (Benedito et al., 2010).

6.4.3 Arbuscular mycorrhiza induced the expression of genes in roots of *Medicago truncatula*

A list of *M. truncatula* genes was created that had previously been shown to be induced by arbuscular mycorrhiza (Hohnjec et al., 2006; Liu et al., 2007; Massoumou et al., 2007; Benedito et al., 2010). Two genes showed significantly higher expression in mycorrhizal than in non mycorrhizal roots of *M. truncatula* (Figure 6.7; 6.8). The protease inhibitor (probe set Mtr.35511.1.S1_at) has been reported to be involved in protein degradation and plant defence (Hohnjec et al., 2005). It was suggested that the protein might be involved in fine-tuning protease activity during arbuscule degradation or in plant defence response to the presence of intraradical hyphae (Hohnjec et al., 2005). The induction of plant genes involved in plant defence has been reported in response to colonisation by AM fungi (Journet et al., 2002; Hohnjec et al., 2005; Küster et al., 2007; Liu et al., 2007). The serine carboxypeptidase *MtScp1* (probe set Mtr.40285.1.S1_at) has been shown to be induced by arbuscular mycorrhiza and is expressed in cortical cells containing arbuscules and in adjacent cells (Liu et al., 2003). It was suggested that MtScp1 might be involved in signalling processes during AM establishment (Liu et al., 2003) or in AM specific protein translocation to subcellular compartments (Hohnjec et al.,

2005). The expression of *MtScp1* was induced in response to *Glomus intraradices*, *Gigaspora gigantea* and *Glomus versiforme* (Liu et al., 2007). Seven additional genes seemed to be induced by colonisation with *Glomus* sp. in *M. truncatula* roots although the differences in expression levels were not significant (Table 6.3). The expression of specific tissue protein 2 (probe set Mtr.10562.1.S1_at) was induced by colonisation with *G. intraradices*, *Gi. gigantea* and *G. versiforme* (Liu et al., 2007). An ATP binding cassette transporter (probe set Mtr.46524.1.S1_at) was 42 times up regulated in mycorrhizal roots in comparison to non mycorrhizal roots (Benedito et al., 2010). ATP binding cassette transporters use ATP to transport diverse solutes, but it cannot be predicted if these proteins import or export substrates or which solutes are transported (Benedito et al., 2010). The remaining five genes belong to the arbuscule related AM marker genes in *M. truncatula* (Hohnjec et al., 2006). The germin-like protein MtGlp1 (probe set Mtr.12500.1.S1_at) has been proposed to function as an oxalate oxidase (Hohnjec et al., 2006) and the blue copper binding protein MtBcp1 (probe set Mtr.15627.1.S1_s_at) is involved in electron transfer (Hohnjec et al., 2005). A glutathione-S-transferase MtGst1 (probe set Mtr.15957.1.S1_at) is probably involved in stress response in mycorrhizal root tissue (Wulf et al., 2003). A β-tubulin MtTubb1 (probe set Mtr.17272.1.S1_at), which is a major component of microtubules, is also induced by AM fungi (Manthey et al., 2004). Increased levels of β-tubulin occur due to the rearrangement of microtubules in cortical cells colonised by AM fungi (Manthey et al., 2004). The phosphate transporter MtPt4 (probe set Mtr.43062.1.S1_at) is essential for P uptake in mycorrhizal roots (Harrison et al., 2002; Javot et al., 2007). There are at least three possible reasons why the expression levels of these AM induced genes were not significantly different in mycorrhizal and non mycorrhizal roots of *M. truncatula* (Table 6.3). First, RNA was extracted from the whole root system. Due to non colonised regions of the root, RNA extraction from the whole root system could result in a dilution of mycorrhiza specific signals (Manthey et al., 2004). Second, the RNA was extracted from two independent experiments, but in these experiments the colonisation rate could not be determined (see Chapter 5). A low colonisation rate or differences in colonisation rates between the experiments could have led to the non significant differences in expression. Third, the intensity values of the two microarray replicates were diverse for many probes and the low replication confounded the statistical analysis.

6.5 CONCLUSIONS

- Gene expression in roots and shoots of *Medicago truncatula* differed
- Caesium induced changes in gene expression were related to secondary metabolism
- The presence of caesium significantly down regulated genes encoding three putative Cs^+ transporters in shoots (orthologs to *AtGLR2.1*, *AtAKT1*, *AtCHX19*) and might down regulate the expression of four putative KUPs (two orthologs to *AtHAK5*, and orthologs to *AtKUP10*, *AtKUP11*) in roots
- Arbuscular mycorrhiza influenced the expression of a wide range of genes in both roots and shoots
- Arbuscular mycorrhiza seemed to influence the expression of genes encoding a variety of putative VICCs and putative KUPs in roots or shoots
- Arbuscular mycorrhiza significantly induced the expression of arbuscule related genes (a protease inhibitor and *MtScp1*) and might have induced the expression of seven additional arbuscule related genes (specific tissue protein 2, ABC transporter, *MtGlp1*, *MtBcp1*, *MtGst1*, *MtTubb1*, *MtPt4*) in roots

Chapter 7
General summary and discussion

7.1 AIMS AND OBJECTIVES

Radiocaesium contamination of the environment has been caused by global fallout from nuclear weapon testing, discharge from nuclear power plants, waste disposal and accidents at nuclear installations. Radiocaesium contamination of agricultural land still exists across Belarus, Russia and Ukraine due to the accident at the nuclear power plant in Chernobyl in 1986 (Smith et al., 2000; Beresford et al., 2001). Two anthropogenic radioisotopes of Cs (^{134}Cs and ^{137}Cs) are of environmental concern due to their relatively long half-lives (2.06 and 30.17 years, respectively) and the emission of harmful β and γ radiation during decay (White and Broadley, 2000). Radiocaesium enters the food chain through soil to plant transfer and thereby impacts on human health (Gillet et al., 2001; White and Broadley, 2000). Most crop species develop symbioses with arbuscular mycorrhizal (AM) fungi, with the exception of a few from non mycorrhizal plant families such as the Brassicaceae (Gosling et al., 2006). Arbuscular mycorrhizal fungi deliver mineral nutrients to their plant hosts (Smith and Read, 2008) and could therefore influence the transfer of radiocaesium from soil to plants (Entry et al., 1996). The effects of arbuscular mycorrhiza on caesium accumulation by plants are variable (see Chapter 1; White et al., 2010). Decreased, increased and similar shoot Cs concentrations of mycorrhizal plants compared to non mycorrhizal plants have been reported (e.g. Joner et al., 2004; Dubchak et al., 2010; Gyuricza et al., 2010b). If arbuscular mycorrhiza increase Cs accumulation by plants they could assist phytoremediation, the removal of radionuclides from soil by plants (Pilon-Smits, 2005). If arbuscular mycorrhiza decrease Cs accumulation by plants they could be used to grow 'safer' crop plants that accumulate less Cs in their edible tissues (Payne et al., 2004; Hampton et al, 2005). Due to the chemical similarity between Cs and K, K^+ transport proteins play a major role in Cs^+ uptake by plants (White et al., 2010). Under K-replete conditions, voltage-insensitive cation channels (VICCs) are mainly responsible for Cs^+ uptake and under K-deficient conditions K^+/H^+ symporters (KUPs) are the dominant pathway of Cs^+ uptake (White et al., 2010). The up regulation of genes encoding

KUPs under K-deficient conditions leads to greater Cs accumulation than under K-replete conditions (Hampton et al., 2004; Qi et al., 2008).

The aim of this thesis was to gain a better understanding of the influence of arbuscular mycorrhiza on Cs accumulation by plants. It was hypothesised that AM fungi improve the K status of plants. Therefore, AM fungi would reduce the abundance of KUPs and Cs uptake by mycorrhizal roots would occur mainly through VICCs. As a result, AM fungi would decrease the accumulation of Cs by plants. These hypotheses were tested by comparing Cs accumulation in mycorrhizal and non mycorrhizal plants. The expression of putative Cs^+ transport proteins, including VICCs and KUPs, was investigated in mycorrhizal and non mycorrhizal *Medicago truncatula* plants.

7.2 ARBUSCULAR MYCORRHIZA AND CAESIUM ACCUMULATION BY PLANTS

The Arbuscular Mycorrhizal–Plant (AM-P) *in vitro* culture system (Dupré de Boulois et al., 2006) was used to investigate the influence of *Glomus* sp. on Cs accumulation by *M. truncatula*. By using an *in vitro* system any potential changes in Cs accumulation would be related to colonisation by *Glomus* sp. since no rhizobia or any other microorganisms were present. To achieve mycorrhizal *M. truncatula* plants in the *in vitro* system the growth conditions had to be optimised (Chapter 3). Although *M. truncatula* has been successfully colonised by *Glomus* sp. on modified Strullu-Romand (MSR) medium (Declerck et al., 1998, modified from Strullu and Romand, 1986) in the *in vitro* system (Dupré de Boulois et al., 2006) no colonisation on MSR medium could be achieved in work undertaken for this thesis. Several factors influence the development of arbuscular mycorrhiza. The most prominent is probably the availability of P, since a major benefit of the symbiosis is improved P nutrition (Smith and Read, 2008). High availability of P can impede fungal colonisation (Thomson et al., 1986; Braunberger et al., 1991) and it was therefore decided to use modified Hoagland's medium (Hoagland and Arnon, 1938) with a reduced P concentration of 20 μM to facilitate AM development. Secondly, carbon allocation to the fungal symbiont is dependent on the photosynthetic activity of the host plants (Azcón-Aguilar and Bago, 1994). Hence, low light intensity can reduce colonisation by AM fungi (Hayman, 1974; Tester et al., 1986; Son and Smith, 1988; Smith and Gianinazzi-Pearson, 1990). Low phosphorus availability and high light

intensity were found to be important for successful colonisation of *M. truncatula* by *Glomus* sp. in the *in vitro* system (Chapter 3).

Caesium accumulation can be toxic to plants and reduce plant growth (Hampton et al., 2004; Isaure et al., 2006). To avoid changes in plant development due to Cs toxicity, it was decided to investigate the influence of *Glomus* sp. on Cs accumulation by *M. truncatula* under non toxic conditions. Otherwise a comparison between plants that had been grown in the absence of Cs and plants that had been grown in the presence of Cs would have been difficult. Therefore, Cs toxicity in *M. truncatula* was studied (Chapter 4). The plants were grown under K-deficient (1.65 mM K) or K-replete (21.65 mM K) conditions with different concentrations of Cs. High concentrations of Cs above 0.15 mM in the presence of 1.65 mM K in the medium and above 0.3 mM Cs in the presence of 21.65 mM K in the medium led to decreased plant height and finally death of the plants (Section 4.3.1). Higher tolerance to Cs availability in the rhizosphere of K-replete plants in comparison to K-deficient plants was also observed in *Arabidopsis thaliana* (Hampton et al., 2004). The availability of K in the medium also influenced Cs accumulation. Potassium-replete plants had lower Cs concentrations in their shoots at the same external Cs supply than K-deficient plants (Section 4.3.2). That increasing K availability decreases Cs accumulation in plant shoots has been shown in several previous studies (Davis, 1963; Smolders et al., 1996a,b; Zhu et al., 2000, 2002; Tsukada and Hasegawa, 2002; Hampton et al., 2004; Le Lay et al., 2006). Increasing Cs concentrations in the medium decreased K concentrations in plant shoots (Section 4.3.3), which is also in agreement with previous studies (Hampton et al., 2004; Le Lay et al., 2006). Shoot fresh weight was inversely related to the Cs_{shoot}:K_{shoot} ratio in both K-deficient and K-replete plants, which suggests that Cs toxicity was related to the Cs_{shoot}:K_{shoot} ratio (Section 4.3.3). This relationship was also observed by Hampton et al. (2004), who suggested that toxicity occurs because Cs competes with K for a biochemical function.

Medicago truncatula was grown in the presence of *Glomus intraradices* at different Cs concentrations in the medium (Section 5.3.3.2). Mycorrhizal colonisation rate and arbuscule abundance decreased at high external Cs supply (Table 5.6). In parts of the roots where arbuscule development occurred in the presence of high Cs concentrations several adjacent cells contained arbuscules. Furthermore, it seemed that the abundance of hyphae growing on the root surface

and the formation of fungal storage vesicles within the root were increased when Cs supply was high (Figure 5.16). These findings suggest that the initiation of the symbiosis is sensitive to Cs. To the best of my knowledge, this is the first time that an inhibition of arbuscular mycorrhiza formation by Cs has been shown.

Three experiments were performed to investigate the influence of arbuscular mycorrhiza on caesium accumulation by plants (Chapters 2 and 5). As mentioned above the hypothesis was tested that AM fungi increase K concentrations in plants and thereby reduce Cs concentrations.

For the first experiment, *M. truncatula* was grown under K-deficient conditions (Section 5.3.1) in symbiosis with *Glomus* sp. with an external Cs supply of 0.05 mM in an *in vitro* system (Section 5.3.2). The mycorrhizal colonisation rate was around 10% and was slightly lower in plants that had been grown with Cs in the medium than in plants that had been grown without Cs in the medium (Section 5.3.2.2). Fresh weights of mycorrhizal and non mycorrhizal plants did not differ and therefore any potential changes in tissue element concentrations were not related to differences in biomass. Potassium concentrations in non mycorrhizal plants did not differ from K concentrations in mycorrhizal plants (Section 5.3.2.3).

For the second experiment, six different plant species (*Hordeum vulgare*, *Beta vulgaris*, *Brassica napus*, *Medicago truncatula*, *Solanum tuberosum* and *Helianthus annuus*) were grown in fertilised non sterile and sterile soil with a Cs supply of 3 mg kg^{-1} soil. The plants that had been grown in non sterile soil were colonised by soil arbuscular mycorrhizal fungi (with the exception of the non mycorrhizal species *Beta vulgaris* and *Brassica napus*) but the colonisation rates could not be determined. All plants that had been grown in sterile soil were non mycorrhizal (Section 2.3.3.2). Potassium concentrations in roots were higher in mycorrhizal than in non mycorrhizal plants but it could not be determined whether the K was stored in mycorrhizal structures or had been delivered to the plant. Shoot K concentrations did not differ between mycorrhizal and non mycorrhizal plants (Section 2.3.4). These results suggest that AM fungi do not influence K concentrations in plants. It has previously been observed that mycorrhizal plants have lower (Kothari et al., 1990) or higher (Clark and Zeto, 2000) shoot K concentrations than non mycorrhizal plants. The reasons for these contrasting results are unknown.

When *M. truncatula* was grown under *in vitro* conditions, Cs accumulation in both non mycorrhizal and mycorrhizal plants varied significantly between experiments (Section 5.3.2.3). This might indicate that Cs accumulation is highly sensitive to environmental factors and thus, results obtained using *in vitro* systems might differ from these obtained under field conditions. Generally, Cs concentrations in shoots of mycorrhizal plants were slightly lower than in shoots of non mycorrhizal plants, but Cs concentrations in roots did not differ between mycorrhizal and non mycorrhizal plants (Section 5.3.2.3). The same observations were made when the plants were grown in non sterile or sterile soil. Generally, Cs concentrations in shoots of mycorrhizal plants were lower than in shoots of non mycorrhizal plants, and Cs concentrations in roots did not differ (Section 2.3.4).

The third experiment was performed to investigate whether external Cs supply influences the effect of AM fungi on Cs accumulation by plants. *Medicago truncatula* was grown in symbiosis with *Glomus intraradices* under K-replete conditions with different external Cs supplies (Section 5.3.3). Caesium concentrations in roots did not differ between mycorrhizal or non mycorrhizal plants independent of the external Cs supply. In shoots of mycorrhizal plants Cs concentrations were increased at the lowest Cs supply, decreased at the second lowest Cs supply and similar at the three highest Cs supplies in comparison to non mycorrhizal plants (Section 5.3.3).

The results of the three different experiments (Chapters 2 and 5) show that Cs concentrations in roots are not influenced by AM fungi, but their influence on Cs concentrations in shoots is variable. Previously, it has been observed that arbuscular mycorrhiza decrease Cs concentrations in shoots (Dighton and Terry, 1996; Berreck and Haselwandter, 2001; Gyuricza et al., 2010b), increase Cs concentrations in shoots (Rogers and Williams, 1986; Entry et al., 1999; Rosén et al., 2005; Dubchak et al., 2010) or have no effect on Cs concentrations in shoots (Rogers and Williams, 1986; Joner et al., 2004; Rosén et al., 2005).

Caesium accumulation differs between plant species (Chapter 2; Andersen, 1967; Broadley et al., 1999a). Caesium concentrations in plant shoots measured in the experiment in the thesis presented here did not correlate with Cs concentrations in shoots measured by Andersen (1967) or reviewed by Broadley et al. (1999a). This again demonstrates that the accumulation of Cs is highly environmentally dependent. Caesium accumulation by plants is determined by soil type, Cs and K concentrations

in soil and plant species specific interactions with soil type are also likely to occur (Andersen, 1967; Hampton et al., 2004; Le Lay et al., 2006; Waegeneers et al., 2009). In experiments investigating the influence of AM fungi on Cs accumulation by plants, stable Cs and radioactive Cs have been used (e.g. Berreck and Haselwandter, 2001; Joner et al., 2004). The accumulation of stable Cs and radioactive Cs has been shown to be correlated in various plant species (Salt et al., 2004; Yoshida et al., 2004; Soudek et al., 2006). It is therefore unlikely that differences in the influence of AM fungi on Cs accumulation by plants are related to the Cs isotope used in experiments.

It seems likely that plant and fungal species, plant/symbiosis age and availability of Cs and K determine the influence of AM fungi on Cs accumulation by plants. Under field conditions, legumes are also colonised by rhizobia. Douka and Xenoulis (1991) showed that Cs concentration in nodulated *Medicago sativa* were lower than in ryegrass and the authors suggested that the rhizobial symbiosis might lower Cs accumulation. However, Cs accumulation varies greatly between plant species (Broadley et al., 1999a) therefore it remains unclear if rhizobia influence Cs accumulation by plants. It has been proposed that AM fungi could be used for phytoremediation purposes or for growth of 'safer' crop plants that accumulate less Cs (Entry et al., 1996; Dubchak et al., 2010; Gyuricza et al., 2010b). However, Entry et al. (1996) pointed out that it cannot be controlled which fungus colonises plants under field conditions. Most of the experiments investigating the effect of AM fungi on Cs accumulation by plants have used *Glomus* species (Table 1.2) but under field conditions plants are colonised by a spectrum of Glomeromycota (Oehl et al., 2010). Arbuscular mycorrhiza have varying effects on Cs concentrations in plant shoots (Table 1.2) probably dependent on plant and fungal species, soil type and other potential factors. Therefore, the use of arbuscular mycorrhiza to improve phytoremediation or to cultivate 'safer' crop plants does not seem feasible.

7.3 GENE EXPRESSION IN *MEDICAGO TRUNCATULA* IN RESPONSE TO CAESIUM AND ARBUSCULAR MYCORRHIZA

Gene expression in roots and shoots of *M. truncatula* changed in response to Cs in the rhizosphere and to colonisation by arbuscular mycorrhizal fungi (Chapter 6). Caesium seemed to influence the expression of genes that are involved in oxidative stress with influence on secondary metabolism (Section 6.3.3) which is in

agreement with Sahr et al. (2005a) who showed that ^{133}Cs induced oxidative stress in *A. thaliana*. It must be noted that the analysis performed in this thesis was based on *A. thaliana* orthologs of the *M. truncatula* genes that responded to Cs. The orthologs were compared against the whole genome of *A. thaliana* on the assumption that the genomes of *M. truncatula* and *A. thaliana* are similar enough to allow the determination of overrepresented biological processes. A similar approach was applied by Widodo et al. (2010) where the authors used a Basic Local Alignment Search Tool (BLAST) to identify *A. thaliana* orthologs of rice (*Oryza sativa*) genes to determine gene categories involved in Zn acquisition and transport.

Caesium influenced the expression of putative Cs^+ transport proteins (Section 6.3.4); a putative glutamate receptor cation channel orthologous to *AtGLR2.1*, a putative K^+ channel orthologous to *AtAKT1* and a putative Na^+/H^+ exchanger orthologous to *AtCHX19* were significantly down regulated in shoots of *M. truncatula* in response to Cs. Changes in gene expression of putative Cs^+ transport proteins due to the presence of Cs was previously shown by Hampton et al. (2004) and Sahr et al. (2005a) in *A. thaliana*. These changes are very likely to be influenced by K status of the plants and the concentration of Cs in the rhizosphere. Hampton et al. (2004) and Sahr et al. (2005a) exposed K-replete plants to Cs whereas the *M. truncatula* plants exposed to Cs in this thesis were K-deficient. Non toxic Cs concentrations were used in this thesis and by Sahr et al. (2005a), whereas Hampton et al. (2004) used toxic concentrations of Cs. The transcriptional responses to K starvation and Cs intoxication are not independent of each other, but show distinct differences (Hampton et al., 2004). Differences in K status and Cs concentrations might explain why the expressions of genes encoding different putative Cs^+ transport proteins were affected in response to Cs in different studies (Chapter 6; Hampton et al., 2004; Sahr et al., 2005a).

Arbuscular mycorrhiza influenced gene expression in roots and shoots of *M. truncatula*. That arbuscular mycorrhiza not only influence gene expression in roots but also in shoots has also been shown by Liu et al. (2007). The expression of two arbuscule related marker genes of *M. truncatula* (Hohnjec et al., 2006), of a protease inhibitor probably involved in protein degradation and plant defence (Hohnjec et al., 2005) and of the serine carboxypeptidase *MtScp1* probably involved in signalling processes during AM establishment (Liu et al., 2003), were significantly induced in mycorrhizal roots (Section 6.3.5). Several other genes in roots and shoots responded

to arbuscular mycorrhiza in their expression but these could not be classified into functional groups (Section 6.3.3). The use of microarray technology revealed that AM fungi influence the expression of genes in roots involved in various processes, such as signal transduction, transport, cell structure, primary and secondary metabolism, protein binding, and plant defence (Hohnjec et al., 2005; Liu et al., 2007; Gomez et al., 2009; Benedito et al., 2010). Formation of arbuscular mycorrhiza caused genes located in the chloroplast to change their expression in shoots (Section 6.3.3). This might suggest that AM fungi influence plant photosynthesis (Augé, 2001; Smith and Read, 2008; Dubchak et al., 2010). The expression of some genes encoding putative Cs^+ transport proteins (mainly voltage insensitive cation channels (VICCs) and K^+/H^+ symporters (KUPs)) changed in response to arbuscular mycorrhiza (Section 6.3.3). Changes in expression of putative VICCs and KUPs in *M. truncatula* due to AM fungi has been shown previously (Hohnjec et al., 2005; Liu et al., 2007; Siciliano et al., 2007; Benedito et al., 2010). Since changes in gene expression of putative VICCs and KUPs occurred in roots and shoots, and genes were up and down regulated, no prediction can be made if, or in which way, AM fungi might influence K or Cs accumulation by plants.

7.4 AREAS FOR FUTURE RESEARCH

Caesium accumulation is influenced by the K status of plants (White et al., 2010). To find explanations for the varying effects of AM fungi on Cs accumulation by plants, the role of arbuscular mycorrhiza in K nutrition of plants, which is not clear yet (Smith and Read, 2008), needs to be better understood. It is known that K^+ transport proteins also mediate Cs^+ transport (White and Broadley, 2000) but in which way the abundance or activity of these proteins is influenced by the presence of Cs or by arbuscular mycorrhizal fungi is unknown. The expression of genes encoding VICCs and KUPs can change when Cs is present but no consistent conclusions can yet be drawn (Chapter 6; Hampton et al., 2004; Sahr et al., 2005a). For example, the expression of *AtHAK5* and its orthologs in *M. truncatula* were down regulated in the presence of Cs in some studies (Chapter 6; Sahr et al., 2005a) but the expression of *AtHAK5* was not affected by Cs in others (Hampton et al., 2004). It has also been shown that AM fungi can change the expression of VICCs and KUPs (Chapter 6; Benedito et al., 2010) but no general pattern has been revealed. Investigating the expression of K^+ transport proteins in response to AM

fungi in different plant species might give insight into the different effects of AM fungi on Cs accumulation. It would be important to study the effect of several AM fungal species on the expression of K^+ transport proteins since plant gene expression can respond differently to different fungal strains (Feddermann et al., 2008). Furthermore, not much is known about fungal gene expression in arbuscular mycorrhiza. Proteomic studies of the extraradical mycelium of *Glomus intraradices* and of fungal proteins expressed in mycorrhizal roots of *M. truncatula* did not identify any fungal proteins related to ion transport (Recorbet et al., 2009, 2010). The identification of AM fungal proteins involved in K^+ or Cs^+ transport could be a direction of future work. Proteins present in the extra- and intraradical mycelia of AM fungi could be extracted and their sequences compared to protein databases. Plant genes encoding putative K^+ or Cs^+ transport proteins could be used to search for AM fungal orthologs and the expression of these fungal genes could be investigated in the presence or absence of Cs. The investigation of the expression of fungal transport proteins in symbiosis might help to predict transport of K^+ or Cs^+ to plant root cells.

Caesium accumulation varies between and within plant species (Chapter 2; Broadley et al., 1999a; Payne et al., 2004). One possible strategy to identify 'safer' crop plants could be to grow various crop cultivars under field conditions and to measure differences in tissue Cs concentrations. Plants should be grown in agricultural soil contaminated with Cs in symbiosis with indigenous fungi. Fungal strains isolated from heavy metal contaminated sites were most effective in phytoremediation suggesting that these fungi were adapted to heavy metal contamination (Orłowska et al., 2005; Göhre and Paszkowski, 2006). It seems therefore possible, that AM fungi could also adapt to Cs contamination. Furthermore, information about indigenous fungi is of importance because which fungi colonise roots in the field cannot be controlled (Entry et al., 1996). It seems that Cs accumulation by plants and the influence of AM fungi on Cs accumulation by plants is so variable, that it is possible that conclusions obtained in the laboratory are not yet transferable to field conditions.

During the last decade, molecular studies have increased our knowledge of K^+ uptake and transport by plants. Up until the end of the 1990s, only a few K^+ transport protein families, such as inward-rectifying K^+ channels (KIRCs), outward-rectifying cation channels (KORCs) and K^+/H^+ symporters (KUPs) had been

identified in plants (reviewed by Schachtman, 2000). More recently, studies have shown that K^+ can be transported by several additional plant transport proteins such as cyclic-nucleotide gated channels (CNGCs), glutamate receptors (GLRs) and cation/H^+ antiporters (reviewed by White and Karley, 2010). Since Cs^+ is chemically similar to K^+ and these ions compete for uptake by plant roots, it was long thought that Cs^+ and K^+ shared the same uptake mechanisms; high affinity "active" (proton-coupled) mechanisms at micromolar rhizosphere concentrations and low affinity mechanisms mediated by ion channels at millimolar rhizosphere concentrations (Shaw and Bell, 1989; Zhu and Smolders, 2000). The K-deficiency inducible proton-coupled K^+ transporter that contributes to K^+ nutrition at low rhizosphere K concentrations in *A. thaliana*, AtHAK5, is also permeable to Cs (Qi et al., 2008). However, it has been shown that the K^+ channel that contributes most to plant K nutrition in *A. thaliana*, the KIRC AtAKT1, does not mediate Cs^+ uptake (Broadley et al., 2001). Transcriptomic and functional studies have provided additional insight into common and distinct components of K^+ and Cs^+ uptake (Hampton et al., 2004; Sahr et al., 2005a,b; Qi et al., 2008). Previously, Cs^+/K^+ uptake mechanisms were investigated in non mycorrhizal plants. However, AM fungi induce changes in plant gene expression (Gomez et al., 2009; Benedito et al., 2010). Here, for the first time, mycorrhizal *M. truncatula* plants have been used to examine the expression of genes encoding putative Cs^+ transport proteins. The expression of genes encoding four putative Cs^+ transport proteins (orthologs of *AtHKT1*, *AtGLR2.1*, *AtAKT1* and *AtCHX19*) were significantly influenced by the presence of Cs and/or arbuscular mycorrhiza. Some additional genes encoding VICCs and KUPs also appeared to be influenced by the presence of Cs and/or arbuscular mycorrhiza. A challenge for the next decade will be to understand the complex processes of Cs^+ uptake and transport in mycorrhizal plants.

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Appendix

Table S1: Raw expression values of genes in roots of non mycorrhizal (non myc) and mycorrhizal (myc) *Medicago truncatula* plants that had been grown without or with caesium in the medium. The gene expression was significantly influenced by the presence of Cs in the medium (Analysis of Variance, $p < 0.05$). The *Medicago* probes refer to the *Medicago truncatula* or *M. sativa* probe name for the gene on the Affymetrix GeneChip® Medicago genome array and the AGI ID is the *Arabidopsis thaliana* gene identification. The annotation was based on local translated BLAST alignment. The MtGEA Target Description is the annotation for the gene obtained from the *Medicago truncatula* Gene Expression Atlas and the TAIR Target Description is the annotation of the *Arabidopsis thaliana* genes (*A. thaliana* annotations in italics).

	Raw expression values in roots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.43585.1.S1_at	525.86	644.42	326.89	277.26	At4g15560	CLA1	4.00E-15	1-deoxy-D-xylulose 5-phosphate synthase
Mtr.40891.1.S1_at	1570.59	1990.27	1449.96	1369.63	At5g60600	HDS	3.00E-51	*1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase*
Mtr.15436.1.S1_at	206.76	360.79	98.32	199.62	At1g52800		3.00E-41	2OG-Fe(II) oxygenase
Mtr.50800.1.S1_at	1122.03	1501.98	800.59	706.79	At5g59540		3.00E-42	2OG-Fe(II) oxygenase
Mtr.762.1.S1_at	173.59	257.05	360.95	458.81	At5g59540		9.00E-28	2OG-Fe(II) oxygenase family
Mtr.37564.1.S1_at	5554.13	8233.85	3866.43	4448.95	At1g52820		2.00E-50	2-oxoglutarate-dependent dioxygenase
Mtr.41872.1.S1_at	36.66	53.01	58.06	65.81	At5g59530		9.00E-52	*2-oxoglutarate-dependent dioxygenase*
Mtr.34391.1.S1_at	103.20	107.43	251.05	259.60				AAA-type ATPase
Mtr.23052.1.S1_at	21.88	26.93	71.61	53.16	At4g25835	GA20OX1	2.00E-39	*AAA-type ATPase family protein*
Mtr.35992.1.S1_at	94.27	94.38	137.39	151.96				ABC transporter subunit
Mtr.13167.1.S1_at	335.89	430.12	425.04	619.31	At1g49430	LACS2	2.00E-47	Acyl CoA synthetase
Mtr.42425.1.S1_at	146.10	108.92	109.30	94.19	At3g55480		3.00E-34	Adaptor protein
Mtr.37912.1.S1_at	630.47	942.38	437.66	598.69	At5g16970	AT-AER	5.00E-40	Allyl alcohol dehydrogenase
Mtr.51204.1.S1_at	1254.16	1755.42	989.85	1031.38	At3g04870	ZDS	9.00E-72	Amine oxidase / *Zeta-carotene desaturase*
Mtr.13820.1.S1_at	28.77	39.64	43.01	58.00				Anion transport protein
Mtr.46957.1.S1_at	258.87	284.45	201.36	184.77	At2g45720		2.00E-47	*Armadillo/beta-catenin repeat family protein*
Mtr.41053.1.S1_at	125.51	78.52	189.12	258.17	At5g49700		7.00E-05	AT-hook DNA-binding protein
Mtr.41894.1.S1_at	25.22	25.03	54.87	100.28	At5g49700		7.00E-43	AT-hook DNA-binding protein
Mtr.20120.1.S1_at	740.23	731.74	444.14	473.07	At4g34760		1.00E-39	Auxin responsive SAUR protein
Mtr.41038.1.S1_at	91.50	126.79	64.44	94.33	At1g51140		5.00E-05	*Basic helix-loop-helix (bHLH) family protein*
Mtr.44189.1.S1_at	876.34	1261.26	644.17	752.43	At5g41410		5.00E-09	BEL1-related homeotic protein
Mtr.13491.1.S1_at	353.94	210.13	432.47	490.45				Beta xylosidase
Mtr.12769.1.S1_at	374.97	522.13	317.78	405.02	At5g17560		8.00E-18	BolA-like
Mtr.40801.1.S1_at	194.79	208.96	274.60	432.24	At2g30600		2.00E-27	*BTB/POZ domain-containing protein*

Table S1 (continued)

	Raw expression values in roots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.9066.1.S1_at	711.78	981.23	299.02	414.31	At1g49320		1.00E-29	*BURP domain-containing protein*
Mtr.35295.1.S1_at	55.02	64.95	39.99	39.86	At1g22070		1.00E-09	*bZIP50 protein*
Mtr.35253.1.S1_at	119.31	101.51	98.99	85.04	At5g51510		2.00E-12	*CAAX amino terminal protease family*
Mtr.8857.1.S1_at	93.21	130.38	118.68	212.04	At1g08080	ACA7	4.00E-25	*Carbonic anhydrase family protein*
Mtr.5789.1.S1_s_at	125.58	128.00	173.28	194.37	At1g15950	CCR1	4.00E-16	Cinnamoyl CoA reductase
Mtr.17308.1.S1_at	451.31	488.67	317.39	442.34	At1g29390		2.00E-43	Cold acclimation
Mtr.16199.1.S1_at	247.49	250.65	179.87	170.23	At2g23430		1.00E-06	Cyclin-dependent kinase inhibitor
Mtr.12486.1.S1_at	67.28	82.99	80.35	29.51				Cystatin
Mtr.13213.1.S1_at	2403.90	2635.20	1247.31	1400.36	At2g29090	CYP707A2	1.00E-18	Cytochrome P450
Mtr.13566.1.S1_at	595.12	802.82	566.55	379.30	At4g31950	CYP82C3	2.00E-39	Cytochrome P450
Mtr.1858.1.S1_at	170.43	145.98	110.44	60.02	At2g02580	CYP71B9	4.00E-49	Cytochrome P450
Mtr.23217.1.S1_at	75.12	118.86	53.17	73.23	At3g26290	CYP71B26	2.00E-60	Cytochrome P450
Mtr.39968.1.S1_at	294.91	374.92	233.37	183.51	At3g26300	CYP71B34	3.00E-45	Cytochrome P450
Mtr.41821.1.S1_at	201.16	276.60	162.03	116.39	At4g36220	FAH1	4.00E-41	Cytochrome P450
Mtr.42011.1.S1_at	491.68	586.97	373.99	371.41	At3g14680		3.00E-51	Cytochrome P450
Mtr.42647.1.S1_at	147.16	152.26	67.68	32.21	At2g24180	CYP71B6	7.00E-25	Cytochrome P450
Mtr.47491.1.S1_s_at	599.25	981.15	335.74	559.47	At3g26290	CYP71B26	1.00E-55	Cytochrome P450
Mtr.33930.1.S1_at	98.28	124.68	77.95	78.10	At5g24910	CYP714A1	6.00E-43	*Cytochrome P450 family protein*
Msa.1818.1.S1_at	287.89	257.68	256.54	375.52	At2g46950		8.00E-33	*Cytochrome P450 family protein,*
Msa.1890.1.S1_at	32.32	21.02	40.37	55.91	At5g25610		7.00E-05	*Dehydration-responsive protein (RD22)*
Mtr.31269.1.S1_at	568.76	873.88	414.00	523.63	At5g58240	FHIT	8.00E-34	Diadenosine tetraphosphate hydrolase
Mtr.39486.1.S1_at	77.64	116.22	47.98	30.66	At2g45440	DHDPS2	1.00E-60	Dihydrodipicolinate synthase
Mtr.37123.1.S1_s_at	1621.49	1939.64	1063.89	975.99	At4g32810	CCD8	9.00E-40	Dioxygenase
Mtr.41049.1.S1_at	22.49	34.41	41.32	51.54				Disease resistance gene
Mtr.24731.1.S1_at	115.10	128.93	133.28	197.59	At1g58170		2.00E-10	*Disease resistance-responsive protein-related*
Mtr.28150.1.S1_at	57.20	60.47	49.41	40.28	At1g79050		1.00E-87	*DNA repair protein recA*
Mtr.28330.1.S1_at	2532.53	2516.95	2264.47	1706.83	At4g12080		1.00E-33	*DNA-binding family protein*
Mtr.11636.1.S1_at	1318.62	1976.14	1189.21	1311.94	At1g29160		6.00E-35	Dof zinc finger protein DOF1.5
Mtr.41050.1.S1_at	217.61	181.04	291.83	238.05				Dolichol-phosphate mannosyltransferase
Mtr.11947.1.S1_at	206.00	207.10	323.78	266.83	At2g27310		8.00E-06	Early growth response protein / *F-box family protein*
Mtr.44757.1.S1_at	1029.97	1573.75	788.08	886.01	At5g25900	GA3	3.00E-71	Ent-kaurene oxidase
Mtr.43313.1.S1_at	541.66	605.85	611.19	1246.12				ERF-like protein

Table S1 (continued)

	Raw expression values in roots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.11208.1.S1_at	38.51	36.52	56.43	74.33	At3g22970		1.00E-23	*Expressed protein*
Mtr.13710.1.S1_at	39.40	48.61	47.30	65.00	At3g25590		6.00E-08	*Expressed protein*
Mtr.14652.1.S1_at	1337.54	1464.53	1225.75	711.09	At2g12905		7.00E-15	*Expressed protein*
Mtr.1532.1.S1_at	68.57	73.11	144.67	142.42	At5g12010		7.00E-46	*Expressed protein*
Mtr.17735.1.S1_at	307.72	388.61	216.82	199.67	At4g27900		1.00E-32	*Expressed protein*
Mtr.18857.1.S1_at	120.81	96.41	158.29	108.05	At3g48660		4.00E-22	*Expressed protein*
Mtr.27434.1.S1_at	60.30	62.47	45.11	34.27	At2g34160		2.00E-08	*Expressed protein*
Mtr.27710.1.S1_at	124.62	163.51	70.78	90.21	At4g04745		1.00E-10	*Expressed protein*
Mtr.28306.1.S1_at	135.32	155.88	100.21	74.94	At5g63500		6.00E-20	*Expressed protein*
Mtr.29327.1.S1_at	46.01	44.08	66.77	58.82	At1g74055		3.00E-14	*Expressed protein*
Mtr.3052.1.S1_at	506.61	802.28	341.33	240.58	At2g41660		1.00E-37	*Expressed protein*
Mtr.33659.1.S1_at	197.24	242.57	122.01	162.91	At1g79510		1.00E-41	*Expressed protein*
Mtr.39860.1.S1_at	53.42	78.58	40.71	55.34	At1g67050		3.00E-11	*Expressed protein*
Mtr.40178.1.S1_at	1504.09	1300.26	2858.18	2143.37	At3g55840		2.00E-14	*Expressed protein*
Mtr.43604.1.S1_s_at	275.41	370.46	252.23	287.97	At1g10020		3.00E-35	*Expressed protein*
Mtr.45985.1.S1_at	73.13	94.78	59.62	52.18	At5g21940		2.00E-24	*Expressed protein*
Mtr.9904.1.S1_at	58.47	59.01	58.24	115.00	At5g37840		9.00E-09	*Expressed protein*
Msa.1174.1.S1_at	697.05	655.59	1323.76	940.43	At3g55840		2.00E-40	*Expressed protein*
Mtr.8559.1.S1_at	69.75	63.10	104.95	148.62	At1g03220		1.00E-21	*Extracellular dermal glycoprotein*
Mtr.37043.1.S1_at	596.34	537.93	736.92	959.55	At5g19100		1.00E-20	*Extracellular dermal glycoprotein-related*
Mtr.1408.1.S1_at	909.89	753.05	646.72	629.71	At5g03970		9.00E-25	*F-box family protein*
Mtr.24235.1.S1_at	165.51	195.22	134.59	123.47	At2g16365		6.00E-12	*F-box family protein*
Mtr.42010.1.S1_at	189.38	138.07	151.96	132.57	At3g23880		4.00E-07	*F-box family protein*
Mtr.5478.1.S1_at	156.62	171.58	100.99	142.18	At3g23880		7.00E-06	*F-box family protein*
Mtr.43831.1.S1_at	273.65	225.36	370.39	365.25	At1g76920		8.00E-47	F-box protein
Mtr.38045.1.S1_at	80.65	81.16	154.29	110.32	At4g40080		3.00E-09	Fiber protein Fb19
Mtr.1169.1.S1_s_at	1616.62	1808.47	1544.96	982.31				Gag/pol polyprotein
Mtr.37369.1.S1_at	2441.80	3155.97	1923.86	1614.46				Germacrene D synthase
Mtr.24203.1.S1_at	60.36	87.45	47.41	38.14	At4g25420		3.00E-39	Gibberellin 20-oxidase
Mtr.37455.1.S1_at	244.01	227.51	412.97	578.20	At4g01070	GT72B1	2.00E-12	Glucosyltransferase-13
Mtr.50846.1.S1_at	79.19	64.39	98.33	94.94	At4g10630		4.00E-50	*Glutaredoxin family protein*
Mtr.37396.1.S1_at	920.11	1081.48	995.41	1379.47	At2g29470	ATGSTU3	5.00E-10	Glutathione S-transferase GST 15

Table S1 (continued)

	Raw expression values in roots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.2288.1.S1_at	180.91	212.49	241.92	292.85	At1g02850	BGLU11	2.00E-25	*Glycosyl hydrolase family 1 protein*
Mtr.40320.1.S1_at	1078.44	1105.25	1432.08	1107.12	At2g28110	FRA8	2.00E-05	*Glycosyl transferase family*
Mtr.34734.1.S1_at	145.63	118.49	111.10	57.42	At4g10310		2.00E-23	High affinity potassium transporter 2 / *Sodium transporter (HKT1)*
Mtr.9928.1.S1_at	168.57	197.72	57.19	45.83	At1g22150		8.00E-25	High affinity sulphate transporter 1
Mtr.5415.1.S1_s_at	50.70	57.25	40.16	40.71	At1g08460	HDA08	5.00E-52	*Histone deacetylase family protein*
Mtr.14819.1.S1_x_at	74.49	48.73	60.16	40.73				Hypothetical protein
Mtr.20650.1.S1_at	52.69	53.12	46.83	31.19				Hypothetical protein
Mtr.21184.1.S1_at	223.23	260.58	154.65	186.91				Hypothetical protein
Mtr.44009.1.S1_at	2965.29	2696.20	5033.83	3343.53	AtMg00030		1.00E-23	*Hypothetical protein*
Mtr.49845.1.S1_at	845.02	785.58	654.40	544.81				Hypothetical protein
Mtr.50277.1.S1_at	3690.40	3373.60	3570.32	4859.33				Hypothetical protein
Msa.3150.1.S1_at	132.10	142.26	272.58	126.08	AtMg00030		7.00E-28	*Hypothetical protein*
Mtr.46631.1.S1_at	39.42	21.34	43.01	324.25	At5g27760		9.00E-14	*Hypoxia-responsive family protein*
Mtr.10707.1.S1_at	116.14	76.72	153.94	136.57	At1g02820		7.00E-07	Indole-3-acetic acid induced protein / *Late embryogenesis abundant 3 family*
Mtr.8632.1.S1_s_at	1402.17	1518.20	1342.19	2404.39	At1g14890		7.00E-37	*Invertase/pectin methylesterase inhibitor family protein*
Mtr.37751.1.S1_at	224.27	206.58	244.41	371.20				Isoflavone-7-O-methytransferase 9
Mtr.9802.1.S1_at	290.25	295.74	203.11	251.53	At3g27150		1.00E-34	*Kelch repeat-containing F-box family protein*
Mtr.40433.1.S1_at	554.68	539.00	782.50	771.19	At1g18270		6.00E-43	*Ketose-bisphosphate aldolase class-II family protein*
Mtr.37273.1.S1_at	47.20	53.44	38.23	42.04	At1g03880		1.00E-08	Legumin A precursor / *12S seed storage protein*
Mtr.35520.1.S1_at	178.50	271.70	357.21	307.02	At5g25930		2.00E-17	*Leucine-rich repeat family protein*
Mtr.24183.1.S1_at	105.10	98.04	72.69	50.33	At3g47580		3.00E-42	*Leucine-rich repeat transmembrane protein kinase*
Mtr.35414.1.S1_at	134.18	182.86	95.53	80.18	At5g53320		6.00E-08	*Leucine-rich repeat transmembrane protein kinase*
Mtr.42879.1.S1_at	637.76	592.10	733.05	813.65				Light-induced protein
Mtr.46870.1.S1_at	223.84	441.66	127.47	177.62	At3g22400	LOX5	1.00E-36	Lipoxygenase
Mtr.13559.1.S1_s_at	2229.89	2564.68	1074.08	1648.43	At3g49940		2.00E-40	LOB domain protein
Mtr.27052.1.S1_at	60.57	45.95	39.47	40.35	At5g16300		3.00E-47	Low density lipoprotein B-like protein
Mtr.38444.1.S1_at	243.99	288.89	371.94	431.16	At2g39420		7.00E-52	Lysophospholipase
Mtr.36367.1.S1_at	98.67	64.28	112.10	125.90	At1g70890		4.00E-14	*Major latex protein-related*
Mtr.11121.1.S1_at	145.83	143.92	205.77	242.26	At3g54110		4.00E-67	Mitochondrial uncoupling protein
Mtr.33426.1.S1_at	54.11	67.29	62.15	33.72				MRP-like ABC transporter
Mtr.37527.1.S1_at	45.47	61.78	64.89	107.71	At1g41830		2.00E-38	*Multi-copper oxidase type I family protein*
Mtr.32750.1.S1_at	110.03	103.17	163.16	171.78	At3g23560		4.00E-22	*Multidrug and toxic compound extrusion (MATE) family*

Table S1 (continued)

	Raw expression values in roots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.9331.1.S1_at	196.64	270.27	193.71	203.18				MYB transcription factor
Mtr.11021.1.S1_at	321.67	429.36	565.84	604.11	At1g69490		7.00E-05	NAC domain protein NAC2
Mtr.39502.1.S1_at	582.12	408.40	647.83	625.61	At5g37980		9.00E-24	*NADP-dependent oxidoreductase*
Mtr.2375.1.S1_at	1390.52	1281.38	1003.81	808.35				Neuraminidase B
Mtr.41063.1.S1_at	66.00	45.56	125.20	138.54				Nine-cis-epoxycarotenoid dioxygenase
Mtr.44730.1.S1_at	243.71	331.85	120.34	171.47	At2g26690		1.00E-62	Nitrate transporter
Mtr.44604.1.S1_at	250.77	261.03	163.14	192.05	At2g36540		4.00E-15	*NLI interacting factor (NIF) family protein*
Mtr.8820.1.S1_at	30.46	51.73	77.42	70.32	At1g28470		1.00E-20	*No apical meristem (NAM) family protein*
Mtr.47977.1.S1_at	50.02	76.76	33.41	25.07	At2g39210		6.00E-64	Nodule-specific protein
Mtr.47908.1.S1_at	99.26	97.94	159.90	161.03	At1g68170		4.00E-39	*Nodulin MtN21 family protein*
Mtr.4041.1.S1_at	432.52	412.28	555.46	493.71	At1g47490		1.00E-11	Nucleic acid binding protein
Mtr.43526.1.S1_at	3188.00	3719.25	2428.75	2233.29				O-antigen translocase
Mtr.45021.1.S1_at	71.39	83.17	41.25	42.23	At1g30220		3.00E-60	Organic-cation transporter / *Sugar transporter family protein*
Mtr.30704.1.S1_at	84.72	93.34	136.03	135.19	At5g19730		4.00E-14	*Pectinesterase family protein*
Mtr.22665.1.S1_at	68.23	65.39	55.74	39.46	At1g52640		4.00E-53	*Pentatricopeptide (PPR) repeat-containing protein*
Mtr.47230.1.S1_at	175.65	132.83	141.59	106.40	At1g08610		2.00E-23	Pentatricopeptide (PPR) repeat-containing protein
Mtr.24000.1.S1_at	248.77	231.12	213.78	140.17	At5g23070		2.00E-54	Pentatricopeptide (PPR) repeat-containing protein / *Thymidine kinase*
Mtr.37112.1.S1_at	136.75	225.23	106.17	90.44	At5g46050		1.00E-16	Peptide transporter
Mtr.40970.1.S1_at	171.09	207.48	418.16	435.07	At5g05340		6.00E-31	Peroxidase
Mtr.49999.1.S1_at	37.71	29.01	60.08	71.99	At1g09155		2.00E-20	Phloem-specific lectin / *SKP1 interacting partner 3-related*
Mtr.34902.1.S1_s_at	106.83	102.64	52.56	63.16	At1g53310	ATPPC1	7.00E-55	Phosphoenolpyruvate carboxylase
Mtr.13592.1.S1_at	63.58	79.75	57.55	43.55	At3g15354		4.00E-48	Photomorphogenesis repressor protein-like / *WD-40 repeat family protein*
Mtr.12375.1.S1_at	91.51	60.26	123.55	346.75	At1g31330		5.00E-44	Photosystem I reaction center subunit III
Mtr.46047.1.S1_at	35.51	61.09	64.63	80.01	At5g42180		4.00E-48	Plant peroxidase
Mtr.23575.1.S1_x_at	120.87	117.28	272.16	280.00	At5g06860		2.00E-32	Polygalacturonase inhibitor protein
Mtr.12213.1.S1_at	2085.79	1882.46	3077.40	2525.47	At3g53980		2.00E-37	*Protease inhibitor*
Mtr.15516.1.S1_at	51.06	45.58	75.07	98.04	At1g23550	SRO2	7.00E-12	*Protein with similarity to RCD1 but without the WWE domain*
Msa.3096.1.S1_at	258.86	249.45	282.61	383.86	At5g01320		4.00E-25	*Pyruvate decarboxylase*
Mtr.32262.1.S1_at	109.97	131.17	80.66	84.96	At2g33170		4.00E-30	Receptor-like protein kinase
Mtr.43418.1.S1_s_at	262.37	411.89	575.31	565.04				Receptor-like protein kinase
Mtr.42979.1.S1_at	46.88	42.49	60.70	102.25				Repetitive proline-rich cell wall protein 1 precursor
Mtr.41287.1.S1_at	50.69	66.46	38.65	53.88	At2g39140	SVR1	8.00E-37	Ribosomal large subunit pseudouridine synthase B

Table S1 (continued)

	Raw expression values in roots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.45966.1.S1_at	293.65	625.85	180.99	276.79	At5g17230	PSY	3.00E-49	Ribosomal protein S2 / *Phytoene synthase*
Mtr.47463.1.S1_at	51.95	75.46	31.43	23.63	At3g49950		2.00E-38	*Scarecrow transcription factor family protein*
Mtr.51973.1.S1_at	64.64	75.48	61.56	56.89	At3g16990		3.00E-42	Seed maturation protein / *TENA/THI-4 family protein*
Mtr.35871.1.S1_at	39.07	73.26	23.06	26.68	At4g37650		6.00E-14	Short-root protein
Mtr.7886.1.S1_at	78.68	116.11	43.82	46.77	At4g37650		4.00E-39	Short-root protein
Mtr.32746.1.S1_at	67.42	90.42	99.32	135.24				Somatic embryogenesis receptor kinase-like protein
Mtr.1872.1.S1_at	608.69	647.53	1514.37	2037.59				Specific tissue protein 2
Mtr.32685.1.S1_at	47.64	42.98	65.88	47.17	At2g46090		8.00E-11	*Sphingosine kinase (SphK)*
Mtr.44565.1.S1_at	24.95	27.15	41.13	76.62	At1g43800		3.00E-61	Stearoyl acyl carrier protein desaturase
Mtr.45469.1.S1_at	33.82	31.20	51.23	100.06	At1g43800		1.00E-71	Stearoyl acyl carrier protein desaturase
Msa.1276.1.S1_at	1255.84	1362.01	1075.76	1000.70	At4g02280		2.00E-77	*Sucrose synthase*
Mtr.2082.1.S1_at	70.53	91.69	49.70	69.71	At5g17570		7.00E-35	*TatD-related deoxyribonuclease family protein*
Mtr.33281.1.S1_s_at	18.47	24.20	68.65	79.13	At5g23960	TPS21	2.00E-18	Terpenoid synthetase
Mtr.12895.1.S1_at	828.96	750.00	1134.73	849.52				Tetratricopeptide repeat protein-like
Mtr.12366.1.S1_s_at	1985.49	2193.99	1617.93	1470.58	At1g74840		8.00E-36	Transcription factor Myb1
Mtr.51252.1.S1_s_at	116.12	124.92	181.69	216.49	At5g42830		8.00E-64	Transferase
Mtr.33621.1.S1_at	193.10	161.16	152.59	128.55	At2g37020		2.00E-57	Translin-like protein
Mtr.24439.1.S1_at	84.58	85.00	74.44	45.45				Tubulin family
Mtr.2218.1.S1_at	85.26	105.09	69.78	71.13	At5g04460		2.00E-08	Ubiquitin-protein ligase-like
Mtr.34715.1.S1_at	134.53	155.86	110.02	104.62	At4g36550		2.00E-14	*U-box domain-containing protein*
Mtr.40333.1.S1_x_at	480.99	773.98	982.32	880.15	At2g44790	UCC2	3.00E-07	Uclacyanin II precursor (Blue copper-binding protein II)
Mtr.40991.1.S1_at	65.45	95.06	93.83	111.35	At1g05680		9.00E-39	UDP-glucosyltransferase
Mtr.37847.1.S1_at	60.25	19.98	65.70	398.81	At1g22370	AtUGT85A5	7.00E-29	UDP-glycosyltransferase
Mtr.42709.1.S1_at	52.45	92.30	42.93	43.20	At1g22360	AtUGT85A2	5.00E-33	UDP-glycosyltransferase 85A8
Msa.1328.1.S1_at	42.87	36.70	50.40	94.93	At4g10270		1.00E-17	*Wound-responsive family protein*
Mtr.42336.1.S1_at	35.45	48.82	56.81	76.76	At4g18170		3.00E-15	WRKY transcription factor 48
Mtr.10910.1.S1_at	35.48	32.74	37.95	61.26	At1g10550	XTH33	9.00E-14	Xyloglucan endotransglucosylase
Mtr.40928.1.S1_at	1433.18	1371.38	2122.86	1630.56	At5g25560		8.00E-35	*Zinc finger (C3HC4-type RING finger) family protein*
Mtr.38300.1.S1_at	75.91	85.76	56.88	54.01	At1g48570		3.00E-41	*Zinc finger (Ran-binding) family protein*
Mtr.10450.1.S1_at	27.31	23.29	55.67	52.06				No annotation
Mtr.12123.1.S1_at	76.04	75.86	112.47	96.35				No annotation
Mtr.1566.1.S1_at	82.29	130.67	46.53	68.92				No annotation

Table S1 (continued)

	Raw expression values in roots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.1950.1.S1_at	29.06	49.24	53.37	113.48				No annotation
Mtr.27040.1.S1_s_at	58.67	67.02	50.08	47.60				No annotation
Mtr.27223.1.S1_at	386.45	339.83	154.13	174.51				No annotation
Mtr.28840.1.S1_at	49.23	45.34	75.97	58.75				No annotation
Mtr.29163.1.S1_at	78.69	78.99	59.10	57.31				No annotation
Mtr.29418.1.S1_at	150.60	192.91	326.67	348.69				No annotation
Mtr.30257.1.S1_at	90.52	65.74	116.16	94.69				No annotation
Mtr.31248.1.S1_s_at	64.89	60.17	75.27	137.06				No annotation
Mtr.31505.1.S1_at	174.14	164.82	235.58	199.55				No annotation
Mtr.32877.1.S1_at	305.71	269.20	513.54	286.78				No annotation
Mtr.34598.1.S1_at	516.30	675.86	474.98	373.51				No annotation
Mtr.3505.1.S1_at	62.39	50.93	81.13	72.21				No annotation
Mtr.3548.1.S1_at	51.97	50.66	89.30	55.50				No annotation
Mtr.35926.1.S1_at	296.32	259.46	270.96	171.78				No annotation
Mtr.3967.1.S1_at	43.04	34.21	61.67	48.99				No annotation
Mtr.40295.1.S1_at	196.61	207.34	214.12	291.91				No annotation
Mtr.4078.1.S1_at	120.56	116.64	128.93	191.22				No annotation
Mtr.42148.1.S1_at	121.88	93.40	159.93	119.01				No annotation
Mtr.4260.1.S1_at	102.06	76.12	143.92	152.00				No annotation
Mtr.44211.1.S1_at	56.03	63.94	104.16	112.40				No annotation
Mtr.44741.1.S1_at	198.92	156.25	108.90	77.44				No annotation
Mtr.8171.1.S1_at	170.06	120.75	239.65	150.90				No annotation
Mtr.8172.1.S1_at	651.69	372.82	907.47	615.65				No annotation
Mtr.8880.1.S1_at	42.85	42.38	88.79	70.54				No annotation
Msa.812.1.S1_at	1353.02	1222.04	2100.22	1182.59				No annotation
Msa.832.1.S1_at	648.86	580.31	1080.63	615.02				No annotation

Table S2: Raw expression values of genes in roots of non mycorrhizal (non myc) and mycorrhizal (myc) *Medicago truncatula* plants that had been grown without or with caesium in the medium. The gene expression was significantly influenced by arbuscular mycorrhiza (Analysis of Variance, $p < 0.05$). The *Medicago* probes refer to the *Medicago truncatula* or *M. sativa* probe name for the gene on the Affymetrix GeneChip® Medicago genome array and the AGI ID is the *Arabidopsis thaliana* gene identification. The annotation was based on local translated BLAST alignment. The MtGEA Target Description is the annotation for the gene obtained from the *Medicago truncatula* Gene Expression Atlas and the TAIR Target Description is the annotation of the *Arabidopsis thaliana* genes (*A. thaliana* annotations in italics).

	Raw expression values in roots						
	Without caesium		**With caesium**				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.15436.1.S1_at	206.76	360.79	98.32	199.62	At1g52800	3.00E-41	2OG-Fe(II) oxygenase
Msa.1308.1.S1_at	47.84	65.49	44.98	56.26	At5g35910	3.00E-62	*3'-5' exonuclease domain-containing protein*
Mtr.43421.1.S1_at	235.37	381.38	222.24	299.98	At1g20480	5.00E-40	*4-coumarate-CoA ligase family protein*
Msa.2547.1.S1_at	59.47	59.95	49.01	74.86	At2g33450	3.00E-27	*50S ribosomal protein L28*
Mtr.36163.1.S1_at	189.13	104.19	196.58	146.63			60S ribosomal protein
Mtr.18815.1.S1_at	239.37	329.55	174.55	288.41	At1g15520	5.00E-28	AAA ATPase; ABC transporter related
Mtr.34622.1.S1_at	238.56	179.95	273.09	185.14			Abscisic stress ripening protein
Mtr.13167.1.S1_at	335.89	430.12	425.04	619.31	At1g49430	2.00E-47	Acyl CoA synthetase
Mtr.42425.1.S1_at	146.10	108.92	109.30	94.19	At3g55480	3.00E-34	Adaptor protein, adaptin-like
Mtr.43862.1.S1_at	39.54	58.22	35.88	88.60	At4g04880	7.00E-39	*Adenosine/AMP deaminase family protein*
Mtr.12034.1.S1_at	160.66	147.08	200.78	140.35			ADP/ATP translocase-like protein
Mtr.10593.1.S1_at	1056.07	1641.32	1181.40	1276.03	At1g13280	3.00E-40	Allene oxide cyclase precursor
Mtr.37912.1.S1_at	630.47	942.38	437.66	598.69	At5g16970	5.00E-40	Allyl alcohol dehydrogenase
Mtr.3632.1.S1_at	73.64	61.61	81.01	53.85			Angiotensinogen
Mtr.23461.1.S1_at	51.96	71.90	60.67	82.54	At3g18400	3.00E-11	Apical meristem (NAM) protein family
Mtr.43281.1.S1_at	270.73	359.52	215.17	401.88	At4g18910	2.00E-20	*Aquaglyceroporin*
Mtr.27738.1.S1_at	32.99	56.86	45.47	116.79	At1g01490	7.00E-16	ATP-dependent molecular chaperone / *Heavy-metal-associated domain-containing protein*
Mtr.44128.1.S1_at	94.84	94.32	60.67	111.38	At3g07360	4.00E-32	Avr9/Cf-9 rapidly elicited protein-like
Mtr.37400.1.S1_at	425.56	481.35	254.88	471.42	At3g07340	8.00E-20	*Basic helix-loop-helix (bHLH) family protein*
Mtr.41038.1.S1_at	91.50	126.79	64.44	94.33	At1g51140	5.00E-05	*Basic helix-loop-helix (bHLH) family protein*
Mtr.50730.1.S1_at	410.35	334.71	539.00	273.21			Basic-leucine zipper (bZIP) transcription factor
Mtr.12161.1.S1_at	395.38	562.28	357.42	756.71	At3g60130	2.00E-45	Beta-glucosidase precursor isoform AH I
Mtr.50900.1.S1_at	866.09	779.54	433.79	1107.88	At5g60490	2.00E-24	Beta-Ig-H3/fasciclin
Mtr.17361.1.S1_at	822.43	758.75	349.96	1301.13	At5g03170	2.00E-30	Beta-Ig-H3/fasciclin
Mtr.37837.1.S1_s_at	67.33	100.01	52.88	89.60	At5g08130	3.00E-36	BHLH protein family-like
Mtr.12769.1.S1_at	374.97	522.13	317.78	405.02	At5g17560	8.00E-18	BolA-like family protein

Table S2 (continued)

Medicago probes	Raw expression values in roots: Without caesium Non myc	Without caesium Myc	With caesium Non myc	With caesium Myc	AGI ID	e-value	MtGEA Target Description / *TAIR Target Description*
Mtr.7101.1.S1_at	58.28	79.51	49.33	78.50	At4g16610	1.00E-10	C2H2 zinc-finger protein
Mtr.2219.1.S1_x_at	162.04	120.69	198.91	135.94			Ca^{2+}/Na^{+} antiporter
Mtr.40942.1.S1_at	847.99	905.87	736.96	1122.30	At4g34050	1.00E-15	Caffeoyl-CoA O-methyltransferase 5
Mtr.11249.1.S1_s_at	51.47	80.79	53.46	90.36	At4g33000	3.00E-18	Calcineurin B-like protein 10
Mtr.10823.1.S1_s_at	47.10	42.55	29.63	56.94	At3g56760	1.00E-66	Calcium-dependent protein kinase-like
Mtr.10272.1.S1_at	40.85	61.60	39.56	68.43	At3g22930	8.00E-61	Calmodulin
Mtr.42008.1.S1_s_at	216.47	255.90	178.19	269.37	At2g02790	1.00E-15	*Calmodulin-binding family protein*
Mtr.8857.1.S1_at	93.21	130.38	118.68	212.04	At1g08080	4.00E-25	*Carbonic anhydrase family protein*
Mtr.34859.1.S1_at	82.59	64.43	70.25	50.75	At3g47060	9.00E-68	Cell division protein FtsH
Mtr.44799.1.S1_at	173.70	335.50	202.44	306.68	At1g55120	5.00E-29	Cell wall invertase II / *Beta-fructosidase*
Mtr.40676.1.S1_at	183.32	206.79	142.62	266.62	At3g18490	8.00E-58	Chloroplast nucleoid DNA binding / *Aspartyl protease family protein*
Mtr.12985.1.S1_at	563.89	464.91	640.94	482.27	At1g62880	2.00E-18	*Cornichon family protein*
Mtr.13774.1.S1_at	120.08	94.33	160.81	98.45			Cytochrome b
Mtr.37029.1.S1_at	125.81	90.70	156.18	85.35			Cytochrome b
Mtr.42015.1.S1_at	238.12	329.76	219.45	291.73	At5g09680	4.00E-32	*Cytochrome b5 domain-containing protein*
Mtr.47491.1.S1_s_at	599.25	981.15	335.74	559.47	At3g26290	1.00E-55	Cytochrome P450
Msa.1818.1.S1_at	287.89	257.68	256.54	375.52	At2g46950	8.00E-33	*Cytochrome P450 family protein*
Mtr.8651.1.S1_a_at	279.47	31.64	45.04	12.14			Dehydrin-like protein
Mtr.30372.1.S1_at	169.26	147.38	171.85	103.52	At3g11670	1.00E-06	Digalactosyldiacylglycerol synthase 1
Mtr.23370.1.S1_at	78.27	127.60	74.22	110.89			Disease resistance protein
Mtr.23370.1.S1_s_at	198.23	236.98	155.10	240.93			Disease resistance protein
Mtr.4738.1.S1_s_at	66.12	137.30	110.23	160.12	At2g28670	1.00E-14	*Disease resistance-responsive family protein*
Mtr.24731.1.S1_at	115.10	128.93	133.28	197.59	At1g58170	2.00E-10	*Disease resistance-responsive protein-related*
Mtr.50411.1.S1_at	92.59	75.84	68.45	142.13	At1g13635	9.00E-36	DNA glycosylase
Mtr.38994.1.S1_at	224.81	262.81	143.41	285.21	At1g69780	5.00E-34	DNA-binding protein
Mtr.11636.1.S1_at	1318.62	1976.14	1189.21	1311.94	At1g29160	6.00E-35	Dof zinc finger protein
Mtr.6125.1.S1_s_at	500.51	648.61	439.26	747.43	At4g19120	2.00E-43	*Early-responsive to dehydration stress protein (ERD3)*
Mtr.34086.1.S1_at	227.21	212.82	289.69	188.30	At5g07710	6.00E-09	*Exonuclease family protein*
Mtr.37813.1.S1_s_at	34.37	77.30	35.01	47.60	At1g68470	6.00E-71	*Exostosin family protein*
Mtr.6079.1.S1_at	30.72	51.58	34.75	43.63	At4g22580	1.00E-34	*Exostosin family protein*
Msa.1613.1.S1_s_at	84.52	134.02	81.91	116.27	At1g09310	2.00E-51	*Expressed protein*
Mtr.11390.1.S1_at	160.61	214.65	163.61	208.57	At3g55990	7.00E-61	*Expressed protein*

Table S2 (continued)

	Raw expression values in roots						
	Without caesium		With caesium				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.13710.1.S1_at	39.40	48.61	47.30	65.00	At3g25590	6.00E-08	*Expressed protein*
Mtr.14652.1.S1_at	1337.54	1464.53	1225.75	711.09	At2g12905	7.00E-15	*Expressed protein*
Mtr.18857.1.S1_at	120.81	96.41	158.29	108.05	At3g48660	4.00E-22	*Expressed protein*
Mtr.18858.1.S1_at	990.87	869.22	1282.33	785.25	At3g48660	2.00E-14	*Expressed protein*
Mtr.20398.1.S1_at	355.50	557.71	323.35	561.80	At3g23090	7.00E-39	*Expressed protein*
Mtr.21612.1.S1_at	479.72	525.19	351.74	557.08	At4g36660	6.00E-53	*Expressed protein*
Mtr.250.1.S1_at	67.97	94.15	60.27	112.46	At2g35290	3.00E-11	Expressed protein
Mtr.26508.1.S1_at	151.15	113.91	165.09	97.34	At4g13740	1.00E-43	*Expressed protein*
Mtr.28713.1.S1_at	47.77	36.42	52.53	36.28	At2g20585	8.00E-17	*Expressed protein*
Mtr.32117.1.S1_at	205.10	247.36	184.39	297.66	At3g48980	5.00E-75	*Expressed protein*
Mtr.32435.1.S1_at	79.18	49.48	80.37	44.63	At3g61690	6.00E-74	*Expressed protein*
Mtr.33829.1.S1_at	214.07	223.85	164.93	365.90	At2g35880	3.00E-44	*Expressed protein*
Mtr.35312.1.S1_at	243.41	324.31	211.54	397.72	At5g41050	3.00E-16	*Expressed protein*
Mtr.35565.1.S1_at	59.52	91.95	75.62	81.38	At5g61670	4.00E-07	*Expressed protein*
Mtr.36203.1.S1_at	249.82	182.77	200.79	155.55	At1g13360	7.00E-09	*Expressed protein*
Mtr.37377.1.S1_at	1602.59	1071.31	1586.16	1342.17			Expressed protein
Mtr.39860.1.S1_at	53.42	78.58	40.71	55.34	At1g67050	3.00E-11	*Expressed protein*
Mtr.40229.1.S1_at	178.40	114.18	192.70	120.03	At4g29735	7.00E-20	*Expressed protein*
Mtr.42939.1.S1_at	1585.48	1557.37	2278.35	1376.81	At1g28135	1.00E-05	*Expressed protein*
Mtr.43458.1.S1_s_at	365.32	560.55	309.24	531.04	At3g23090	6.00E-08	*Expressed protein*
Mtr.43604.1.S1_s_at	275.41	370.46	252.23	287.97	At1g10020	3.00E-35	*Expressed protein*
Mtr.4424.1.S1_at	99.80	96.05	126.73	71.39	At1g49405	4.00E-22	Expressed protein
Mtr.44722.1.S1_at	313.20	239.58	336.15	239.84	At1g53035	2.00E-40	*Expressed protein*
Mtr.47992.1.S1_at	34.95	44.82	34.98	59.98	At2g25735	1.00E-06	*Expressed protein*
Mtr.5386.1.S1_at	51.53	80.43	47.68	70.96	At2g17550	8.00E-05	*Expressed protein*
Mtr.9010.1.S1_at	1154.75	686.36	1059.97	638.76	At5g05250	2.00E-15	*Expressed protein*
Mtr.9169.1.S1_at	248.88	273.26	228.85	385.47	At5g67210	1.00E-66	*Expressed protein*
Mtr.9500.1.S1_at	349.62	227.18	393.94	262.41	At1g33055	2.00E-09	*Expressed protein*
Mtr.9904.1.S1_at	58.47	59.01	58.24	115.00	At5g37840	9.00E-09	*Expressed protein*
Mtr.47022.1.S1_s_at	20.49	86.05	20.42	104.66	At5g01740	1.00E-13	Expressed wound-inducible protein
Mtr.13136.1.S1_at	646.32	585.86	293.13	929.21	At5g60490	1.00E-10	Fasciclin-like AGP 10
Mtr.13136.1.S1_s_at	251.51	255.01	96.69	345.72	At5g60490	2.00E-33	Fasciclin-like AGP 10

Table S2 (continued)

	Raw expression values in roots						
	Without caesium		With caesium				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Msa.2535.1.S1_at	618.99	1254.59	743.68	1086.72	At2g20520	8.00E-28	*Fasciclin-like arabinogalactan-protein (FLA6)*
Mtr.13033.1.S1_at	522.24	1183.04	661.31	1017.22	At2g20520	8.00E-38	*Fasciclin-like arabinogalactan-protein (FLA6)*
Mtr.37610.1.S1_at	3623.88	3952.98	3301.57	4901.75			Fasciclin-like arabinogalactan-protein 2
Mtr.42010.1.S1_at	189.38	138.07	151.96	132.57	At3g23880	4.00E-07	*F-box family protein*
Mtr.5478.1.S1_at	156.62	171.58	100.99	142.18	At3g23880	7.00E-06	*F-box family protein*
Mtr.24699.1.S1_at	72.26	103.13	74.33	81.16	At5g39450	2.00E-31	F-box protein family-like
Mtr.7403.1.S1_at	369.58	256.10	468.37	330.11			Fe(II) transport protein 3
Mtr.19637.1.S1_s_at	662.86	769.80	660.07	966.43	At1g50480	1.00E-82	Formate-tetrahydrofolate ligase
Mtr.24871.1.S1_s_at	52.73	100.09	60.67	114.80	At3g13790	3.00E-33	Fructan 1-exohydrolase IIa precursor
Mtr.1989.1.S1_at	107.82	136.70	98.60	114.24	At5g13810	4.00E-20	*Glutaredoxin family protein*
Mtr.12560.1.S1_at	44.75	69.89	56.28	82.80			Glutathione S-transferase
Mtr.37396.1.S1_at	920.11	1081.48	995.41	1379.47	At2g29470	5.00E-10	Glutathione S-transferase
Mtr.43490.1.S1_at	2589.67	2294.10	3054.82	2296.23			Glycine/proline-rich protein
Mtr.43182.1.S1_at	1121.03	1758.64	786.54	1366.99	At1g61820	3.00E-47	*Glycosyl hydrolase family 1 protein*
Mtr.38003.1.S1_at	102.16	152.80	103.00	136.06	At4g16660	3.00E-67	Growth regulator like protein / *Heat shock protein 70*
Mtr.44959.1.S1_at	40.27	52.73	33.42	58.73			Guanine nucleotide releasing factor 1
Mtr.47603.1.S1_at	173.74	142.51	170.54	106.31			Haloacid dehalogenase-like hydrolase family
Mtr.10662.1.S1_at	278.52	163.03	246.74	128.28	At4g25200	1.00E-33	Heat shock 22 kDa protein
Mtr.40057.1.S1_at	925.07	842.04	983.90	615.43	At5g09590	2.00E-34	Heat shock 70 kDa protein
Msa.1899.1.S1_at	1422.57	1210.25	1756.99	1207.24	At5g56030	6.00E-86	*Heat shock protein 81-2 (HSP81-2)*
Mtr.36259.1.S1_at	94.23	68.98	96.57	46.51			Heat shock protein PIR1 homolog
Mtr.44719.1.S1_at	84.28	46.53	84.83	54.43	At2g26150	3.00E-21	Heat shock transcription factor
Mtr.9270.1.S1_at	808.29	1109.38	601.49	1011.14	At4g08570	4.00E-47	*Heavy-metal-associated domain-containing protein*
Mtr.34734.1.S1_at	145.63	118.49	111.10	57.42	At4g10310	2.00E-23	High affinity potassium transporter 2 / *Sodium transporter (HKT1)*
Mtr.42733.1.S1_at	42.65	54.77	46.03	76.12			Histone H1
Mtr.50674.1.S1_at	61.13	117.40	59.59	90.67			Homeodomain-like; Myb, DNA-binding
Mtr.43748.1.S1_s_at	88.20	103.69	72.19	103.32	At1g77420	3.00E-39	*Hydrolase*
Mtr.8442.1.S1_at	1112.18	1681.89	1577.26	2796.21			Hydroxyproline-rich glycoprotein
Mtr.17436.1.S1_at	28.85	43.82	27.21	55.02	At1g72790	2.00E-13	*Hydroxyproline-rich glycoprotein family protein*
Msa.3150.1.S1_at	132.10	142.26	272.58	126.08	AtMg00030	7.00E-28	*Hypothetical protein*
Mtr.14819.1.S1_x_at	74.49	48.73	60.16	40.73			Hypothetical protein
Mtr.14994.1.S1_at	233.99	477.38	287.85	439.19			Hypothetical protein

Table S2 (continued)

Medicago probes	Raw expression values in roots: Without caesium Non myc	Without caesium Myc	With caesium Non myc	With caesium Myc	AGI ID	e-value	MtGEA Target Description / *TAIR Target Description*
Mtr.17844.1.S1_at	105.58	160.85	93.10	161.67			Hypothetical protein
Mtr.18605.1.S1_at	62.41	123.09	86.17	119.07			Hypothetical protein
Mtr.18605.1.S1_x_at	97.86	192.73	123.97	182.09			Hypothetical protein
Mtr.21430.1.S1_at	65.97	57.54	94.10	48.77			Hypothetical protein
Mtr.44009.1.S1_at	2965.29	2696.20	5033.83	3343.53	AtMg00030	1.00E-23	*Hypothetical protein*
Mtr.49373.1.S1_at	80.57	59.88	75.67	46.19			Hypothetical protein
Mtr.49667.1.S1_at	293.70	404.02	302.14	330.09			Hypothetical protein
Mtr.50115.1.S1_at	142.26	130.68	184.99	92.21			Hypothetical protein
Mtr.50487.1.S1_at	49.13	26.42	43.79	29.70			Hypothetical protein
Mtr.37387.1.S1_s_at	107.80	174.09	114.68	129.90	At1g79110	2.00E-12	Inhibitor of apoptosis-like protein
Mtr.43211.1.S1_at	1435.33	1171.79	1565.24	1171.33	At1g16350	2.00E-17	Inosine monophosphate dehydrogenase
Mtr.8632.1.S1_s_at	1402.17	1518.20	1342.19	2404.39	At1g14890	7.00E-37	*Invertase/pectin methylesterase inhibitor family protein*
Mtr.13235.1.S1_at	401.95	477.63	368.36	624.45			Isoprenylated protein
Mtr.14066.1.S1_s_at	904.03	488.24	697.54	438.23	AtCg00490	6.00E-101	*Large subunit of RUBISCO*
Mtr.41347.1.S1_at	91.65	152.38	69.13	118.88	At5g15900	7.00E-38	Leaf senescence protein-like
Mtr.10005.1.S1_at	84.52	96.24	87.78	133.49	At1g07650	5.00E-55	*Leucine-rich repeat transmembrane protein kinase*
Mtr.24183.1.S1_at	105.10	98.04	72.69	50.33	At3g47580	3.00E-42	*Leucine-rich repeat transmembrane protein kinase*
Mtr.18715.1.S1_at	56.59	45.95	71.47	21.86	At4g08850	8.00E-28	Leucine-rich repeat; Protein kinase
Mtr.43246.1.S1_s_at	92.22	118.72	74.29	108.36	At4g36860	2.00E-75	LIM domain containing protein-like
Msa.1835.1.S1_at	39.66	48.29	41.92	69.08	At1g49430	2.00E-47	*Long-chain-fatty-acid-CoA ligase*
Mtr.40285.1.S1_at	87.17	226.43	77.09	197.85			Lysosomal protective protein precurso
Mtr.35647.1.S1_at	266.19	440.94	238.08	329.76			Major allergen
Mtr.19730.1.S1_at	32.96	46.26	34.79	51.81			Microtubule-associated protein
Mtr.7160.1.S1_at	34.87	57.08	33.90	53.57	At2g34660	2.00E-62	MRP-like ABC transporter
Mtr.11148.1.S1_at	472.80	536.12	444.02	703.15			Multi resistance protein homolog
Mtr.37527.1.S1_at	45.47	61.78	64.89	107.71	At1g41830	2.00E-38	*Multi-copper oxidase type I family protein*
Mtr.9331.1.S1_at	196.64	270.27	193.71	203.18			MYB transcription factor
Msa.1055.1.S1_at	2075.95	1380.84	1773.47	1096.72	AtMg00580	6.00E-36	*NADH dehydrogenase subunit 4*
Mtr.11042.1.S1_at	665.77	837.49	512.45	908.34	At1g12110	1.00E-30	Nitrate transporter
Mtr.44730.1.S1_at	243.71	331.85	120.34	171.47	At2g26690	1.00E-62	Nitrate transporter
Mtr.42072.1.S1_at	129.85	345.00	119.34	235.55	At5g25250	4.00E-31	Nodulin
Mtr.15545.1.S1_at	90.80	76.12	87.48	50.04	At4g28940	5.00E-15	*Nucleosidase-related*

Table S2 (continued)

	Raw expression values in roots						
	Without caesium		With caesium				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.3864.1.S1_at	55.25	44.77	63.09	46.92	At2g34410	3.00E-07	*O-acetyltransferase family protein*
Mtr.8606.1.S1_at	1640.38	1803.76	1562.23	2820.29	At5g54160	6.00E-09	O-methyltransferase
Mtr.35511.1.S1_at	22.46	355.81	27.83	272.54	At2g38870	5.00E-12	Pathogenesis-related protein / *Protease inhibitor*
Mtr.1107.1.S1_at	30.67	50.20	28.14	53.13	At1g67750	2.00E-78	Pectate lyase
Msa.1350.1.S1_at	18.82	38.59	16.90	48.18	At3g49220	8.00E-61	*Pectinesterase family protein*
Mtr.22665.1.S1_at	68.23	65.39	55.74	39.46	At1g52640	4.00E-53	*Pentatricopeptide (PPR) repeat-containing protein*
Mtr.47230.1.S1_at	175.65	132.83	141.59	106.40	At1g08610	2.00E-23	Pentatricopeptide (PPR) repeat-containing protein
Mtr.9254.1.S1_at	87.04	131.81	110.00	204.61	At2g37040	5.00E-42	Phenylalanine ammonia lyase
Mtr.17725.1.S1_at	37.86	52.95	29.99	98.97	At3g49220	5.00E-96	Plant invertase / *Pectinesterase family protein*
Msa.2731.1.S1_at	189.79	278.57	168.46	226.83	At1g08500	7.00E-53	*Plastocyanin-like domain-containing protein*
Mtr.23580.1.S1_at	18.64	47.00	33.75	78.69	At5g06860	1.00E-45	Polygalacturonase inhibitor protein
Mtr.38202.1.S1_at	98.82	146.42	118.04	233.88			Probable WRKY transcription factor 28
Mtr.11599.1.S1_at	81.04	56.53	109.37	54.69	At1g04870	1.00E-22	*Protein arginine N-methyltransferase family protein*
Mtr.3066.1.S1_s_at	26.16	65.43	40.64	55.27	At2g17220	3.00E-37	*Protein kinase*
Mtr.24165.1.S1_at	42.14	115.82	31.20	51.04	At2g28930	2.00E-21	*Protein kinase (APK1b)*
Mtr.50390.1.S1_at	79.91	70.54	97.46	68.19	At2g42390	9.00E-47	*Protein kinase C substrate*
Mtr.1770.1.S1_at	44.80	58.91	44.53	67.37	At3g44610	4.00E-60	Protein kinase-like protein
Mtr.31497.1.S1_at	43.94	60.80	40.64	68.25	At4g11530	2.00E-35	Protein kinase-like protein
Mtr.5506.1.S1_at	374.04	280.25	426.61	249.88	At2g32720	1.00E-10	Protein phosphatase / *Cytochrome b5*
Mtr.35025.1.S1_at	52.79	44.73	70.35	39.81			protein-tyrosine kinase
Mtr.35208.1.S1_at	264.89	293.52	169.93	300.27			P-selectin glycoprotein ligand 1
Mtr.44281.1.S1_at	293.60	450.53	224.58	386.10	At2g01880	2.00E-60	Purple acid phosphatase
Mtr.5541.1.S1_at	86.41	132.55	77.46	112.11			R2R3-MYB transcription factor
Mtr.29863.1.S1_at	145.54	92.88	141.79	57.54			Repetitive proline-rich cell wall protein 1 precursor
Mtr.40069.1.S1_at	1630.49	2011.98	1567.60	2827.41			Repetitive proline-rich cell wall protein 2 precursor
Mtr.39757.1.S1_at	46.76	27.99	48.26	26.32			Retinitis pigmentosa GTPase regulator-like protein
Mtr.48952.1.S1_s_at	16.79	16.34	13.73	58.32	At5g66170	1.00E-35	Rhodanese-like / *Senescence-associated family protein*
Mtr.41287.1.S1_at	50.69	66.46	38.65	53.88	At2g39140	8.00E-37	Ribosomal large subunit pseudouridine synthase B
Mtr.39857.1.S1_at	87.18	69.15	112.89	71.17			Ribosomal protein S14
Msa.1532.1.S1_at	121.17	52.76	105.34	59.67	At2g34520	9.00E-42	*Ribosomal protein S14 mitochondrial family protein*
Mtr.20925.1.S1_s_at	1135.47	1092.78	1384.62	996.24	At1g55310	6.00E-13	RNA-binding region RNP-1 / *SC35-like splicing factor*
Mtr.50912.1.S1_at	16.48	65.32	18.30	40.91	At5g60520	2.00E-53	Root cap / *Late embryogenesis abundant protein-related*

Table S2 (continued)

Medicago probes	Raw expression values in roots: Without caesium Non myc	Without caesium Myc	With caesium Non myc	With caesium Myc	AGI ID	e-value	MtGEA Target Description / *TAIR Target Description*
Mtr.43089.1.S1_at	377.41	517.07	455.87	849.20	At5g06760	3.00E-12	Seed maturation protein LEA 4
Mtr.32128.1.S1_at	646.89	711.79	514.43	836.24	At2g27920	1.00E-45	*Serine carboxypeptidase S10 family protein*
Mtr.32685.1.S1_at	47.64	42.98	65.88	47.17	At2g46090	8.00E-11	*Sphingosine kinase (SphK)*
Mtr.43944.1.S1_at	291.64	162.96	174.24	162.25			Sporulation-specific glucan 1,3-beta-glucosidase precursor
Mtr.11185.1.S1_at	42.70	46.58	36.27	58.37	At1g10760	4.00E-71	Starch excess protein (SEX1)
Mtr.2286.1.S1_at	29.13	69.41	33.55	96.48	At1g50720	2.00E-35	*Stigma-specific Stig1 family protein*
Mtr.13963.1.S1_at	19.58	82.19	23.58	47.38	At2g05920	1.00E-16	*Subtilase family protein*
Mtr.32626.1.S1_at	140.34	260.15	118.17	147.40	At2g45680	4.00E-40	Syntaxin binding protein 4 / *TCP family transcription factor*
Mtr.2082.1.S1_at	70.53	91.69	49.70	69.71	At5g17570	7.00E-35	*TatD-related deoxyribonuclease family protein*
Mtr.34190.1.S1_at	75.81	59.18	97.47	54.19			Telomere binding protein TBP1
Mtr.12895.1.S1_at	828.96	750.00	1134.73	849.52			Tetratricopeptide repeat protein-like
Mtr.40572.1.S1_at	1256.70	881.42	1219.93	1016.25	At2g02180	4.00E-43	*Tobamovirus multiplication protein 3 (TOM3)*
Mtr.9771.1.S1_at	250.86	222.86	296.88	197.43	At5g14530	2.00E-33	*Transducin family protein*
Mtr.51660.1.S1_at	256.10	420.17	258.42	339.29	At5g07860	4.00E-12	Transferase
Mtr.7001.1.S1_at	82.70	126.25	59.13	144.84			Transmembrane GTPase
Mtr.30085.1.S1_at	73.03	64.41	105.14	58.35	At2g44520	2.00E-06	*UbiA prenyltransferase family protein*
Mtr.40991.1.S1_at	65.45	95.06	93.83	111.35	At1g05680	9.00E-39	UDP-glucosyltransferase
Mtr.35914.1.S1_at	237.77	235.98	253.99	167.63	At3g08790	3.00E-05	*VHS domain-containing protein*
Msa.1954.1.S1_at	50.78	70.80	54.72	62.14	At2g47260	1.00E-16	*WRKY family transcription factor*
Mtr.9902.1.S1_s_at	48.38	69.82	44.78	72.87	At4g26640	3.00E-13	*WRKY family transcription factor*
Mtr.12483.1.S1_at	49.23	95.56	44.24	127.38	At5g24110	6.00E-14	WRKY transcription factor 41
Mtr.42336.1.S1_at	35.45	48.82	56.81	76.76	At4g18170	3.00E-15	WRKY transcription factor 48
Mtr.43969.1.S1_s_at	82.35	94.14	50.90	91.61	At3g13810	7.00E-05	*Zinc finger (C2H2 type) family protein*
Mtr.24268.1.S1_s_at	354.37	311.66	407.05	262.46	At4g17910	3.00E-16	*Zinc finger (C3HC4-type RING finger) family protein*
Mtr.43697.1.S1_at	1471.82	1240.64	1534.20	1104.61	At2g23780	4.00E-14	*Zinc finger (C3HC4-type RING finger) family protein*
Mtr.7890.1.S1_at	402.14	347.57	467.58	363.59	At3g43700	2.00E-05	Zinc finger POZ domain protein
Mtr.19759.1.S1_at	55.81	38.82	59.03	32.38	At4g13010	3.00E-63	Zinc-containing alcohol dehydrogenase superfamily
Mtr.18387.1.S1_at	27.56	28.83	19.29	53.73			Zn-finger, C2H2 type
Msa.1828.1.S1_at	50.25	43.86	51.37	32.81			No annotation
Msa.2597.1.S1_at	934.57	1290.23	1135.85	1545.55			No annotation
Msa.2805.1.S1_at	1821.90	2384.95	1936.85	3136.82			No annotation
Msa.713.1.S1_at	40.46	34.71	53.17	30.10			No annotation

Table S2 (continued)

	Raw expression values in roots						
	Without caesium		With caesium				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Msa.812.1.S1_at	1353.02	1222.04	2100.22	1182.59			No annotation
Msa.832.1.S1_at	648.86	580.31	1080.63	615.02			No annotation
Mtr.10542.1.S1_at	1743.25	2586.34	1871.54	2538.95			No annotation
Mtr.10978.1.S1_at	2021.63	1674.02	2297.14	1647.24			No annotation
Mtr.11800.1.S1_at	735.41	642.91	927.37	659.24			No annotation
Mtr.12246.1.S1_at	4104.73	5240.34	4905.49	7300.46			No annotation
Mtr.12664.1.S1_at	48.27	95.92	76.17	88.25			No annotation
Mtr.1505.1.S1_at	40.11	58.62	29.50	55.29			No annotation
Mtr.1566.1.S1_at	82.29	130.67	46.53	68.92			No annotation
Mtr.19044.1.S1_at	61.43	45.67	53.42	43.08			No annotation
Mtr.26426.1.S1_at	74.03	75.15	98.56	55.52			No annotation
Mtr.26822.1.S1_at	313.74	297.95	410.53	198.35			No annotation
Mtr.26822.1.S1_s_at	438.50	360.83	536.44	313.93			No annotation
Mtr.27006.1.S1_at	314.70	196.95	299.02	208.83			No annotation
Mtr.27533.1.S1_s_at	807.83	608.99	1039.90	657.16			No annotation
Mtr.27673.1.S1_at	276.04	249.79	364.62	237.97			No annotation
Mtr.27688.1.S1_at	445.42	356.46	476.35	300.35			No annotation
Mtr.28744.1.S1_at	555.28	418.78	528.75	393.57			No annotation
Mtr.28748.1.S1_at	68.21	54.51	92.18	57.68			No annotation
Mtr.2932.1.S1_at	103.33	70.86	91.65	67.51			No annotation
Mtr.29657.1.S1_at	110.41	105.26	151.08	93.14			No annotation
Mtr.29708.1.S1_at	117.70	116.33	145.07	96.41			No annotation
Mtr.30102.1.S1_at	167.81	102.00	187.53	92.77			No annotation
Mtr.30611.1.S1_at	92.50	80.13	113.90	66.19			No annotation
Mtr.30651.1.S1_at	325.76	151.74	375.27	212.81			No annotation
Mtr.30654.1.S1_at	58.25	34.05	82.73	45.17			No annotation
Mtr.30655.1.S1_at	51.69	27.34	62.46	30.73			No annotation
Mtr.30866.1.S1_at	131.47	119.81	181.47	104.34			No annotation
Mtr.3154.1.S1_s_at	62.01	55.70	66.86	36.58			No annotation
Mtr.3216.1.S1_at	13985.36	9388.89	13790.28	10641.58			No annotation
Mtr.3229.1.S1_at	361.13	297.30	410.98	265.42			No annotation
Mtr.32877.1.S1_at	305.71	269.20	513.54	286.78			No annotation

Table S2 (continued)

	Raw expression values in roots						
	Without caesium		With caesium				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.3348.1.S1_at	514.15	378.18	556.56	307.83			No annotation
Mtr.3357.1.S1_at	99.71	89.86	134.79	77.53			No annotation
Mtr.3376.1.S1_at	52.71	32.78	66.60	42.06			No annotation
Mtr.33795.1.S1_at	134.98	75.39	100.06	80.52			No annotation
Mtr.33999.1.S1_at	313.37	172.35	243.87	185.45			No annotation
Mtr.3406.1.S1_at	72.61	55.87	95.49	50.33			No annotation
Mtr.3434.1.S1_at	156.70	126.31	212.56	102.02			No annotation
Mtr.35477.1.S1_at	66.78	56.24	79.00	37.97			No annotation
Mtr.3548.1.S1_at	51.97	50.66	89.30	55.50			No annotation
Mtr.35926.1.S1_at	296.32	259.46	270.96	171.78			No annotation
Mtr.37025.1.S1_a_at	48.26	29.10	51.42	28.04			No annotation
Mtr.37095.1.S1_at	805.98	514.35	724.98	510.47			No annotation
Mtr.37852.1.S1_at	27.21	31.06	12.44	140.91			No annotation
Mtr.38324.1.S1_at	548.65	450.79	568.32	396.89			No annotation
Mtr.39230.1.S1_at	486.47	409.09	573.46	397.99			No annotation
Mtr.39253.1.S1_at	98.69	56.24	83.21	74.39			No annotation
Mtr.39848.1.S1_at	74.20	58.19	90.92	59.07			No annotation
Mtr.40295.1.S1_at	196.61	207.34	214.12	291.91			No annotation
Mtr.42130.1.S1_at	751.94	640.37	827.63	620.35			No annotation
Mtr.42148.1.S1_at	121.88	93.40	159.93	119.01			No annotation
Mtr.4231.1.S1_at	203.13	142.69	240.50	152.56			No annotation
Mtr.4250.1.S1_at	103.31	89.91	123.58	51.87			No annotation
Mtr.42802.1.S1_at	495.81	388.58	545.52	385.93			No annotation
Mtr.4298.1.S1_at	61.26	49.97	80.91	52.42			No annotation
Mtr.43313.1.S1_at	541.66	605.85	611.19	1246.12			No annotation
Mtr.44042.1.S1_at	283.39	267.94	325.98	185.83			No annotation
Mtr.44470.1.S1_at	122.72	116.88	124.46	72.78			No annotation
Mtr.45600.1.S1_s_at	1182.43	954.38	1422.87	814.28			No annotation
Mtr.45601.1.S1_s_at	1797.63	1633.50	1917.63	1189.22			No annotation
Mtr.46577.1.S1_s_at	143.18	95.34	125.49	106.91			No annotation
Mtr.47257.1.S1_at	3585.76	3466.11	2591.85	5666.52			No annotation
Mtr.47257.1.S1_s_at	3698.02	3924.70	2608.69	5348.17			No annotation

Table S2 (continued)

	Raw expression values in roots						
	Without caesium		With caesium				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.4738.1.S1_at	53.79	89.13	76.85	114.31			No annotation
Mtr.4988.1.S1_at	362.23	239.21	407.51	247.29			No annotation
Mtr.7281.1.S1_at	111.74	94.05	142.08	96.86			No annotation
Mtr.7740.1.S1_at	88.53	65.69	93.24	50.59			No annotation
Mtr.8171.1.S1_at	170.06	120.75	239.65	150.90			No annotation
Mtr.8172.1.S1_at	651.69	372.82	907.47	615.65			No annotation
Mtr.8363.1.S1_at	443.93	247.49	483.21	293.24			No annotation
Mtr.9288.1.S1_at	102.87	79.88	116.06	63.47			No annotation
Mtr.9299.1.S1_at	79.04	48.52	81.82	52.48			No annotation
Mtr.9377.1.S1_at	63.38	68.20	35.89	110.33			No annotation
Mtr.9665.1.S1_at	44.91	48.16	37.86	58.16			No annotation
Mtr.9762.1.S1_at	432.96	341.07	533.41	367.42			No annotation

Table S3: Raw expression values of genes in roots of non mycorrhizal (non myc) and mycorrhizal (myc) *Medicago truncatula* plants that had been grown without or with caesium in the medium. The gene expression was significantly influenced by an interaction of Cs in the medium and arbuscular mycorrhiza (Analysis of Variance, $p < 0.05$). The Medicago probes refer to the *Medicago truncatula* probe name for the gene on the Affymetrix GeneChip® Medicago genome array and the AGI ID is the *Arabidopsis thaliana* gene identification. The annotation was based on local translated BLAST alignment. The MtGEA Target Description is the annotation for the gene obtained from the *Medicago truncatula* Gene Expression Atlas and the TAIR Target Description is the annotation of the *Arabidopsis thaliana* genes (*A. thaliana* annotations in italics).

	Raw expression values in roots						
	Without caesium		**With caesium**				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Msa.2547.1.S1_at	59.47	59.95	49.01	74.86	At2g33450	3.00E-27	*50S ribosomal protein L28, chloroplast*
Mtr.37444.1.S1_at	2715.69	2387.48	2404.21	3043.52	At1g77120	7.00E-15	Alcohol dehydrogenase 1
Mtr.1076.1.S1_at	975.82	766.34	502.11	858.05	At1g73590	1.00E-67	Auxin efflux carrier protein
Mtr.760.1.S1_s_at	144.89	230.56	200.71	111.87	At2g36210	4.00E-15	Auxin-induced protein family
Mtr.44128.1.S1_at	94.84	94.32	60.67	111.38	At3g07360	4.00E-32	Avr9/Cf-9 rapidly elicited protein-like / *Armadillo/beta-catenin repeat family protein*
Mtr.50730.1.S1_at	410.35	334.71	539.00	273.21			Basic-leucine zipper (bZIP) transcription factor
Mtr.17361.1.S1_at	822.43	758.75	349.96	1301.13	At5g03170	2.00E-30	Beta-Ig-H3/fasciclin
Mtr.50900.1.S1_at	866.09	779.54	433.79	1107.88	At5g60490	2.00E-24	Beta-Ig-H3/fasciclin
Mtr.40942.1.S1_at	847.99	905.87	736.96	1122.30	At4g34050	1.00E-15	Caffeoyl-CoA O-methyltransferase 5
Mtr.10823.1.S1_s_at	47.10	42.55	29.63	56.94	At3g56760	1.00E-66	Calcium-dependent protein kinase-like
Msa.888.1.S1_at	157.44	346.00	277.15	67.26	At3g22840	2.00E-45	*Chlorophyll A-B binding family protein*
Mtr.12569.1.S1_at	1418.52	1637.63	2256.26	1554.22	At1g64660	2.00E-45	*Cys/Met metabolism pyridoxal-phosphate-dependent enzyme family protein*
Mtr.12486.1.S1_at	67.28	82.99	80.35	29.51			Cystatin
Mtr.10175.1.S1_at	697.20	1069.19	944.26	721.06	At3g48290	4.00E-40	Cytochrome P450
Mtr.13153.1.S1_at	52.97	40.44	34.77	52.37	At3g14610	3.00E-34	*Cytochrome P450*
Mtr.13566.1.S1_at	595.12	802.82	566.55	379.30	At4g31950	2.00E-39	Cytochrome P450
Msa.1818.1.S1_at	287.89	257.68	256.54	375.52	At2g46950	8.00E-33	*Cytochrome P450 family protein*
Mtr.30372.1.S1_at	169.26	147.38	171.85	103.52	At3g11670	1.00E-06	Digalactosyldiacylglycerol synthase 1
Mtr.28166.1.S1_at	192.25	219.81	241.23	140.07			Dihydrolipoamide dehydrogenase
Mtr.50411.1.S1_at	92.59	75.84	68.45	142.13	At1g13635	9.00E-36	DNA glycosylase
Mtr.38994.1.S1_at	224.81	262.81	143.41	285.21	At1g69780	5.00E-34	DNA-binding protein
Mtr.34086.1.S1_at	227.21	212.82	289.69	188.30	At5g07710	6.00E-09	*Exonuclease family protein*
Mtr.14652.1.S1_at	1337.54	1464.53	1225.75	711.09	At2g12905	7.00E-15	*Expressed protein*
Mtr.27434.1.S1_at	60.30	62.47	45.11	34.27	At2g34160	2.00E-08	*Expressed protein*
Mtr.27849.1.S1_at	471.55	349.24	335.57	593.93	At5g24920	6.00E-06	*Expressed protein*
Mtr.33829.1.S1_at	214.07	223.85	164.93	365.90	At2g35880	3.00E-44	*Expressed protein*

Table S3 (continued)

	Raw expression values in roots						
	Without caesium		With caesium				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.42939.1.S1_at	1585.48	1557.37	2278.35	1376.81	At1g28135	1.00E-05	*Expressed protein*
Mtr.7227.1.S1_at	37.24	58.37	54.00	33.93	At1g17455	3.00E-37	*Expressed protein*
Mtr.9655.1.S1_at	148.63	187.10	153.66	123.92	At1g17210	3.00E-52	*Expressed protein*
Mtr.9904.1.S1_at	58.47	59.01	58.24	115.00	At5g37840	9.00E-09	*Expressed protein*
Mtr.13136.1.S1_at	646.32	585.86	293.13	929.21	At5g60490	1.00E-10	Fasciclin-like
Mtr.13136.1.S1_s_at	251.51	255.01	96.69	345.72	At5g60490	2.00E-33	Fasciclin-like
Mtr.9854.1.S1_at	93.00	83.22	64.13	113.32	At3g58790	7.00E-73	*Galacturonosyltransferase activity*
Mtr.40734.1.S1_at	619.85	953.11	904.05	507.62	At2g36800	6.00E-65	Glucosyltransferase-14
Mtr.40320.1.S1_at	1078.44	1105.25	1432.08	1107.12	At2g28110	2.00E-05	*Glycosyl transferase family*
Mtr.44590.1.S1_s_at	33.36	25.58	31.36	53.48	At2g37090	2.00E-44	Glycuronosyltransferase-like protein
Mtr.20183.1.S1_s_at	161.72	156.04	117.00	199.81	At1g80160	4.00E-34	Glyoxalase/extradiol ring-cleavage dioxygenase / *Lactoylglutathione lyase family protein*
Mtr.44959.1.S1_at	40.27	52.73	33.42	58.73			Guanine nucleotide releasing factor 1
Mtr.40057.1.S1_at	925.07	842.04	983.90	615.43	At5g09590	2.00E-34	Heat shock 70 kDa protein
Mtr.31518.1.S1_at	399.08	262.78	382.14	521.61			Hydrolase, alpha/beta fold family
Mtr.37437.1.S1_at	72.75	90.31	101.20	69.90			Hypersensitive-induced response protein
Msa.3150.1.S1_at	132.10	142.26	272.58	126.08	AtMg00030	7.00E-28	*Hypothetical protein*
Mtr.49328.1.S1_at	52.66	23.27	18.22	119.56			Hypothetical protein
Mtr.50277.1.S1_at	3690.40	3373.60	3570.32	4859.33			Hypothetical protein
Mtr.46631.1.S1_at	39.42	21.34	43.01	324.25	At5g27760	9.00E-14	*Hypoxia-responsive family protein*
Mtr.4424.1.S1_at	99.80	96.05	126.73	71.39	At1g49405	4.00E-22	*Integral membrane protein*
Mtr.8632.1.S1_s_at	1402.17	1518.20	1342.19	2404.39	At1g14890	7.00E-37	*Invertase/pectin methylesterase inhibitor family protein*
Mtr.35520.1.S1_at	178.50	271.70	357.21	307.02	At5g25930	2.00E-17	*Leucine-rich repeat family protein*
Mtr.11809.1.S1_at	336.74	261.16	243.30	445.70	At4g24120	8.00E-46	Metal-nicotinamine transporter / *Oligopeptide transporter OPT family protein*
Mtr.33467.1.S1_at	227.82	163.49	149.98	181.72			MLP-like protein 43
Mtr.33426.1.S1_at	54.11	67.29	62.15	33.72			MRP-like ABC transporter
Mtr.11148.1.S1_at	472.80	536.12	444.02	703.15			Multi resistance protein homolog
Mtr.9331.1.S1_at	196.64	270.27	193.71	203.18			MYB transcription factor
Mtr.14314.1.S1_at	73.25	51.74	37.62	100.42	At5g64700	2.00E-39	*Nodulin MtN21 family protein*
Mtr.22897.1.S1_at	269.03	217.60	212.18	271.25	At3g62110	4.00E-61	Polygalacturonase
Mtr.8212.1.S1_at	191.52	143.40	144.19	276.07	At1g18390	1.00E-11	Protein kinase
Mtr.45613.1.S1_s_at	1800.68	2289.61	2018.41	1172.16	AtCg00700	7.00E-15	*PSII low MW protein*
Mtr.38555.1.S1_at	171.72	122.50	90.71	281.59	At1g17250	1.00E-11	Receptor-kinase like protein / *Leucine-rich repeat family protein*

Table S3 (continued)

	Raw expression values in roots						
	Without caesium		With caesium				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.42037.1.S1_at	209.63	176.34	111.29	184.69	At5g16000	8.00E-46	Receptor-kinase like protein / *Leucine-rich repeat family protein*
Mtr.48952.1.S1_at	50.69	43.41	46.61	148.55			Rhodanese-like
Mtr.48952.1.S1_s_at	16.79	16.34	13.73	58.32	At5g66170	1.00E-35	Rhodanese-like / *Senescence-associated family protein*
Mtr.50074.1.S1_at	22.51	10.11	13.15	67.98			RmlC-like cupin
Mtr.11185.1.S1_at	42.70	46.58	36.27	58.37	At1g10760	4.00E-71	Starch excess protein (SEX1)
Mtr.27746.1.S1_at	41.40	59.19	42.82	30.14			SUPERMAN like protein
Mtr.3548.1.S1_at	51.97	50.66	89.30	55.50	At5g51910	2.00E-04	*TCP family transcription factor*
Mtr.30085.1.S1_at	73.03	64.41	105.14	58.35	At2g44520	2.00E-06	*UbiA prenyltransferase family protein*
Mtr.8345.1.S1_at	44.80	50.21	65.41	40.31	At4g17895	1.00E-18	*Ubiquitin-specific protease 20*
Mtr.37847.1.S1_at	60.25	19.98	65.70	398.81	At1g22370	7.00E-29	UDP-glycosyltransferase 85A8
Mtr.35914.1.S1_at	237.77	235.98	253.99	167.63	At3g08790	3.00E-05	*VHS domain-containing protein*
Mtr.13171.1.S1_at	226.97	116.70	139.72	443.48	At4g10270	2.00E-06	*Wound-responsive family protein*
Mtr.7286.1.S1_at	65.01	54.95	35.78	66.74	At1g72220	7.00E-44	*Zinc finger (C3HC4-type RING finger) family protein*
Mtr.18387.1.S1_at	27.56	28.83	19.29	53.73			Zn-finger, C2H2 type
Msa.2945.1.S1_at	770.00	840.79	1163.44	738.05			No annotation
Msa.713.1.S1_at	40.46	34.71	53.17	30.10			No annotation
Msa.812.1.S1_at	1353.02	1222.04	2100.22	1182.59			No annotation
Mtr.26426.1.S1_at	74.03	75.15	98.56	55.52			No annotation
Mtr.26822.1.S1_at	313.74	297.95	410.53	198.35			No annotation
Mtr.27252.1.S1_at	60.75	65.13	63.99	44.61			No annotation
Mtr.28657.1.S1_s_at	44.77	35.60	29.54	56.24			No annotation
Mtr.29657.1.S1_at	110.41	105.26	151.08	93.14			No annotation
Mtr.29708.1.S1_at	117.70	116.33	145.07	96.41			No annotation
Mtr.30866.1.S1_at	131.47	119.81	181.47	104.34			No annotation
Mtr.32877.1.S1_at	305.71	269.20	513.54	286.78			No annotation
Mtr.3434.1.S1_at	156.70	126.31	212.56	102.02			No annotation
Mtr.36065.1.S1_at	104.29	79.59	76.57	150.24			No annotation
Mtr.39253.1.S1_at	98.69	56.24	83.21	74.39			No annotation
Mtr.45041.1.S1_at	49.29	28.07	18.74	49.58			No annotation
Mtr.47257.1.S1_at	3585.76	3466.11	2591.85	5666.52			No annotation
Mtr.47257.1.S1_s_at	3698.02	3924.70	2608.69	5348.17			No annotation
Mtr.8264.1.S1_at	69.62	100.38	104.49	48.66			No annotation

Table S3 (continued)

	Raw expression values in roots						
	Without caesium		With caesium				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.8273.1.S1_at	919.68	1540.81	1181.57	952.08			No annotation
Mtr.9377.1.S1_at	63.38	68.20	35.89	110.33			No annotation

Table S4: Raw expression values of genes in shoots of non mycorrhizal (non myc) and mycorrhizal (myc) *Medicago truncatula* plants that had been grown without or with caesium in the medium. The gene expression was significantly influenced by the presence of Cs in the medium (Analysis of Variance, $p < 0.05$). The *Medicago* probes refer to the *Medicago truncatula* or *M. sativa* probe name for the gene on the Affymetrix GeneChip® Medicago genome array and the AGI ID is the *Arabidopsis thaliana* gene identification. The annotation was based on local translated BLAST alignment. The MtGEA Target Description is the annotation for the gene obtained from the *Medicago truncatula* Gene Expression Atlas and the TAIR Target Description is the annotation of the *Arabidopsis thaliana* genes (*A. thaliana* annotations in italics).

	Raw expression values in shoots							
	Without caesium		**With caesium**					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.5963.1.S1_at	1088.60	1600.45	946.83	893.39				AAA-type ATPase-like protein
Mtr.40695.1.S1_s_at	804.45	651.33	499.56	333.43	At1g65890	AAE12	5.00E-26	*Acyl-activating enzyme 12 (AAE12)*
Mtr.43580.1.S1_at	16.87	77.22	41.24	10.79	At1g20560		2.00E-05	Adenosine monophosphate binding protein 1
Mtr.14426.1.S1_at	66.91	53.47	29.11	29.55	At1g59960		2.00E-14	Aldo/keto reductase
Mtr.45133.1.S1_at	76.05	84.85	147.44	90.22	At4g12270		6.00E-62	Amine oxidase
Msa.1814.1.S1_at	1294.02	1270.42	664.43	1416.05	At1g35720	ANNAT1	2.00E-62	*Annexin gene family*
Mtr.40663.1.S1_at	240.33	463.71	268.15	213.90	At1g31280		2.00E-08	*Argonaute genePAZ domain-containing protein*
Mtr.21172.1.S1_at	125.83	144.65	194.23	174.13	At4g11400		2.00E-40	AT-rich interaction region
Mtr.43097.1.S1_at	411.64	917.54	568.35	1551.85	At5g18670		2.00E-21	Beta-amylase
Mtr.13752.1.S1_at	521.34	338.22	360.08	349.00	At4g33000	CBL10	5.00E-22	Calcineurin B-like protein 10
Mtr.38079.1.S1_at	32.25	64.22	27.59	15.58	At2g36220		6.00E-13	Calcium/calmodulin protein kinase 1
Mtr.9580.1.S1_at	73.61	161.90	52.23	64.94	At5g64220		2.00E-18	*Calmodulin-binding protein*
Mtr.10055.1.S1_at	362.76	453.23	256.47	226.68				cAMP-dependent protein kinase type 3
Mtr.35147.1.S1_s_at	426.65	300.62	262.99	92.86	At1g11190	BFN1	3.00E-12	CEL I mismatch endonuclease
Mtr.38029.1.S1_at	6385.65	4632.52	5149.92	4257.20	At1g75350	emb2184	1.00E-40	Chloroplast 50S ribosomal protein L31-like
Mtr.33424.1.S1_s_at	104.74	130.71	97.92	63.94				Condensin complex component
Mtr.2712.1.S1_at	1089.24	722.40	825.60	514.41				Cyclin delta-3
Mtr.1456.1.S1_at	191.31	272.83	169.67	210.09	At2g31270	CDT1A	4.00E-07	*Cyclin-dependent protein kinase*
Mtr.11364.1.S1_at	100.04	281.77	56.63	52.29	At2g46960	CYP709B1	1.00E-41	Cytochrome P450
Msa.1818.1.S1_at	266.81	647.07	178.64	144.63	At2g46950	CYP709B2	8.00E-33	*Cytochrome P450 family protein*
Mtr.38799.1.S1_s_at	331.78	377.78	438.70	81.63	At2g41510	CKX1	1.00E-33	Cytokinin oxidase-like protein
Mtr.44219.1.S1_at	76.90	73.09	69.92	18.41	At3g63440	CKX6	6.00E-64	Cytokinin oxidase-like protein
Mtr.43949.1.S1_at	198.58	227.38	295.68	215.22	At2g26830	emb1187	8.00E-23	Ethanolamine kinase
Mtr.13061.1.S1_at	396.67	271.33	246.18	224.17	At1g11440		1.00E-09	*Expressed protein*
Mtr.18422.1.S1_at	108.78	178.68	106.95	24.25	At5g04550		2.00E-33	*Expressed protein*
Mtr.24811.1.S1_at	84.68	95.04	129.48	107.74	At2g26200		6.00E-34	*Expressed protein*

Table S4 (continued)

	Raw expression values in shoots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.27397.1.S1_at	647.94	368.75	389.74	337.54	At2g04360		1.00E-49	*Expressed protein*
Mtr.38228.1.S1_at	443.99	816.74	596.01	1042.04	At5g59050		5.00E-06	*Expressed protein*
Mtr.38291.1.S1_at	1909.86	2395.71	1393.44	1812.16	At2g46490		4.00E-12	*Expressed protein*
Mtr.38672.1.S1_at	1334.26	857.17	908.73	724.39	At1g51100		2.00E-26	*Expressed protein*
Mtr.40792.1.S1_at	245.52	346.55	264.85	168.99	At1g53380		4.00E-42	*Expressed protein*
Mtr.41288.1.S1_at	793.29	532.83	591.05	492.23	At3g23760		8.00E-70	*Expressed protein*
Mtr.27793.1.S1_s_at	336.37	187.29	190.48	184.09				Galactosidase like protein
Mtr.43170.1.S1_s_at	135.18	144.46	251.13	208.15	At5g18170	GDH1	2.00E-36	Glutamate dehydrogenase 1
Mtr.12412.1.S1_at	389.71	471.23	724.63	620.03	At5g18170		3.00E-28	Glutamate dehydrogenase 1
Mtr.6324.1.S1_at	50.52	44.90	32.50	27.63	At5g27100		2.00E-39	Glutamate receptor 2.4 precursor
Mtr.43300.1.S1_at	2653.44	1836.02	2124.23	1750.79	At4g28730		1.00E-26	Glutaredoxin protein family-like
Mtr.13661.1.S1_at	423.88	354.28	303.24	254.08	At1g66970	SVL2	7.00E-38	Glycerophosphoryl diester phosphodiesterase 2 precursor
Msa.2885.1.S1_at	9641.88	6826.59	7143.95	7008.88	At1g32470		2.00E-43	*Glycine cleavage system H protein*
Mtr.8969.1.S1_at	284.44	187.88	137.62	160.79	At2g27500		3.00E-56	*Glycosyl hydrolase family 17 protein*
Mtr.37156.1.S1_at	295.19	344.73	226.57	247.87	At5g05460		4.00E-24	*Glycosyl hydrolase family 85 protein*
Mtr.37584.1.S1_at	1706.10	1411.62	1262.97	1164.88	At3g11660		5.00E-25	*Harpin-induced family protein*
Mtr.50930.1.S1_at	88.17	31.68	23.79	20.21	At3g50330		1.00E-32	Helix-loop-helix DNA-binding
Mtr.19029.1.S1_s_at	187.54	247.27	178.91	182.86				Hypothetical protein
Mtr.17966.1.S1_at	43.46	72.66	24.85	23.29				Leucine-rich repeat
Mtr.7591.1.S1_at	54.00	106.21	71.41	25.43	At4g29880		3.00E-23	*Leucine-rich repeat family protein*
Mtr.35681.1.S1_at	218.33	141.32	102.79	117.28				Metal-dependent amidase/aminoacylase/carboxypeptidase
Mtr.44870.1.S1_at	536.88	799.49	358.84	345.81				MLO-like protein 10
Mtr.13060.1.S1_at	169.52	212.29	133.36	93.65	At2g35660	CTF2A	3.00E-68	*Monooxygenase family protein*
Mtr.17230.1.S1_at	19.19	60.12	34.35	11.13	At3g17630		2.00E-16	Na+/H+ antiporter-like protein
Mtr.2375.1.S1_at	26.87	64.65	33.02	13.33				Neuraminidase B
Mtr.6666.1.S1_at	245.10	405.29	190.05	301.85	At2g39510		4.00E-04	*Nodulin MtN21 family protein*
Mtr.27937.1.S1_at	185.59	291.56	124.36	206.92	At1g68170		1.00E-28	Nodulin-like protein
Msa.2628.1.S1_at	153.17	93.69	94.52	93.25	At4g33490		9.00E-57	*Nucellin protein*
Mtr.37374.1.S1_at	2005.63	1739.91	1514.85	1107.02	At4g37050	PLP4	3.00E-12	Patatin-like protein 1
Mtr.50768.1.S1_at	284.69	147.97	101.02	119.88	At2g14095		2.00E-13	Peptidase A11B
Mtr.39868.1.S1_at	35.33	54.20	55.05	56.01	At1g06900		8.00E-61	*Peptidase M16 family protein*
Mtr.9748.1.S1_at	641.12	370.38	487.21	308.78	At3g15520		1.00E-73	Peptidyl-prolyl cis-trans isomerase

Table S4 (continued)

Medicago probes	Raw expression values in shoots: Without caesium Non myc	Without caesium Myc	With caesium Non myc	With caesium Myc	AGI ID	Gene name	e-value	MtGEA Target Description / *TAIR Target Description*
Mtr.22013.1.S1_s_at	2330.46	897.88	1141.31	723.26	At3g26060	ATPRX Q	2.00E-19	Peroxiredoxin Q
Mtr.6484.1.S1_at	951.23	1824.33	978.62	360.21	At2g32830		3.00E-05	Phosphate transporter 2
Mtr.38209.1.S1_at	1276.40	928.65	676.55	1357.58				Polysaccharide ABC transporter
Mtr.51989.1.S1_s_at	34.68	60.10	39.86	23.84	At2g26650		7.00E-34	Potassium channel
Mtr.43279.1.S1_at	112.49	314.91	353.10	290.95	At3g30775	ERD5	3.00E-50	Proline dehydrogenase
Mtr.39238.1.S1_at	2590.93	909.95	1058.60	1101.40	At3g16370		1.00E-61	Proline-rich protein APG-like / *GDSL-motif lipase*
Msa.1037.1.S1_at	67.85	90.12	110.33	104.41	At1g07870		1.00E-93	*Protein kinase family protein*
Mtr.5291.1.S1_at	336.23	120.57	178.32	127.01	At1g45207		2.00E-15	*Remorin family protein*
Mtr.48460.1.S1_at	193.23	202.62	253.77	213.28	At3g12280		2.00E-26	Retinoblastoma-related protein
Mtr.19517.1.S1_at	15702.92	10707.55	11264.72	11010.34	At5g38410		8.00E-05	Ribulose bisphosphate carboxylase
Mtr.40987.1.S1_at	3522.41	2203.64	2463.78	2136.79	At1g71500		2.00E-30	*Rieske (2Fe-2S) domain-containing protein*
Mtr.39708.1.S1_at	1375.93	975.38	681.40	694.15				Serine/threonine kinase protein-like
Mtr.38550.1.S1_at	36.73	40.30	83.01	52.65	At4g34980	SLP2	4.00E-67	Subtilisin-like proteinase
Mtr.38540.1.S1_at	55.61	39.75	16.58	22.27	At5g23960	TPS21	3.00E-28	Terpene synthase
Mtr.12493.1.S1_at	1552.23	947.53	1179.10	877.06	At2g41680	NTRC	1.00E-29	*Thioredoxin reductase*
Mtr.15318.1.S1_at	1077.39	660.85	853.11	676.67	At1g76760	ATY1	6.00E-47	Thioredoxin-related
Mtr.33389.1.S1_at	99.60	145.63	138.16	145.15	At1g16800		2.00E-05	*tRNA-splicing endonuclease positive effector-related*
Mtr.45215.1.S1_at	338.16	581.87	407.21	265.72	At2g41560	ACA4	4.00E-22	Type IIB calcium ATPase
Mtr.5950.1.S1_s_at	1786.03	1715.98	1102.05	1046.52	At5g25560		7.00E-75	Zinc finger protein
Mtr.10267.1.S1_at	606.77	500.91	472.51	441.97				No annotation
Mtr.12664.1.S1_at	68.04	88.48	46.23	36.53				No annotation
Mtr.12886.1.S1_at	1078.70	742.55	739.84	662.97				No annotation
Mtr.27107.1.S1_at	170.88	115.89	62.07	97.66				No annotation
Mtr.28477.1.S1_at	127.11	110.32	49.25	95.74				No annotation
Mtr.35562.1.S1_at	31.79	90.32	20.38	16.43				No annotation
Mtr.38803.1.S1_at	401.78	638.75	371.01	226.27				No annotation
Mtr.40695.1.S1_at	797.41	733.40	618.62	375.50				No annotation
Mtr.4597.1.S1_at	651.51	1066.40	644.58	588.16				No annotation
Mtr.9377.1.S1_at	156.59	119.42	77.07	139.70				No annotation
Mtr.9483.1.S1_at	1927.79	1136.32	1293.89	1137.47				No annotation

Table S5: Raw expression values of genes in shoots of non mycorrhizal (non myc) and mycorrhizal (myc) *Medicago truncatula* plants that had been grown without or with caesium in the medium. The gene expression was significantly influenced by arbuscular mycorrhiza (Analysis of Variance, $p < 0.05$). The *Medicago* probes refer to the *Medicago truncatula* or *M. sativa* probe name for the gene on the Affymetrix GeneChip® Medicago genome array and the AGI ID is the *Arabidopsis thaliana* gene identification. The annotation was based on local translated BLAST alignment. The MtGEA Target Description is the annotation for the gene obtained from the *Medicago truncatula* Gene Expression Atlas and the TAIR Target Description is the annotation of the *Arabidopsis thaliana* genes (*A. thaliana* annotations in italics).

	Raw expression values in shoots							
	Without caesium		**With caesium**					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Msa.1080.1.S1_at	1354.81	598.63	763.21	565.12	At3g11630		3.00E-54	*2-cys peroxiredoxin*
Msa.1307.1.S1_at	1650.66	770.01	973.01	707.75	At3g11630		3.00E-59	*2-cys peroxiredoxin*
Mtr.12260.1.S1_at	8371.31	4872.31	6268.22	4830.64	At5g06290	2-Cys Prx B	1.00E-72	2-Cys peroxiredoxin
Mtr.25633.1.S1_at	5478.64	2830.84	3712.58	2799.43	At5g06290	2-Cys Prx B	4.00E-106	2-Cys peroxiredoxin
Msa.2517.1.S1_at	962.86	656.23	818.05	751.63	At4g33510	DHS2	1.00E-80	*2-dehydro-3-deoxyphosphoheptonate aldolase 2*
Mtr.8920.1.S1_at	205.07	152.57	256.02	164.90	At5g64290		2.00E-31	2-oxoglutarate/malate translocator
Mtr.28252.1.S1_at	48.02	57.65	33.38	58.68	At1g06620		7.00E-40	*2-oxoglutarate-dependent dioxygenase*
Mtr.13458.1.S1_at	177.44	95.28	161.92	99.78	At4g05090		9.00E-59	3(2),5-Bisphosphate nucleotidase-like protein
Mtr.34741.1.S1_at	341.03	244.57	324.82	224.87	At4g05090		2.00E-44	3(2),5-Bisphosphate nucleotidase-like protein
Mtr.13096.1.S1_at	1199.82	654.15	815.60	591.15	At5g18660	PCB2	8.00E-69	*3,8-divinyl protochlorophyllide a 8-vinyl reductase*
Mtr.41249.1.S1_at	3778.88	1990.81	2407.71	2061.38	At5g14320		3.00E-59	30S ribosomal protein S13
Mtr.9095.1.S1_at	5385.81	3278.53	3900.41	3509.05	At1g74970	RPS9	4.00E-71	30S ribosomal protein S9
Mtr.11893.1.S1_at	825.10	481.23	588.69	460.27	At2g02500	ISPD	5.00E-74	4-Diphosphocytidyl-2C-methyl-D-erythritol synthase
Mtr.16367.1.S1_at	27.10	41.90	21.70	47.37				AAA ATPase
Mtr.21293.1.S1_at	121.41	88.53	106.55	68.19	At2g14750	APK1	4.00E-19	AAA ATPase / *APS kinaseadenylylsulfate kinase*
Mtr.9795.1.S1_at	886.96	555.79	660.76	643.62	At1g17840	WBC11	6.00E-79	ABC transporter
Mtr.8752.1.S1_at	956.13	353.48	537.85	532.81	At3g51440		6.00E-21	ABC transporter permease protein / *Strictosidine synthase family protein*
Mtr.4028.1.S1_at	762.71	384.26	697.12	374.47	At2g36840		5.00E-39	*ACT domain-containing protein*
Mtr.9843.1.S1_at	598.48	245.46	414.46	253.41	At2g36840		2.00E-73	*ACT domain-containing protein*
Mtr.37684.1.S1_at	1790.66	2393.69	1735.11	2659.42	At2g16700	ADF5	4.00E-21	Actin-depolymerizing factor 5
Mtr.40695.1.S1_s_at	804.45	651.33	499.56	333.43	At1g65890	AAE12	5.00E-26	*Acyl-activating enzyme 12 (AAE12)*
Mtr.45535.1.S1_at	127.66	73.15	88.78	70.54				Adenylyl-sulfate kinase
Mtr.45535.1.S1_s_at	162.83	123.57	134.05	101.97	At2g14750	APK1	1.00E-05	Adenylyl-sulfate kinase
Mtr.19660.1.S1_at	59.45	44.49	69.59	42.79	At4g32440		1.00E-14	*Agenet domain-containing protein*
Mtr.18939.1.S1_at	23.85	35.24	20.12	74.57	At1g25530		1.00E-35	*Amino acid/polyamine transporter II*
Msa.1814.1.S1_at	1294.02	1270.42	664.43	1416.05	At1g35720	ANNAT1	2.00E-62	*Annexin gene family*

Table S5 (continued)

	Raw expression values in shoots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.37757.1.S1_at	1763.67	2147.85	1299.98	3037.87	At5g01210		1.00E-52	Anthranilate N-benzoyltransferase-like protein
Msa.1751.1.S1_at	371.05	87.44	101.02	105.90	At4g18910		6.00E-41	*Aquaglyceroporin / NOD26-like major intrinsic protein 2*
Mtr.40663.1.S1_at	240.33	463.71	268.15	213.90	At1g31280		2.00E-08	*Argonaute gene PAZ domain-containing protein*
Mtr.12736.1.S1_at	2207.80	999.02	1500.74	1262.31	At4g09010	APX4	1.00E-54	*Ascorbate peroxidase*
Mtr.32351.1.S1_s_at	2429.71	940.48	1420.90	1257.92	At4g09010	APX4	1.00E-15	*Ascorbate peroxidase APX4*
Mtr.7842.1.S1_at	4457.73	2374.83	3550.73	2903.71	At4g09010	APX4	3.00E-19	*Ascorbate peroxidase APX4*
Msa.2600.1.S1_at	55.37	172.19	83.86	168.56	At1g66180		4.00E-35	*Aspartyl protease family protein*
Mtr.41053.1.S1_at	304.96	627.05	370.89	621.29	At5g49700		7.00E-05	AT-hook DNA-binding protein
Mtr.20763.1.S1_s_at	406.38	213.11	379.58	203.72	At1g19920	APS2	3.00E-17	ATP sulfurylase-related
Mtr.45729.1.S1_s_at	780.56	405.54	667.71	376.96	At1g19920	APS2	6.00E-37	ATP sulfurylase-related
Mtr.41682.1.S1_at	775.18	509.33	636.22	505.55	At1g32500	ATNAP6	3.00E-33	*ATP-binding-cassette transporter*
Mtr.10708.1.S1_at	2921.53	2022.93	2409.02	2179.02	At1g12410		2.00E-44	ATP-dependent Clp proteinase catalytic chain
Mtr.25341.1.S1_at	79.44	21.84	30.80	25.63	At2g17500		4.00E-51	*Auxin efflux carrier family protein*
Mtr.25341.1.S1_x_at	55.19	14.07	21.10	16.49	At2g17500		4.00E-51	*Auxin efflux carrier family protein*
Mtr.10432.1.S1_at	2451.49	2563.94	1853.53	3258.57				Auxin-induced protein 22B
Mtr.25939.1.S1_at	301.24	266.66	99.79	458.41	At4g38840		4.00E-25	Auxin-induced protein 6B
Mtr.10431.1.S1_at	733.77	802.60	607.70	972.04	At1g04240	SHY2	2.00E-31	Auxin-induced protein IAA4
Mtr.702.1.S1_at	186.19	155.61	82.49	237.71	At4g38840		8.00E-26	Auxin-induced protein X10A
Mtr.705.1.S1_x_at	69.86	48.49	14.86	100.83	At4g38840		4.00E-12	Auxin-induced protein X10A
Mtr.10147.1.S1_at	133.02	200.31	19.37	184.89	At4g34760		5.00E-16	Auxin-induced SAUR-like protein
Mtr.10701.1.S1_at	451.07	897.12	365.71	1292.48				Axi 1 protein-like protein
Mtr.21519.1.S1_at	75.47	73.28	93.23	72.70	At2g32590		3.00E-47	*Barren family protein*
Mtr.44005.1.S1_at	11.85	48.31	14.91	53.61	At5g65640	bHLH093	1.00E-54	*Basic helix-loop-helix (bHLH) family protein*
Mtr.14223.1.S1_at	385.83	278.31	304.98	203.14	At2g42300		3.00E-54	Basic helix-loop-helix dimerisation region bHLH
Mtr.12848.1.S1_at	268.99	410.14	267.96	572.10	At5g44380		4.00E-20	Berberine bridge enzyme-like protein / *FAD-binding domain*
Msa.1635.1.S1_at	987.35	1380.65	604.93	1607.67	At1g70850		1.00E-15	*Bet v I allergen family protein*
Mtr.43097.1.S1_at	411.64	917.54	568.35	1551.85	At5g18670	BMY3	2.00E-21	Beta-amylase
Mtr.13426.1.S1_at	303.27	426.37	368.67	429.41	At5g15870		6.00E-55	Beta-glucan-elicitor receptor
Mtr.44504.1.S1_at	61.37	135.05	53.71	127.19	At5g15870		1.00E-16	Beta-glucan-elicitor receptor
Mtr.12519.1.S1_at	1283.15	773.64	909.95	791.51	At2g26640	KCS11	1.00E-15	*beta-ketoacyl-CoA synthase*
Mtr.27641.1.S1_at	93.94	468.69	227.06	491.71	At2g30600		4.00E-08	*BTB/POZ domain-containing protein*
Mtr.37023.1.S1_s_at	4458.05	8347.50	5946.90	8476.21	At2g30600		2.00E-20	*BTB/POZ domain-containing protein*

Table S5 (continued)

	Raw expression values in shoots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.40801.1.S1_at	1142.42	3961.57	2172.47	4107.60	At2g30600		2.00E-27	*BTB/POZ domain-containing protein*
Mtr.8953.1.S1_s_at	73.31	36.46	60.53	56.98	At2g46270		1.00E-25	BZip transcription factor / *G-box binding factor 3*
Mtr.2631.1.S1_at	840.82	394.93	728.59	282.00	At5g54160	ATOMT1	1.00E-37	Caffeic acid O-methyltransferase II
Mtr.13752.1.S1_at	521.34	338.22	360.08	349.00	At4g33000	CBL10	5.00E-22	Calcineurin B-like protein 10
Mtr.38175.1.S1_at	784.06	382.67	570.72	340.28	At5g62070		4.00E-18	*Calmodulin-binding family protein*
Mtr.41220.1.S1_at	35.87	59.34	27.20	66.43	At2g24300		2.00E-71	*Calmodulin-binding protein*
Mtr.9580.1.S1_at	73.61	161.90	52.23	64.94	At5g64220		2.00E-18	*Calmodulin-binding protein*
Mtr.2092.1.S1_at	222.47	56.33	95.25	25.18				Carbonic anhydrase
Mtr.11409.1.S1_at	498.81	349.24	390.63	165.04	At1g11190	BFN1	5.00E-26	CEL I mismatch endonuclease
Mtr.35147.1.S1_s_at	426.65	300.62	262.99	92.86	At1g11190		3.00E-12	CEL I mismatch endonuclease
Mtr.43442.1.S1_at	2624.07	1438.74	2115.82	1486.19	At1g75690		1.00E-51	*Chaperone protein dnaJ-related*
Mtr.51755.1.S1_at	115.12	162.89	122.30	131.26	At1g23100		1.00E-14	Chaperonin
Mtr.13324.1.S1_at	2844.23	1456.83	1804.05	1404.28	At1g19150	LHCA6	1.00E-94	*Chlorophyll A-B binding protein*
Mtr.38029.1.S1_at	6385.65	4632.52	5149.92	4257.20	At1g75350		1.00E-40	Chloroplast 50S ribosomal protein L31-like
Mtr.13460.1.S1_at	3808.95	2182.44	2513.34	2052.32	At5g13510		6.00E-54	Chloroplast ribosomal protein L10
Mtr.35138.1.S1_at	1071.72	741.85	892.70	737.17	At2g47450	CAO	1.00E-53	*Chloroplast signal recognition particle component (CAO)*
Mtr.27628.1.S1_at	102.61	191.86	102.07	241.97	At4g27430	CIP7	7.00E-15	*COP1-interacting protein 7 (CIP7)*
Mtr.2712.1.S1_at	1089.24	722.40	825.60	514.41				Cyclin delta-3
Mtr.1456.1.S1_at	191.31	272.83	169.67	210.09	At2g31270	CDT1A	4.00E-07	*Cyclin-dependent protein kinase*
Mtr.21518.1.S1_at	926.46	410.60	660.69	463.45	At4g15440	HPL1	1.00E-10	Cytochrome P450 / *Hydroperoxide lyase (HPL1)*
Mtr.21518.1.S1_s_at	2347.25	1120.10	1944.24	1459.66	At4g15440		2.00E-56	Cytochrome P450 / *Hydroperoxide lyase (HPL1)*
Mtr.38799.1.S1_at	189.77	295.07	337.35	58.81				Cytokinin oxidase-like protein
Mtr.38799.1.S1_s_at	331.78	377.78	438.70	81.63	At2g41510	CKX1	1.00E-33	Cytokinin oxidase-like protein
Mtr.51672.1.S1_at	2577.22	1221.79	1767.00	1292.47	At1g69740		1.00E-88	Delta-aminolevulinic acid dehydratase / *Porphobilinogen synthase*
Mtr.33588.1.S1_at	37.48	46.26	32.41	61.41	At3g04220		2.00E-08	*Disease resistance protein*
Mtr.31300.1.S1_at	720.11	457.74	624.77	501.71	At1g65870		4.00E-41	Disease resistance response protein
Mtr.45367.1.S1_at	70.87	122.11	71.37	126.62				DNA binding with one finger 7 protein
Mtr.9768.1.S1_at	116.05	124.03	97.08	159.54	At2g31970	RAD50	9.00E-28	*DNA repair-recombination protein*
Mtr.1977.1.S1_at	210.00	132.94	157.60	158.41	At1g76880		9.00E-05	DNA-binding protein DF1
Mtr.39563.1.S1_at	79.61	96.30	76.89	119.88	At3g55560	AGF2	1.00E-15	*DNA-binding protein-related*
Mtr.24891.1.S1_at	360.70	816.18	245.46	636.62	At2g47440		2.00E-38	DnaJ domain-containing protein
Mtr.38240.1.S1_s_at	21.85	46.86	21.29	53.71	At1g01250		5.00E-09	*DREB subfamily*

Table S5 (continued)

	Raw expression values in shoots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.10192.1.S1_at	493.54	187.47	326.35	232.00	At1g01250		1.00E-36	DREB-like protein
Mtr.32790.1.S1_at	613.63	710.97	484.73	1469.82	At1g54070		2.00E-11	Drm3
Mtr.32790.1.S1_s_at	780.37	1014.86	626.59	1649.61				Drm3
Mtr.44506.1.S1_x_at	283.21	444.26	281.03	436.22	At3g57240	BG3	2.00E-32	Endo-1,3-beta-glucanase
Msa.3121.1.S1_at	174.01	803.78	257.47	1562.87	At5g61590		2.00E-31	*ERF (ethylene response factor) subfamily*
Mtr.11873.1.S1_at	66.06	88.10	54.00	138.29	At3g51770		7.00E-73	Ethylene-overproduction protein / *Tetratricopeptide repeat (TPR)*
Msa.1766.1.S1_at	2698.36	1605.64	1850.29	1662.08	At3g61870		1.00E-15	*Expressed protein*
Msa.989.1.S1_s_at	821.60	458.36	591.49	469.99	At3g61870		3.00E-62	*Expressed protein*
Mtr.1101.1.S1_s_at	32.29	53.95	43.84	54.46	At1g13120	emb1745	3.00E-31	*Expressed protein*
Mtr.11829.1.S1_at	497.28	343.62	461.95	358.77	At1g10660		1.00E-46	*Expressed protein*
Mtr.12188.1.S1_at	352.01	173.31	184.48	161.82	At2g04360		1.00E-10	*Expressed protein*
Mtr.12227.1.S1_s_at	66.47	143.47	102.40	165.20	At3g29575		2.00E-30	Expressed protein
Mtr.12418.1.S1_at	1627.06	1024.49	1386.10	778.60	At1g16080		1.00E-36	*Expressed protein*
Mtr.13061.1.S1_at	396.67	271.33	246.18	224.17	At1g11440		1.00E-09	*Expressed protein*
Mtr.13441.1.S1_at	218.55	171.66	216.24	131.12	At1g28510		1.00E-46	*Expressed protein*
Mtr.14799.1.S1_at	343.18	209.37	251.66	199.53	At5g63050		9.00E-28	*Expressed protein*
Mtr.15457.1.S1_at	34.71	46.13	28.85	66.98	At2g47480		3.00E-17	*Expressed protein*
Mtr.18237.1.S1_at	5688.91	3648.98	4094.88	3644.36	At3g61870		3.00E-74	*Expressed protein*
Mtr.21922.1.S1_at	91.31	70.97	105.02	72.78	At3g18295		9.00E-14	Expressed protein
Mtr.23325.1.S1_at	65.75	32.08	53.90	33.69	At1g02700		2.00E-31	*Expressed protein*
Mtr.24755.1.S1_at	165.95	142.70	195.68	70.36	At1g52565		5.00E-06	Expressed protein
Mtr.25425.1.S1_at	208.04	103.28	123.81	110.88	At5g03120		6.00E-13	*Expressed protein*
Mtr.25634.1.S1_at	112.95	68.25	98.08	81.91	At3g52520		4.00E-10	Expressed protein
Mtr.27068.1.S1_at	103.32	156.19	55.18	203.76	At5g14090		3.00E-25	*Expressed protein*
Mtr.27397.1.S1_at	647.94	368.75	389.74	337.54	At2g04360		1.00E-49	*Expressed protein*
Mtr.29273.1.S1_at	41.17	142.53	44.73	163.75	At4g12690		8.00E-19	*Expressed protein*
Mtr.31763.1.S1_at	367.45	199.74	236.14	226.28	At4g40045		2.00E-13	*Expressed protein*
Mtr.34752.1.S1_s_at	88.87	132.52	54.93	146.36	At3g13950		4.00E-06	*Expressed protein*
Mtr.36171.1.S1_s_at	117.18	183.82	121.11	179.84	At5g05840		6.00E-24	*Expressed protein*
Mtr.37079.1.S1_at	90.44	112.69	57.85	137.12	At2g45450		2.00E-09	*Expressed protein*
Mtr.38228.1.S1_at	443.99	816.74	596.01	1042.04	At5g59050		5.00E-06	*Expressed protein*
Mtr.38291.1.S1_at	1909.86	2395.71	1393.44	1812.16	At2g46490		4.00E-12	*Expressed protein*

Table S5 (continued)

	Raw expression values in shoots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.38615.1.S1_at	653.52	1308.81	770.10	1025.03	At4g19700		4.00E-28	*Expressed protein*
Mtr.38661.1.S1_at	728.40	412.44	488.28	379.71	At5g44650		1.00E-31	*Expressed protein*
Mtr.38672.1.S1_at	1334.26	857.17	908.73	724.39	At1g51100		2.00E-26	*Expressed protein*
Mtr.39059.1.S1_at	77.12	79.58	51.41	101.76	At4g14380		3.00E-13	*Expressed protein*
Mtr.39209.1.S1_at	338.23	231.78	252.60	237.33	At5g43150		4.00E-09	*Expressed protein*
Mtr.39327.1.S1_at	801.53	321.14	474.41	323.40	At2g35450		2.00E-37	*Expressed protein*
Mtr.40236.1.S1_s_at	3881.89	2668.21	5412.00	1295.53	At1g17710		4.00E-27	*Expressed protein*
Mtr.41288.1.S1_at	793.29	532.83	591.05	492.23	At3g23760		8.00E-70	*Expressed protein*
Mtr.42067.1.S1_s_at	236.08	1012.56	464.66	1052.73	At3g19680		2.00E-15	*Expressed protein*
Mtr.42139.1.S1_at	69.47	85.00	50.37	102.21	At2g42610		9.00E-65	*Expressed protein*
Mtr.42257.1.S1_at	54.64	79.91	27.93	99.91	At2g31160		3.00E-54	*Expressed protein*
Mtr.42374.1.S1_at	324.99	152.35	210.76	169.98	At3g28760		2.00E-73	*Expressed protein*
Mtr.43494.1.S1_at	271.42	480.04	329.45	516.25	At1g56020		3.00E-09	*Expressed protein*
Mtr.43604.1.S1_s_at	147.06	633.91	311.57	768.55	At1g10020		3.00E-35	*Expressed protein*
Mtr.44533.1.S1_at	203.68	102.52	109.65	100.32	At3g20680		5.00E-32	*Expressed protein*
Mtr.44854.1.S1_at	1097.51	749.27	859.26	680.61	At4g28080		4.00E-07	*Expressed protein*
Mtr.45095.1.S1_at	410.94	255.94	323.60	228.44	At3g27050		8.00E-49	*Expressed protein*
Mtr.46118.1.S1_at	932.49	822.01	1042.69	669.73	At5g53045		5.00E-13	*Expressed protein*
Mtr.4993.1.S1_at	119.83	228.55	213.40	208.43	At2g28130		4.00E-16	*Expressed protein*
Mtr.50974.1.S1_at	578.18	340.94	413.01	356.74	At4g31560	HCF153	2.00E-16	*Expressed protein*
Mtr.51053.1.S1_at	146.26	97.22	122.36	83.13	At5g64480		8.00E-14	*Expressed protein*
Mtr.5840.1.S1_at	21.37	66.57	19.15	64.79	At2g04220		8.00E-21	*Expressed protein*
Mtr.691.1.S1_at	189.30	198.49	143.40	241.41	At4g21500		2.00E-10	*Expressed protein*
Mtr.8670.1.S1_at	1637.75	1307.30	1779.70	1224.64	At2g12400		1.00E-35	*Expressed protein*
Mtr.9012.1.S1_s_at	841.01	491.15	628.63	575.91	At5g24060		1.00E-35	*Expressed protein*
Mtr.928.1.S1_at	169.23	101.29	113.36	84.11	At4g34090		3.00E-17	Expressed protein
Mtr.8968.1.S1_at	3955.55	4444.72	5045.72	1980.58	At1g17710		2.00E-19	*Expressed protein*
Mtr.8559.1.S1_at	268.40	792.17	427.57	1207.69	At1g03220		1.00E-21	*Extracellular dermal glycoprotein*
Mtr.9322.1.S1_at	112.44	314.76	51.15	378.14	At3g23840		6.00E-46	Fatty acid elongase-like protein / *Transferase family protein*
Mtr.42010.1.S1_at	37.21	53.75	44.40	60.16	At3g23880		4.00E-07	*F-box family protein*
Mtr.24508.1.S1_at	8688.68	4392.57	6534.30	4829.75	At1g60950	FED A	1.00E-37	*Ferredoxin*
Mtr.49132.1.S1_at	2839.29	1947.06	2260.84	1887.53	At4g14890		1.00E-47	Ferredoxin

Table S5 (continued)

	Raw expression values in shoots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.33883.1.S1_at	23.54	39.32	28.66	59.63	At5g48460		1.00E-58	Fimbrin-like protein AtFim2
Mtr.32723.1.S1_at	417.40	207.82	243.54	211.62	At2g20830		5.00E-29	Formiminotransferase-cyclodeaminase-like
Mtr.23357.1.S1_at	37.78	72.00	44.48	68.94				Functional candidate resistance protein KR1
Mtr.27793.1.S1_s_at	336.37	187.29	190.48	184.09				Galactosidase like protein
Mtr.9233.1.S1_at	316.44	444.12	246.61	384.99	At3g54810	BME3	3.00E-06	*GATA type zinc finger domain*
Mtr.6808.1.S1_at	162.46	111.03	143.10	105.28	At2g30330		3.00E-34	GCN5-like protein 1
Mtr.40268.1.S1_at	289.31	199.92	225.33	195.54	At2g39770	CYT1	8.00E-90	*GDP-mannose pyrophosphorylase (GMP1)*
Mtr.8725.1.S1_at	164.84	327.57	153.73	398.09	At5g14470		1.00E-32	*GHMP kinase-related*
Mtr.32124.1.S1_at	175.46	142.53	251.39	27.67	At1g30040	GA2OX2	2.00E-44	Gibberellin 2-beta-dioxygenase
Mtr.38883.1.S1_at	78.36	72.50	49.69	110.64	At1g78440	ATGA2OX1	3.00E-34	Gibberellin 2-oxidase
Mtr.9997.1.S1_at	168.24	345.51	209.31	267.55	At5g20870		1.00E-05	Glucan endo-1,3-beta-glucosidase precursor
Mtr.2057.1.S1_s_at	68.18	47.25	89.72	61.87	At5g64290	DIT2.1	5.00E-24	Glutamate/malate translocator
Msa.3145.1.S1_s_at	257.68	305.77	208.87	332.23	At5g37600	GSR1	1.00E-70	*Glutamine synthetase*
Mtr.22927.1.S1_at	733.78	931.84	666.54	887.41	At5g37600	GSR1	6.00E-99	Glutamine synthetase
Mtr.33674.1.S1_at	78.33	12.78	67.24	19.51				Glutamyl-tRNA reductase 1
Mtr.19479.1.S1_at	810.70	534.50	777.18	493.22	At2g38270	CXIP2	4.00E-79	Glutaredoxin / *CAX-interacting protein*
Mtr.43300.1.S1_at	2653.44	1836.02	2124.23	1750.79	At4g28730		1.00E-26	Glutaredoxin protein family-like
Mtr.40518.1.S1_at	86.80	54.80	76.03	33.67	At1g02930	GSTF6	5.00E-25	Glutathione S-transferase
Msa.2885.1.S1_at	9641.88	6826.59	7143.95	7008.88	At1g32470		2.00E-43	*Glycine cleavage system H protein*
Mtr.9582.1.S1_at	426.46	312.16	467.95	341.71	At3g01180	AtSS2	8.00E-35	Glycogen(starch) synthase isoform II precursor
Mtr.8679.1.S1_at	2292.66	3584.29	2138.50	2583.13	At1g24170	LGT9	6.00E-21	Glycosyl transferase
Mtr.40320.1.S1_at	624.14	1269.19	771.94	1127.81	At2g28110	FRA8	2.00E-05	*Glycosyl transferase family 47 protein*
Mtr.13030.1.S1_at	341.60	543.62	372.60	555.16	At5g59840		5.00E-20	GTP-binding protein
Mtr.38394.1.S1_at	673.09	359.06	471.22	381.11	At4g28556	RIC7	6.00E-09	Heat-shock protein beta-7 / *P21-rho-binding domain-containing protein*
Msa.1621.1.S1_at	214.95	405.00	130.42	514.65	At3g04720		2.00E-51	*Hevein-like protein (HEL)*
Mtr.11436.1.S1_at	821.78	506.09	568.58	574.84	At1g31860	AT-IE	7.00E-74	Histidine biosynthesis bifunctional protein / P*yrophosphohydrolase*
Mtr.21405.1.S1_at	99.23	162.65	96.31	159.47	At3g61150	HDG1	8.00E-58	Homeobox
Mtr.40459.1.S1_at	745.29	820.25	548.54	1079.34	At4g37790	HAT22	6.00E-39	Homeobox protein HAT9
Mtr.45889.1.S1_at	163.66	243.00	117.02	260.03	At4g16780	ATHB-2	3.00E-53	Homeobox-leucine zipper protein
Mtr.11739.1.S1_s_at	294.28	180.52	224.32	178.23	At2g18950	HPT1	1.00E-39	Homogentisate phytylprenyltransferase
Msa.2909.1.S1_at	320.96	223.06	268.04	206.56	At2g18950	HPT1	1.00E-71	*Homogentisate phytylprenyltransferase family protein (HPT1)*
Mtr.43749.1.S1_s_at	839.67	452.77	654.39	559.63	At1g77420		9.00E-43	*Hydrolase*

Table S5 (continued)

	Raw expression values in shoots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Msa.3143.1.S1_at	2110.51	995.94	1639.42	1155.67	At4g15440	HPL1	1.00E-59	*Hydroperoxide lyase (HPL1)*
Mtr.45669.1.S1_at	454.95	224.45	263.18	233.81	At1g17650	GLYR2	5.00E-08	Hydroxyacid dehydrogenase/reductase
Mtr.41103.1.S1_at	655.18	451.95	500.99	501.53	At1g79870		4.00E-61	Hydroxyphenylpyruvate reductase (HPPR)
Mtr.8442.1.S1_at	39.21	157.96	7.76	358.02				Hydroxyproline-rich glycoprotein
Mtr.12870.1.S1_at	548.02	987.35	390.13	1442.96	At5g65660		6.00E-09	*Hydroxyproline-rich glycoprotein family protein*
Mtr.17626.1.S1_s_at	42.92	90.90	40.35	76.15				Hypothetical protein
Mtr.17706.1.S1_at	509.61	326.48	419.75	316.85				Hypothetical protein
Mtr.19029.1.S1_s_at	187.54	247.27	178.91	182.86				Hypothetical protein
Mtr.49736.1.S1_x_at	390.73	528.40	254.94	782.15				Hypothetical protein
Mtr.51374.1.S1_at	46.29	101.56	41.93	76.52				Hypothetical protein
Mtr.51662.1.S1_at	63.83	77.19	62.31	80.03				Hypothetical protein
Mtr.52057.1.S1_at	34.47	97.26	39.60	59.72				Hypothetical protein
Mtr.13915.1.S1_at	249.08	143.07	194.68	178.63	At1g78620		2.00E-24	*Integral membrane family protein*
Mtr.27878.1.S1_at	870.75	178.13	438.27	210.81	At1g75280		3.00E-41	Isoflavone reductase-like protein
Mtr.5446.1.S1_at	27.37	57.70	35.22	85.03	At1g70510	KNAT2	2.00E-18	KN1-type homeobox protein
Mtr.5446.1.S1_s_at	30.57	76.78	34.71	138.24	At1g70510	KNAT2	2.00E-18	KN1-type homeobox protein
Mtr.34690.1.S1_at	249.85	124.15	179.32	100.04	At1g67280		3.00E-39	Lactoylglutathione lyase
Mtr.428.1.S1_s_at	99.81	117.76	69.40	132.11	At3g05990		9.00E-54	Leucine rich repeat protein family
Mtr.41172.1.S1_s_at	33.33	56.04	35.10	45.99	At3g05990		7.00E-60	*Leucine-rich repeat family protein*
Mtr.44074.1.S1_at	117.67	238.00	137.11	263.51	At4g03390	SRF3	2.00E-57	*Leucine-rich repeat transmembrane protein kinase*
Mtr.12682.1.S1_at	2869.75	1670.78	2148.24	1628.97	At1g45474	LHCA5	5.00E-44	Light-harvesting complex protein
Mtr.13800.1.S1_at	59.32	18.23	28.93	22.51	At3g61680		7.00E-40	Lipase class 3 family protein-like
Mtr.12359.1.S1_at	231.48	369.50	172.98	548.30				M.truncatula enod40 mRNA for non-translatable RNA
Mtr.40508.1.S1_at	5896.93	3888.86	5187.04	3993.31	At5g45930	CHLI2	1.00E-36	Magnesium-chelatase subunit chlI
Mtr.14257.1.S1_at	4515.84	2559.94	3877.73	2338.35	At4g25080	CHLM	3.00E-73	Magnesium-protoporphyrin IX methyltransferase
Mtr.29176.1.S1_at	194.02	128.34	169.37	124.83	At1g26160		7.00E-14	Metal-dependent phosphohydrolase
Mtr.13480.1.S1_at	329.09	216.53	273.26	228.09	At3g59980		4.00E-27	Methionyl-tRNA synthetase
Mtr.34403.1.S1_at	158.64	111.59	114.12	110.12	At2g33820	MBAC1	2.00E-58	Mitochondrial basic amino acid carrier
Mtr.37525.1.S1_at	994.15	147.23	285.03	244.75				Multifunctional aquaporin
Mtr.51597.1.S1_s_at	177.20	126.31	152.59	110.81	At4g11980	ATNUDX14	2.00E-23	*MutT/nudix family protein*
Mtr.11942.1.S1_at	254.98	129.44	173.85	190.81	At3g10760		4.00E-07	*Myb family transcription factor*
Mtr.13636.1.S1_at	48.12	96.27	28.31	112.26	At1g57560		1.00E-09	Myb family transcription factor MYB2

Table S5 (continued)

	Raw expression values in shoots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.44930.1.S1_s_at	56.06	57.76	32.69	68.44	At3g28910		2.00E-29	MYB96 transcription factor-like protein
Mtr.9341.1.S1_at	133.82	72.02	87.22	78.65				NAD(P)H-quinone oxidoreductase chain 4
Mtr.2382.1.S1_at	41.56	73.33	19.96	86.66	At2g47270		3.00E-12	NADH dehydrogenase subunit 2
Mtr.39567.1.S1_at	802.90	993.48	527.95	1229.76	At1g27040		1.00E-05	Nitrate transporter NTL1
Mtr.22598.1.S1_at	118.74	286.99	116.55	324.02	At3g43630		1.00E-47	Nodulin-like protein
Mtr.27937.1.S1_at	185.59	291.56	124.36	206.92	At1g68170		1.00E-28	Nodulin-like protein
Mtr.31296.1.S1_at	35.68	45.19	42.04	64.67	At4g19450		4.00E-50	*Nodulin-related*
Mtr.42611.1.S1_at	68.82	37.72	51.97	47.64	At2g38550		2.00E-09	Non-green plastid inner envelope membrane protein precursor
Mtr.12256.1.S1_at	1010.43	2318.96	265.18	2190.05				Nonspecific lipid-transfer protein precursor
Msa.2628.1.S1_at	153.17	93.69	94.52	93.25	At4g33490		9.00E-57	*Nucellin protein*
Msa.1457.1.S1_at	552.29	303.64	330.87	251.08	At5g20550		9.00E-51	*Oxidoreductase, 2OG-Fe(II) oxygenase family protein*
Mtr.38677.1.S1_at	67.09	43.43	60.23	34.65	At1g17020	SRG1	8.00E-56	*Oxidoreductase, 2OG-Fe(II) oxygenase family protein*
Mtr.38768.1.S1_at	87.29	92.59	44.43	149.04	At4g25310		1.00E-40	*Oxidoreductase, 2OG-Fe(II) oxygenase family protein*
Mtr.31344.1.S1_at	75.15	44.56	64.69	51.05	At3g15090		3.00E-20	Oxidoreductase-like protein
Mtr.35444.1.S1_at	2426.60	1315.20	1819.89	1232.35	At3g55330	PPL1	2.00E-13	Oxygen evolving complex protein-like / *Photosystem II reaction center*
Mtr.6707.1.S1_s_at	135.59	100.38	235.16	114.18	At2g17340		3.00E-59	*Pantothenate kinase-related*
Mtr.12532.1.S1_at	7661.93	4146.89	6397.11	5183.78	At4g37070	PLP1	2.00E-16	Patatin-like protein 1
Mtr.26632.1.S1_at	111.00	134.47	38.55	188.44	At1g78780		8.00E-64	*Pathogenesis-related family protein*
Mtr.43770.1.S1_at	113.11	140.62	80.24	181.73	At3g19690		3.00E-06	Pathogenesis-related PR-1-like protein
Mtr.39139.1.S1_at	289.39	531.71	175.74	703.93	At3g04720		6.00E-50	Pathogenesis-related protein 4A / *Hevein-like protein (HEL)*
Mtr.38897.1.S1_at	496.81	750.83	312.48	700.10	At3g43270		9.00E-54	Pectinesterase PPE8B precursor
Mtr.47230.1.S1_at	39.47	71.25	60.14	60.71	At1g08610		2.00E-23	Pentatricopeptide (PPR) repeat-containing protein
Mtr.39868.1.S1_at	35.33	54.20	55.05	56.01	At1g06900		8.00E-61	*Peptidase M16 family protein*
Mtr.9748.1.S1_at	641.12	370.38	487.21	308.78	At3g15520		1.00E-73	Peptidyl-prolyl cis-trans isomerase
Mtr.39782.1.S1_at	444.09	158.87	242.78	165.68	At1g74070		8.00E-19	*Peptidyl-prolyl cis-trans isomerase cyclophilin-type family protein*
Mtr.10844.1.S1_at	4396.43	2180.89	2864.32	1806.52	At3g26060	ATPRX Q	9.00E-51	Peroxiredoxin Q
Mtr.22013.1.S1_s_at	2330.46	897.88	1141.31	723.26	At3g26060	ATPRX Q	2.00E-19	Peroxiredoxin Q
Mtr.12209.1.S1_at	530.08	2910.12	1187.92	2581.57	At4g08950		2.00E-45	Phi-1 protein
Msa.1875.1.S1_at	86.47	298.16	121.27	267.25	At4g08950		8.00E-40	*Phosphate-responsive protein*
Msa.3094.1.S1_at	884.71	3765.54	1636.50	3538.04	At4g08950		4.00E-58	*Phosphate-responsive protein*
Msa.1910.1.S1_at	9951.99	6616.74	8656.98	6654.03	At1g56190		9.00E-48	*Phosphoglycerate kinase*
Mtr.33734.1.S1_at	145.57	73.78	101.01	96.48	At1g58280		1.00E-15	Phosphoglycerate mutase-like protein

Table S5 (continued)

	Raw expression values in shoots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.41408.1.S1_at	40.59	114.64	84.69	101.63	At1g52700		5.00E-49	*Phospholipase/carboxylesterase family protein*
Mtr.31792.1.S1_at	1090.35	603.39	733.70	552.35	At5g43745		2.00E-15	*Phosphotransferase-related*
Msa.1588.1.S1_at	1430.60	1015.59	1202.02	1032.10	At2g47590	PHR2	9.00E-46	*Photolyase/blue light photoreceptor (PHR2)*
Mtr.223.1.S1_at	2974.20	2005.29	2519.97	2191.93	At2g47590	PHR2	5.00E-61	Photolyase/blue-light receptor (PHR2)
Mtr.34726.1.S1_at	245.60	65.00	231.02	107.59	At4g02770	PSAD-1	2.00E-57	Photosystem I reaction center subunit II
Mtr.43317.1.S1_s_at	4066.33	2343.90	3519.91	2795.43	At1g67740	PSBY	7.00E-15	Photosystem II core complex proteins psbY
Mtr.37092.1.S1_at	1414.28	742.85	981.25	968.41				Photosystem II M protein
Mtr.40683.1.S1_at	2766.96	1651.86	2036.14	1492.91	At4g28660	PSB28	1.00E-52	Photosystem II protein W-like protein
Mtr.12722.1.S1_at	1437.01	788.45	1404.18	784.05	At5g17230	PSY	1.00E-64	*Phytoene synthase (PSY)*
Mtr.20321.1.S1_at	2953.26	682.73	2362.84	765.07	At2g45180		4.00E-30	Plant lipid transfer / *Protease inhibitor*
Mtr.43596.1.S1_at	4714.50	3196.88	3628.62	2993.43	At4g17560		3.00E-44	Plastid ribosomal protein L19
Mtr.49640.1.S1_at	365.09	230.39	327.17	219.13	At2g21280	SULA	2.00E-80	*Plastid-targeted protein*
Mtr.49641.1.S1_at	614.97	474.54	679.41	403.86	At2g21280	SULA	4.00E-38	*Plastid-targeted protein*
Mtr.8772.1.S1_at	2864.69	1721.11	2387.69	1669.51	At3g01480	CYP38	7.00E-49	Poly(A) polymerase
Mtr.8773.1.S1_s_at	924.78	383.42	513.25	397.98	At3g01480	CYP38	6.00E-37	Poly(A) polymerase
Mtr.46184.1.S1_at	1939.48	1338.56	1893.75	1426.19	At4g36810	GGPS1	8.00E-57	Polyprenyl synthetase
Mtr.38209.1.S1_at	1276.40	928.65	676.55	1357.58				Polysaccharide ABC transporter
Mtr.17054.1.S1_s_at	616.50	410.14	505.18	440.92	At5g19370		2.00E-69	PpiC-type peptidyl-prolyl cis-trans isomerase / *Rhodanese-like domain*
Mtr.21270.1.S1_at	1015.47	477.51	688.03	514.75	At5g34930		3.00E-10	Prephenate dehydrogenase
Mtr.13880.1.S1_at	309.86	348.04	221.29	565.65	At1g29195		9.00E-31	Prokineticin 2 precursor (PK2)
Mtr.12290.1.S1_s_at	390.72	1104.93	668.09	762.08				Proline dehydrogenase
Mtr.39238.1.S1_at	2590.93	909.95	1058.60	1101.40	At3g16370		1.00E-61	Proline-rich protein APG-like / *GDSL-motif lipase*
Mtr.40631.1.S1_at	85.68	113.08	70.31	122.68	At4g33950	OST1	1.00E-17	Protein kinase
Mtr.43264.1.S1_at	67.38	78.41	46.40	111.78	At4g33950	OST1	2.00E-69	Protein kinase
Mtr.9552.1.S1_at	183.95	381.68	228.62	206.23	At1g50700	CPK33	5.00E-08	Protein kinase
Mtr.46263.1.S1_at	120.93	190.65	137.35	134.65	At4g14350		9.00E-67	Protein kinase-like
Mtr.2338.1.S1_at	227.77	314.69	193.72	380.31	At1g28230		7.00E-43	*Purine permease (PUP1)*
Mtr.37731.1.S1_at	5672.37	2490.20	4472.96	3209.06	At1g03600	PSB27	5.00E-41	PWWP domain protein-like / *Photosystem II family protein*
Mtr.10984.1.S1_at	248.10	382.84	188.51	331.60	At2g29990	NDA2	4.00E-26	*Pyridine nucleotide-disulphide oxidoreductase family protein*
Mtr.9546.1.S1_at	54.47	69.98	45.13	99.95	At2g29990	NDA2	9.00E-47	*Pyridine nucleotide-disulphide oxidoreductase family protein*
Mtr.44591.1.S1_at	2344.09	1434.76	1732.46	1268.72	At1g23740		1.00E-59	Quinone oxidoreductase-like protein
Mtr.40349.1.S1_at	350.21	264.55	280.39	174.93	At1g22740	RABG3B	9.00E-32	*Ras-related protein (RAB7)*

Table S5 (continued)

Medicago probes	Raw expression values in shoots: Without caesium Non myc	Without caesium Myc	With caesium Non myc	With caesium Myc	AGI ID	Gene name	e-value	MtGEA Target Description / *TAIR Target Description*
Mtr.24464.1.S1_at	20.56	53.41	31.29	43.13	At1g16670		9.00E-38	Receptor-like protein kinase
Mtr.2927.1.S1_at	36.85	67.98	50.27	75.05	At2g33170		1.00E-32	Receptor-like protein kinase
Mtr.5291.1.S1_at	336.23	120.57	178.32	127.01	At1g45207		2.00E-15	*Remorin family protein*
Mtr.28279.1.S1_at	37.06	62.95	39.05	53.38	At3g14460		4.00E-08	Resistance complex protein
Mtr.39812.1.S1_s_at	58.29	128.79	52.47	66.32	At5g47910	RBOHD	6.00E-60	Respiratory burst oxidase homolog
Mtr.24829.1.S1_at	105.72	188.48	10.92	622.87				Retroelement pol polyprotein-related
Mtr.49088.1.S1_at	229.14	280.09	129.49	359.07	At2g17850		5.00E-37	Rhodanese-like / *Senescence-associated family protein*
Mtr.37187.1.S1_at	379.99	357.79	491.19	252.20	At4g24750		2.00E-66	Rhodanese-like domain-containing protein-like
Mtr.16922.1.S1_at	153.25	103.53	128.27	98.30	At4g28706		1.00E-55	Ribokinase / *pfkB-type carbohydrate kinase family protein*
Mtr.27411.1.S1_s_at	237.71	150.05	165.02	143.18	At3g52380	CP33	5.00E-17	Ribonucleoprotein precursor
Mtr.19517.1.S1_at	15702.92	10707.55	11264.72	11010.34	At5g38410		8.00E-05	Ribulose bisphosphate carboxylase, small chain
Mtr.40987.1.S1_at	3522.41	2203.64	2463.78	2136.79	At1g71500		2.00E-30	*Rieske (2Fe-2S) domain-containing protein*
Mtr.24065.1.S1_at	19.94	65.92	12.92	118.80	At1g49230		2.00E-44	RING zinc finger protein
Mtr.37552.1.S1_at	6649.64	3962.80	5391.81	4917.44	At1g60000		3.00E-33	RNA- or ssDNA-binding protein
Mtr.41209.1.S1_at	454.05	252.81	338.40	296.46	At5g17520	RCP1	5.00E-62	Root cap 1 (RCP1)
Mtr.42261.1.S1_at	3304.42	2576.07	2847.61	2152.75				Rubisco activase
Mtr.10881.1.S1_at	2989.98	2011.39	2326.75	1962.21	At1g54500		3.00E-55	*Rubredoxin family protein*
Msa.2701.1.S1_at	772.30	739.95	920.54	344.77	At1g27730	STZ	2.00E-19	*Salt tolerance zinc finger protein*
Mtr.41191.1.S1_at	56.72	75.02	54.75	82.67	At2g43650		1.00E-05	*Sas10/U3 ribonucleoprotein (Utp) family protein*
Mtr.8921.1.S1_at	344.04	220.89	300.50	233.05	At4g08690		6.00E-35	*SEC14 cytosolic factor family protein*
Mtr.43769.1.S1_at	285.17	853.20	416.14	848.70	At5g20250	DIN10	1.00E-20	Seed imbibition protein / *Alkaline alpha galactosidase*
Mtr.13668.1.S1_at	52.54	85.95	38.43	78.09	At5g20700		1.00E-12	*Senescence-associated protein-related*
Mtr.4799.1.S1_at	188.20	251.90	144.49	234.94	At5g20700		2.00E-10	*Senescence-associated protein-related*
Mtr.31632.1.S1_at	268.98	537.22	158.92	548.76				Serine/threonine protein kinase
Mtr.34632.1.S1_s_at	1608.17	6281.94	2984.40	5740.31				Seven transmembrane helix receptor
Mtr.41710.1.S1_at	183.93	234.40	214.90	282.82	At1g28060		2.00E-62	*Small nuclear ribonucleoprotein family protein*
Mtr.28620.1.S1_at	76.53	58.79	58.95	54.17	At5g04610		7.00E-24	*Spermidine synthase-related*
Mtr.31150.1.S1_at	638.63	383.44	455.40	366.71	At1g33290		5.00E-50	*Sporulation protein-related*
Mtr.27443.1.S1_at	356.41	118.39	340.93	246.99	At5g59810	SBT5.4	2.00E-57	Subtilisin-like protease
Mtr.20281.1.S1_at	114.82	77.50	96.68	74.19	At2g46225	ABIL1	3.00E-50	*Subunit of the WAVE complex*
Mtr.10952.1.S1_at	410.43	222.18	346.50	183.85				Sulfate adenylyltransferase
Mtr.31750.1.S1_at	142.82	308.12	119.94	399.45	At3g15990		2.00E-29	Sulfate transporter 3.4

Table S5 (continued)

	Raw expression values in shoots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.12493.1.S1_at	1552.23	947.53	1179.10	877.06	At2g41680	NTRC	1.00E-29	*Thioredoxin reductase*
Mtr.15318.1.S1_at	1077.39	660.85	853.11	676.67	At1g76760	ATY1	6.00E-47	Thioredoxin-related
Mtr.40730.1.S1_at	3421.72	2079.41	2700.45	2102.03	At4g02530		2.00E-35	Thylakoid lumenal 16.5 kDa protein
Mtr.34594.1.S1_s_at	2531.97	1618.37	1905.80	1742.43				Thylakoid lumenal 17.4 kDa protein
Mtr.38403.1.S1_at	3119.30	1522.13	2134.88	1651.07	At3g55330	PPL1	8.00E-35	Thylakoid lumenal 25.6 kDa protein / *Photosystem II reaction center*
Mtr.43702.1.S1_at	109.31	66.45	98.32	57.35	At1g12250		4.00E-39	Thylakoid lumenal protein-like
Mtr.21620.1.S1_at	412.32	290.69	333.96	300.33	At1g29810		2.00E-37	Transcriptional coactivator/pterin dehydratase
Mtr.7001.1.S1_at	59.14	140.17	47.73	142.06				Transmembrane GTPase
Mtr.52340.1.S1_s_at	539.67	800.87	671.57	933.44	At1g06410	ATTPS7	5.00E-80	Trehalose-phosphatase / *Glycosyl transferase family 20 protein*
Mtr.33389.1.S1_at	99.60	145.63	138.16	145.15	At1g16800		2.00E-05	*tRNA-splicing endonuclease positive effector-related*
Msa.1058.1.S1_at	111.21	68.51	87.22	68.21	At3g44620		3.00E-58	*Tyrosine phosphatase-like protein*
Mtr.15385.1.S1_at	537.64	377.96	461.44	354.13	At2g18950	HPT1	7.00E-72	UbiA prenyltransferase
Mtr.13567.1.S1_at	1873.63	1060.55	1375.44	1227.96	At3g11950		5.00E-36	*UbiA prenyltransferase family protein*
Mtr.6887.1.S1_at	97.37	39.54	56.28	30.62	At3g20630	UBP14	2.00E-19	Ubiquitin isopeptidase T
Mtr.24551.1.S1_s_at	1087.37	325.83	685.87	390.28	At3g20630	UBP14	1.00E-13	Ubiquitin-specific protease 14
Mtr.32678.1.S1_s_at	402.98	126.54	258.78	133.99				Ubiquitin-specific protease 14
Mtr.32703.1.S1_at	252.65	75.25	146.87	76.98	At3g20630	UBP14	6.00E-23	Ubiquitin-specific protease 14
Mtr.10389.1.S1_s_at	2294.29	4162.83	2551.78	4978.57	At1g63180	UGE3	2.00E-83	UDP-glucose 4-epimerase
Mtr.21000.1.S1_at	85.16	124.27	81.62	178.84	At3g62550		2.00E-33	Universal stress protein
Mtr.32186.1.S1_at	159.22	113.86	138.93	103.22	At2g26540	HEMD	1.00E-24	*Uroporphyrinogen-III synthase family protein*
Mtr.44993.1.S1_at	366.48	210.55	275.08	213.09	At2g26540	HEMD	2.00E-23	*Uroporphyrinogen-III synthase family protein*
Mtr.33754.1.S1_at	43.73	55.91	40.08	59.22	At5g52990		1.00E-15	*Vesicle-associated membrane protein-related*
Msa.1277.1.S1_s_at	108.38	264.92	131.01	298.56	At4g10270		3.00E-09	*Wound-responsive family protein*
Mtr.43704.1.S1_at	11.09	80.97	23.25	90.32	At4g10270		1.00E-04	*Wound-responsive family protein*
Mtr.42546.1.S1_at	59.56	96.72	72.96	95.49	At4g31805		2.00E-10	*WRKY family transcription factor*
Mtr.10910.1.S1_at	117.17	996.72	143.51	173.23	At1g10550	XTH33	9.00E-14	Xyloglucan endotransglucosylase
Mtr.21678.1.S1_at	335.84	232.74	327.69	259.97	At2g45190	AFO	3.00E-12	YABBY protein
Mtr.38995.1.S1_at	621.65	382.62	609.85	524.15	At2g26580		3.00E-28	YABBY-like transcription factor
Mtr.23979.1.S1_s_at	113.26	170.66	125.97	179.28	At3g50700	AtIDD2	8.00E-18	*Zinc finger (C2H2 type) family protein*
Mtr.9746.1.S1_at	241.39	438.50	116.32	634.36	At4g37890	EDA40	2.00E-19	*Zinc finger (C3HC4-type RING finger) family protein*
Mtr.38590.1.S1_at	81.93	145.77	37.57	252.47	At2g22680		1.00E-15	Zinc finger (C3HC4-type RING finger)-like protein
Mtr.13378.1.S1_at	42.19	68.88	38.83	89.93	At1g66140	ZFP4	2.00E-29	Zinc finger protein 4

Table S5 (continued)

	Raw expression values in shoots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.19131.1.S1_at	451.39	717.36	498.27	972.01	At2g19810		5.00E-23	Zn-finger
Msa.960.1.S1_at	273.00	206.02	236.46	191.42				No annotation
Mtr.10117.1.S1_at	21.79	47.10	35.69	53.09				No annotation
Mtr.11122.1.S1_at	1015.00	578.30	1184.09	269.03				No annotation
Mtr.11744.1.S1_at	116.58	57.97	119.16	54.98				No annotation
Mtr.11844.1.S1_at	250.36	440.71	222.00	842.72				No annotation
Mtr.11949.1.S1_s_at	570.85	933.05	496.69	651.46				No annotation
Mtr.12886.1.S1_at	1078.70	742.55	739.84	662.97				No annotation
Mtr.13245.1.S1_at	17.09	83.53	24.07	93.57				No annotation
Mtr.1505.1.S1_at	25.75	61.01	22.33	61.24				No annotation
Mtr.1752.1.S1_at	147.57	234.78	207.14	233.82				No annotation
Mtr.2190.1.S1_s_at	655.20	374.76	435.89	413.50				No annotation
Mtr.23356.1.S1_at	38.63	86.03	40.57	76.23				No annotation
Mtr.23854.1.S1_at	21.84	97.08	13.37	182.93				No annotation
Mtr.26556.1.S1_at	42.45	48.84	40.72	60.97				No annotation
Mtr.28311.1.S1_s_at	341.13	234.15	168.89	773.47				No annotation
Mtr.28696.1.S1_at	737.48	978.38	737.21	1171.17				No annotation
Mtr.28985.1.S1_at	64.48	39.56	48.09	44.72				No annotation
Mtr.32996.1.S1_at	48.26	71.51	50.86	64.98				No annotation
Mtr.35561.1.S1_at	149.41	243.91	149.79	161.52				No annotation
Mtr.35564.1.S1_at	1061.28	516.68	699.33	483.92				No annotation
Mtr.35849.1.S1_at	327.46	112.68	245.04	130.05				No annotation
Mtr.35926.1.S1_at	122.79	94.19	132.36	57.69				No annotation
Mtr.36886.1.S1_at	180.67	328.38	218.94	396.92				No annotation
Mtr.38183.1.S1_at	637.67	651.08	598.87	884.97				No annotation
Mtr.38957.1.S1_at	696.93	861.10	579.33	1457.41				No annotation
Mtr.39676.1.S1_at	73.41	115.98	83.86	88.10				No annotation
Mtr.39710.1.S1_at	35.64	43.09	32.13	79.57				No annotation
Mtr.40613.1.S1_at	523.88	431.81	304.00	1150.12				No annotation
Mtr.42067.1.S1_at	976.03	3473.67	1947.39	3565.10				No annotation
Mtr.42405.1.S1_at	88.31	371.49	82.33	172.82				No annotation
Mtr.43175.1.S1_s_at	247.18	459.14	317.67	341.76				No annotation

Table S5 (continued)

	Raw expression values in shoots							
	Without caesium		With caesium					
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**Gene name**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.43176.1.S1_s_at	33.03	69.56	40.13	50.56				No annotation
Mtr.43205.1.S1_at	179.81	306.39	241.39	320.04				No annotation
Mtr.43749.1.S1_at	992.29	500.90	706.68	566.40				No annotation
Mtr.44396.1.S1_at	606.49	151.81	265.21	160.05				No annotation
Mtr.44887.1.S1_at	226.59	333.52	200.00	265.14				No annotation
Mtr.45270.1.S1_at	179.89	254.72	196.54	498.42				No annotation
Mtr.4769.1.S1_at	335.67	427.29	306.73	496.14				No annotation
Mtr.5376.1.S1_at	281.27	425.40	282.71	685.22				No annotation
Mtr.5376.1.S1_x_at	289.08	387.49	224.27	549.08				No annotation
Mtr.6123.1.S1_at	672.13	415.62	500.67	394.54				No annotation
Mtr.6193.1.S1_at	269.65	178.49	238.77	198.96				No annotation
Mtr.6666.1.S1_at	245.10	405.29	190.05	301.85				No annotation
Mtr.8314.1.S1_at	106.25	170.14	75.46	174.12				No annotation
Mtr.8588.1.S1_at	4799.82	2565.61	4299.27	3105.07				No annotation
Mtr.8905.1.S1_at	241.26	1012.79	477.83	1064.36				No annotation
Mtr.9377.1.S1_at	156.59	119.42	77.07	139.70				No annotation
Mtr.9483.1.S1_at	1927.79	1136.32	1293.89	1137.47				No annotation
Mtr.9730.1.S1_at	302.66	447.93	309.20	430.86				No annotation

Table S6: Raw expression values of genes in shoots of non mycorrhizal (non myc) and mycorrhizal (myc) *Medicago truncatula* plants that had been grown without or with caesium in the medium. The gene expression was significantly influenced by an interaction of Cs in the medium and arbuscular mycorrhiza (Analysis of Variance, $p < 0.05$). The *Medicago* probes refer to the *Medicago truncatula* or *M. sativa* probe name for the gene on the Affymetrix GeneChip® Medicago genome array and the AGI ID is the *Arabidopsis thaliana* gene identification. The annotation was based on local translated BLAST alignment. The MtGEA Target Description is the annotation for the gene obtained from the *Medicago truncatula* Gene Expression Atlas and the TAIR Target Description is the annotation of the *Arabidopsis thaliana* genes (*A. thaliana* annotations in italics).

	Raw expression values in shoots						
	Without caesium		**With caesium**				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.1751.1.S1_s_at	1136.65	407.00	667.45	851.07			16S rRNA & tRNA-Val chloroplast genes
Mtr.15981.1.S1_at	34.78	73.63	56.47	26.28			AAA ATPase
Mtr.8752.1.S1_at	956.13	353.48	537.85	532.81	At3g51440	6.00E-21	ABC transporter permease protein / *Strictosidine synthase family protein*
Mtr.9795.1.S1_at	886.96	555.79	660.76	643.62	At1g17840	6.00E-79	ABC transporter permease protein
Mtr.43580.1.S1_at	16.87	77.22	41.24	10.79	At1g20560	2.00E-05	Adenosine monophosphate binding protein 1
Mtr.7044.1.S1_at	724.50	240.25	267.68	394.43	At5g42250	8.00E-57	Alcohol dehydrogenase
Mtr.18939.1.S1_at	23.85	35.24	20.12	74.57	At1g25530	1.00E-35	Amino acid/polyamine transporter II
Mtr.52344.1.S1_at	230.07	249.44	276.31	187.81	At2g30970	7.00E-51	Aminotransferase
Mtr.31276.1.S1_at	60.28	89.35	94.41	57.43	At3g05870	1.00E-33	Anaphase promoting complex subunit 11
Mtr.18027.1.S1_at	53.28	133.95	68.07	48.75	At1g10340	1.00E-17	Ankyrin
Msa.1814.1.S1_at	1294.02	1270.42	664.43	1416.05	At1g35720	2.00E-62	*Annexin gene family*
Mtr.38170.1.S1_at	167.12	413.70	283.70	105.63	At3g29670	1.00E-28	Anthocyanin acyltransferase
Msa.1751.1.S1_at	371.05	87.44	101.02	105.90	At4g18910	6.00E-41	*Aquaglyceroporin / NOD26-like major intrinsic protein 2*
Mtr.40663.1.S1_at	240.33	463.71	268.15	213.90	At1g31280	2.00E-08	*Argonaute gene*
Mtr.26509.1.S1_at	100.61	126.56	138.42	77.42	At4g14147	6.00E-56	ARP2/3 complex 20 kDa subunit
Mtr.12736.1.S1_at	2207.80	999.02	1500.74	1262.31	At4g09010	1.00E-54	*Ascorbate peroxidase APX4*
Mtr.32351.1.S1_s_at	2429.71	940.48	1420.90	1257.92	At4g09010	1.00E-15	*Ascorbate peroxidase APX4*
Mtr.49793.1.S1_x_at	122.53	56.92	37.54	107.27	At4g38840	1.00E-26	Auxin responsive
Mtr.19878.1.S1_x_at	294.63	181.68	95.36	285.23	At4g38840	4.00E-25	Auxin responsive SAUR protein
Mtr.19881.1.S1_x_at	232.84	153.63	69.70	243.82			Auxin responsive SAUR protein
Mtr.19891.1.S1_s_at	178.92	102.31	58.04	189.29	At4g38840	3.00E-05	Auxin responsive SAUR protein
Mtr.19897.1.S1_x_at	87.44	40.47	31.23	73.60	At4g38840	2.00E-11	Auxin responsive SAUR protein
Mtr.20626.1.S1_s_at	157.40	120.83	60.06	161.44	At4g38840	1.00E-28	Auxin responsive SAUR protein
Mtr.49795.1.S1_at	1132.97	882.02	421.36	1286.02	At4g38840	4.00E-05	Auxin responsive SAUR protein
Mtr.696.1.S1_at	76.82	72.02	17.63	130.86	At4g34770	3.00E-30	Auxin-induced (indole-3-acetic acid induced) protein family
Mtr.702.1.S1_at	186.19	155.61	82.49	237.71	At4g38840	8.00E-26	Auxin-induced protein

Table S6 (continued)

Medicago probes	Raw expression values in shoots: Without caesium Non myc	Without caesium Myc	With caesium Non myc	With caesium Myc	AGI ID	e-value	MtGEA Target Description / *TAIR Target Description*
Mtr.705.1.S1_x_at	69.86	48.49	14.86	100.83	At4g38840	4.00E-12	Auxin-induced protein
Mtr.10432.1.S1_at	2451.49	2563.94	1853.53	3258.57			Auxin-induced protein 22B
Mtr.25939.1.S1_at	301.24	266.66	99.79	458.41	At4g38840	4.00E-25	Auxin-induced protein 6B
Mtr.10431.1.S1_at	733.77	802.60	607.70	972.04	At1g04240	2.00E-31	Auxin-induced protein IAA4
Mtr.5719.1.S1_at	591.41	403.84	236.19	588.64	At3g15540	6.00E-41	Auxin-induced protein IAA6
Msa.2799.1.S1_at	175.94	127.67	54.92	207.64	At4g38840	4.00E-25	*Auxin-responsive protein*
Mtr.21519.1.S1_at	75.47	73.28	93.23	72.70	At2g32590	3.00E-47	*Barren family protein*
Mtr.47752.1.S1_at	78.57	33.04	49.63	68.13	At4g37850	4.00E-21	bHLH transcription factor
Mtr.23446.1.S1_at	179.54	191.68	239.50	149.44	At3g25410	3.00E-08	*Bile acid:sodium symporter family protein*
Mtr.50053.1.S1_at	259.45	157.74	129.44	227.45	At1g55760	1.00E-53	*BTB/POZ domain-containing protein*
Mtr.8953.1.S1_s_at	73.31	36.46	60.53	56.98	At2g46270	1.00E-25	BZip transcription factor
Mtr.22070.1.S1_s_at	556.84	417.39	344.68	536.78	At1g63220	8.00E-33	*C2 domain-containing protein*
Mtr.13752.1.S1_at	521.34	338.22	360.08	349.00	At4g33000	5.00E-22	Calcineurin B-like protein 10
Msa.884.1.S1_at	93.62	210.24	119.41	119.18	At3g20410	6.00E-50	*Calmodulin-domain protein kinase isoform 9 (CPK9)*
Mtr.13675.1.S1_at	547.80	645.69	691.38	469.92	At1g55090	1.00E-63	*Carbon-nitrogen hydrolase family protein*
Mtr.48649.1.S1_at	164.66	276.72	436.40	128.07	At2g32540	1.00E-15	Cellulose synthase
Mtr.45617.1.S1_s_at	1279.23	503.94	617.79	826.56	AtCg01230	1.00E-12	*Chloroplast gene encoding ribosomal protein s12*
Mtr.38799.1.S1_at	189.77	295.07	337.35	58.81			Cytokinin oxidase-like protein
Mtr.38799.1.S1_s_at	331.78	377.78	438.70	81.63	At2g41510	1.00E-33	Cytokinin oxidase-like protein
Mtr.33677.1.S1_s_at	774.95	1527.87	1290.53	537.40	At4g24890	8.00E-21	Diphosphonucleotide phosphatase-like protein
Mtr.9768.1.S1_at	116.05	124.03	97.08	159.54	At2g31970	9.00E-28	*DNA repair-recombination protein*
Mtr.1977.1.S1_at	210.00	132.94	157.60	158.41	At1g76880	9.00E-05	DNA-binding protein DF1
Mtr.11636.1.S1_at	137.57	374.63	222.47	108.78	At1g29160	6.00E-35	Dof zinc finger protein DOF1.5
Mtr.32790.1.S1_at	613.63	710.97	484.73	1469.82	At1g54070	2.00E-11	*Dormancy/auxin associated protein-related*
Mtr.30698.1.S1_at	215.79	369.09	468.40	193.65	At4g39780	3.00E-11	*DREB subfamily A-6 of ERF/AP2 transcription factor family*
Mtr.12426.1.S1_at	470.77	730.50	612.31	467.51	At3g60190	2.00E-38	Dynamin-like protein
Mtr.40245.1.S1_s_at	1035.42	1744.94	2075.96	570.82	At3g22840	2.00E-24	Early light inducible protein / *Chlorophyll A-B binding family protein*
Mtr.43949.1.S1_at	198.58	227.38	295.68	215.22	At2g26830	8.00E-23	Ethanolamine kinase
Msa.1155.1.S1_at	92.73	143.89	130.07	108.06	At3g26618	5.00E-79	*Eukaryotic release factor 1 family protein*
Mtr.31293.1.S1_s_at	43.47	84.27	64.56	17.27	At1g12920	8.00E-13	*Eukaryotic release factor 1 family protein*
Msa.2711.1.S1_at	62.24	56.59	52.15	74.92	At4g17940	2.00E-35	*Expressed protein*
Mtr.13130.1.S1_at	462.69	521.22	574.07	321.91	At1g67600	2.00E-28	*Expressed protein*

Table S6 (continued)

	Raw expression values in shoots						
	Without caesium		With caesium				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.18422.1.S1_at	108.78	178.68	106.95	24.25	At5g04550	2.00E-33	*Expressed protein*
Mtr.19877.1.S1_at	60.52	121.14	100.52	18.96	At5g54790	6.00E-05	*Expressed protein*
Mtr.26902.1.S1_at	29.91	61.05	59.53	45.02	At1g54990	5.00E-10	*Expressed protein*
Mtr.27397.1.S1_at	647.94	368.75	389.74	337.54	At2g04360	1.00E-49	*Expressed protein*
Mtr.31763.1.S1_at	367.45	199.74	236.14	226.28	At4g40045	2.00E-13	*Expressed protein*
Mtr.33522.1.S1_at	2051.18	1161.24	1145.18	1410.36	At1g55370	1.00E-26	*Expressed protein*
Mtr.33829.1.S1_at	503.53	400.57	292.37	494.60	At2g35880	3.00E-44	*Expressed protein*
Mtr.34633.1.S1_at	164.84	131.96	94.96	153.48	At2g21180	7.00E-19	Expressed protein
Mtr.38615.1.S1_at	653.52	1308.81	770.10	1025.03	At4g19700	4.00E-28	*Expressed protein*
Mtr.39059.1.S1_at	77.12	79.58	51.41	101.76	At4g14380	3.00E-13	*Expressed protein*
Mtr.39079.1.S1_at	205.39	375.10	406.23	58.70	At1g29640	4.00E-16	*Expressed protein*
Mtr.40744.1.S1_at	1383.16	1018.68	939.61	1134.30	At5g65470	6.00E-43	*Expressed protein*
Mtr.40792.1.S1_at	245.52	346.55	264.85	168.99	At1g53380	4.00E-42	*Expressed protein*
Mtr.44054.1.S1_at	109.84	198.89	172.43	142.92	At5g47050	1.00E-25	*Expressed protein*
Mtr.44654.1.S1_at	668.53	355.76	341.10	425.09	At1g55370	2.00E-33	*Expressed protein*
Mtr.4993.1.S1_at	119.83	228.55	213.40	208.43	At2g28130	4.00E-16	*Expressed protein*
Mtr.8968.1.S1_at	3955.55	4444.72	5045.72	1980.58	At1g17710	2.00E-19	*Expressed protein*
Mtr.38045.1.S1_at	67.35	125.89	100.99	66.63	At4g40080	3.00E-09	Fiber protein Fb19 / *Epsin N-terminal homology (ENTH) domain-containing protein*
Mtr.27793.1.S1_s_at	336.37	187.29	190.48	184.09			Galactosidase like protein
Mtr.42348.1.S1_at	54.80	93.40	73.14	38.34	At3g05320	2.00E-19	GDP-fucose protein-O-fucosyltransferase 1
Msa.1132.1.S1_at	161.78	87.93	70.98	191.98	At1g29660	1.00E-43	*GDSL-motif lipase/hydrolase family protein*
Mtr.27387.1.S1_at	593.75	109.29	102.92	120.73	At1g33811	2.00E-76	*GDSL-motif lipase/hydrolase family protein*
Mtr.29989.1.S1_at	89.99	26.28	29.18	30.35	At1g33811	2.00E-43	*GDSL-motif lipase/hydrolase family protein*
Mtr.42392.1.S1_at	122.68	62.75	55.94	124.48	At4g18970	2.00E-54	*GDSL-motif lipase/hydrolase family protein*
Mtr.38883.1.S1_at	78.36	72.50	49.69	110.64	At1g78440	3.00E-34	Gibberellin 2-oxidase
Mtr.1798.1.S1_s_at	407.60	370.63	310.19	518.69	At1g66200	8.00E-34	Glutamine synthetase
Msa.2885.1.S1_at	9641.88	6826.59	7143.95	7008.88	At1g32470	2.00E-43	*Glycine cleavage system H protein*
Mtr.8969.1.S1_at	284.44	187.88	137.62	160.79	At2g27500	3.00E-56	*Glycosyl hydrolase family 17 protein*
Mtr.44959.1.S1_at	49.44	42.10	23.09	49.66			Guanine nucleotide releasing factor 1
Mtr.34734.1.S1_at	59.22	78.81	127.05	32.53	At4g10310	2.00E-23	High affinity potassium transporter 2 / *Sodium transporter (HKT1)*
Mtr.41103.1.S1_at	655.18	451.95	500.99	501.53	At1g79870	4.00E-61	Hydroxyphenylpyruvate reductase
Mtr.14242.1.S1_s_at	180.99	691.99	210.24	141.77			Hypothetical protein

Table S6 (continued)

	Raw expression values in shoots						
	Without caesium		With caesium				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.16694.1.S1_at	39.46	81.99	64.30	30.79			Hypothetical protein
Mtr.17058.1.S1_at	75.76	59.31	48.59	70.50			Hypothetical protein
Mtr.19029.1.S1_s_at	187.54	247.27	178.91	182.86			Hypothetical protein
Mtr.26005.1.S1_at	311.40	537.65	363.98	294.75			Hypothetical protein
Mtr.5470.1.S1_x_at	141.91	107.19	57.59	223.46			Indole-3-acetic acid induced protein
Mtr.41616.1.S1_at	489.45	338.61	355.93	458.66	At4g24220	1.00E-63	Induced upon wounding stress
Mtr.13915.1.S1_at	249.08	143.07	194.68	178.63	At1g78620	2.00E-24	*Integral membrane family protein*
Mtr.1575.1.S1_at	114.95	65.90	69.24	73.99	At5g08100	2.00E-31	L-asparaginase
Mtr.7591.1.S1_at	54.00	106.21	71.41	25.43	At4g29880	3.00E-23	*Leucine-rich repeat family protein*
Mtr.43705.1.S1_at	101.86	129.01	137.93	69.73	At2g19190	4.00E-29	Light repressible receptor protein kinase
Mtr.8462.1.S1_at	1167.93	1487.70	1268.08	832.04	At1g72520	4.00E-40	Lipoxygenase
Mtr.26293.1.S1_s_at	72.68	134.37	113.29	78.18	At3g21630	5.00E-51	LysM domain-containing receptor-like kinase 7
Mtr.11716.1.S1_at	149.95	262.06	273.47	145.18	At5g61640	8.00E-61	Methionine sulfoxide reductase A
Mtr.44950.1.S1_at	468.91	339.64	195.38	416.92	At5g44680	3.00E-05	*Methyladenine glycosylase family protein*
Mtr.34806.1.S1_s_at	115.86	224.24	154.20	69.02	At1g11310	3.00E-16	MLO-like protein 2
Mtr.37525.1.S1_at	994.15	147.23	285.03	244.75			Multifunctional aquaporin
Mtr.11942.1.S1_at	254.98	129.44	173.85	190.81	At3g10760	4.00E-07	*Myb family transcription factor*
Mtr.44930.1.S1_s_at	56.06	57.76	32.69	68.44	At3g28910	2.00E-29	MYB96 transcription factor-like protein
Mtr.17230.1.S1_at	19.19	60.12	34.35	11.13	At3g17630	2.00E-16	Na+/H+ antiporter-like protein
Mtr.11021.1.S1_at	449.67	1236.93	1082.46	583.60	At1g69490	7.00E-05	NAC domain protein NAC2
Mtr.2375.1.S1_at	26.87	64.65	33.02	13.33			Neuraminidase B
Mtr.44141.1.S1_at	38.47	58.26	66.78	37.34	At5g64530	2.00E-13	*No apical meristem (NAM) family protein*
Mtr.2673.1.S1_s_at	1053.02	709.50	788.82	1310.16	At1g75500	7.00E-33	*Nodulin MtN21 family protein*
Mtr.2424.1.S1_at	24.90	66.90	36.37	28.33			Nodulin-like protein
Mtr.31850.1.S1_at	103.39	146.20	161.21	123.37	At1g06560	2.00E-21	*NOL1/NOP2/sun family protein*
Msa.2628.1.S1_at	153.17	93.69	94.52	93.25	At4g33490	9.00E-57	*Nucellin protein*
Mtr.4724.1.S1_at	957.33	1832.35	1527.65	607.07	At3g20660	2.00E-23	Organic anion transporter-like protein / *Sugar transporter family protein*
Mtr.12532.1.S1_at	7661.93	4146.89	6397.11	5183.78	At4g37070	2.00E-16	Patatin-like protein 1
Mtr.47230.1.S1_at	39.47	71.25	60.14	60.71	At1g08610	2.00E-23	Pentatricopeptide (PPR) repeat-containing protein
Mtr.51559.1.S1_at	86.22	50.30	52.53	75.04	At4g33490	6.00E-64	Peptidase aspartic / *Nucellin protein*
Mtr.39868.1.S1_at	35.33	54.20	55.05	56.01	At1g06900	8.00E-61	*Peptidase M16 family protein*
Msa.1821.1.S1_at	81.23	133.26	130.22	74.42	At5g61640	2.00E-45	*Peptide methionine sulfoxide reductase*

Table S6 (continued)

Medicago probes	Raw expression values in shoots: Without caesium Non myc	Without caesium Myc	With caesium Non myc	With caesium Myc	AGI ID	e-value	MtGEA Target Description / *TAIR Target Description*
Msa.1262.1.S1_at	250.65	294.87	301.30	191.71	At2g39970	7.00E-36	*Peroxisomal membrane protein (PMP36)*
Mtr.6484.1.S1_at	951.23	1824.33	978.62	360.21	At2g32830	3.00E-05	Phosphate transporter 2
Mtr.33734.1.S1_at	145.57	73.78	101.01	96.48	At1g58280	1.00E-15	Phosphoglycerate mutase-like protein
Mtr.41408.1.S1_at	40.59	114.64	84.69	101.63	At1g52700	5.00E-49	*Phospholipase/carboxylesterase family protein*
Mtr.37092.1.S1_at	1414.28	742.85	981.25	968.41			Photosystem II M protein
Mtr.38209.1.S1_at	1276.40	928.65	676.55	1357.58			Polysaccharide ABC transporter
Mtr.51989.1.S1_s_at	34.68	60.10	39.86	23.84	At2g26650	7.00E-34	Potassium channel
Mtr.1874.1.S1_s_at	189.87	234.61	203.95	129.24			Potential phospholipid-transporting ATPase 9
Mtr.17054.1.S1_s_at	616.50	410.14	505.18	440.92	At5g19370	2.00E-69	PpiC-type peptidyl-prolyl cis-trans isomerase / *Rhodanese-like domain-containing protein*
Mtr.43279.1.S1_at	112.49	314.91	353.10	290.95	At3g30775	3.00E-50	Proline dehydrogenase
Msa.1490.1.S1_at	224.59	65.24	61.30	140.18	At4g38770	5.00E-18	*Proline-rich family protein (PRP4)*
Mtr.38468.1.S1_at	1146.14	265.34	294.97	711.17	At4g38770	2.00E-31	Proline-rich protein
Mtr.39238.1.S1_at	2590.93	909.95	1058.60	1101.40	At3g16370	1.00E-61	Proline-rich protein APG-like
Mtr.12213.1.S1_at	407.41	342.90	359.49	705.72	At3g53980	2.00E-37	*Protease inhibitor*
Mtr.9552.1.S1_at	183.95	381.68	228.62	206.23	At1g50700	5.00E-08	Protein kinase
Mtr.11345.1.S1_at	77.70	107.33	117.24	63.04			Protein kinase APK1A
Mtr.26005.1.S1_s_at	492.71	860.21	610.85	496.37	At2g45910	2.00E-62	*Protein kinase family protein*
Mtr.33566.1.S1_at	140.08	238.05	192.49	64.41	At5g47740	7.00E-19	*Protein kinase family protein*
Mtr.36136.1.S1_at	494.16	756.13	617.40	330.88	At2g33580	2.00E-23	*Protein kinase family protein*
Mtr.46263.1.S1_at	120.93	190.65	137.35	134.65	At4g14350	9.00E-67	Protein kinase-like
Mtr.9846.1.S1_at	54.23	94.32	77.01	50.28	At1g44750	3.00E-57	*Purine permease family protein*
Mtr.26299.1.S1_at	1537.64	2534.83	2035.39	591.13	At3g52820	2.00E-75	Purple acid phosphatase (PAP22)
Mtr.24207.1.S1_at	107.87	183.85	143.99	26.18	At3g15890	1.00E-29	Receptor protein kinase PERK1-like protein
Mtr.5291.1.S1_at	336.23	120.57	178.32	127.01	At1g45207	2.00E-15	*Remorin family protein*
Mtr.25774.1.S1_x_at	218.16	661.89	249.48	177.57			Reverse transcriptase
Mtr.8645.1.S1_at	180.90	293.97	245.40	202.44			Riboflavin biosynthesis protein ribA
Mtr.52094.1.S1_at	179.51	98.79	103.82	129.28			Ribosomal protein L14
Mtr.34372.1.S1_at	43.66	108.47	72.53	45.08	At2g07725	6.00E-10	Ribosomal protein L5
Mtr.39857.1.S1_at	654.52	360.40	439.01	496.35			Ribosomal protein S14
Mtr.19517.1.S1_at	15702.92	10707.55	11264.72	11010.34	At5g38410	8.00E-05	Ribulose bisphosphate carboxylase, small chain
Mtr.6778.1.S1_at	97.82	72.38	66.48	105.22	At1g32490	5.00E-20	RNA helicase-like
Mtr.14729.1.S1_at	536.19	428.17	379.33	675.37	At4g17940	1.00E-35	RNA-processing protein

Table S6 (continued)

Medicago probes	Raw expression values in shoots: Without caesium Non myc	Without caesium Myc	With caesium Non myc	With caesium Myc	AGI ID	e-value	MtGEA Target Description / *TAIR Target Description*
Msa.2701.1.S1_at	772.30	739.95	920.54	344.77	At1g27730	2.00E-19	*Salt tolerance zinc finger protein*
Mtr.24391.1.S1_at	850.21	791.19	570.51	1038.61	At3g22550	1.00E-15	Senescence-associated protein-related
Mtr.50713.1.S1_at	132.56	142.58	181.07	114.96	At1g09140	6.00E-16	SF2/ASF splicing modulator
Msa.875.1.S1_at	361.96	469.54	456.99	304.92	At3g61160	6.00E-25	*Shaggy-related protein kinase beta*
Mtr.40819.1.S1_at	102.25	300.35	188.97	79.91	At1g53300	3.00E-16	*Thioredoxin family protein*
Mtr.15318.1.S1_at	1077.39	660.85	853.11	676.67	At1g76760	6.00E-47	Thioredoxin-related
Mtr.10644.1.S1_at	1413.18	1687.35	1706.56	1287.52	At1g12230	2.00E-52	Transaldolase ToTAL2
Mtr.40304.1.S1_at	533.57	625.38	668.04	412.12	At4g16820	3.00E-54	Triacylglycerol lipase like protein
Msa.1179.1.S1_at	103.54	72.61	75.45	83.31	At2g45330	3.00E-18	*tRNA 2'phosphotransferase*
Mtr.33389.1.S1_at	99.60	145.63	138.16	145.15	At1g16800	2.00E-05	*tRNA-splicing endonuclease positive effector-related*
Mtr.45215.1.S1_at	338.16	581.87	407.21	265.72	At2g41560	4.00E-22	Type IIB calcium ATPase
Mtr.20215.1.S1_at	22.43	63.10	27.27	12.77			Uncharacterized Cys-rich domain
Mtr.7906.1.S1_at	289.64	802.87	392.80	148.39	At1g30755	9.00E-12	Vasopressin V1b receptor variant
Mtr.21379.1.S1_at	156.20	192.85	232.54	166.44	At5g49430	2.00E-73	WD-40 repeat / *Transducin family protein*
Mtr.49829.1.S1_at	82.65	120.18	112.92	57.80	At1g15750	6.00E-11	*WD-40 repeat family protein*
Mtr.43632.1.S1_at	639.68	919.83	884.67	563.26			Wound-induced GSK-3-like protein
Mtr.10071.1.S1_at	27.34	51.70	58.22	21.81			WRKY transcription factor 47
Mtr.41226.1.S1_at	34.33	59.60	74.04	32.91	At1g29280	8.00E-14	WRKY transcription factor 65
Mtr.41302.1.S1_at	158.31	113.78	109.32	130.06	At3g09770	2.00E-25	*Zinc finger (C3HC4-type RING finger) family protein*
Mtr.8696.1.S1_at	1500.81	1740.12	1906.91	830.08			Zinc finger DNA-binding protein
Mtr.5436.1.S1_at	287.66	142.95	159.67	183.38	At5g60850	4.00E-28	Zinc finger protein
Mtr.18592.1.S1_at	39.08	166.71	117.03	16.85	At2g28710	2.00E-23	Zn-finger, C2H2 type
Mtr.10123.1.S1_at	135.50	196.60	188.11	117.66			No annotation
Mtr.12772.1.S1_at	232.42	424.26	302.41	124.80			No annotation
Mtr.2174.1.S1_at	30.64	91.48	56.57	24.57			No annotation
Mtr.22211.1.S1_at	48.02	104.38	66.52	23.78			No annotation
Mtr.27999.1.S1_at	63.69	25.83	33.00	33.64			No annotation
Mtr.28014.1.S1_at	15.19	66.01	30.54	13.96			No annotation
Mtr.28311.1.S1_s_at	341.13	234.15	168.89	773.47			No annotation
Mtr.28477.1.S1_at	127.11	110.32	49.25	95.74			No annotation
Mtr.29127.1.S1_at	98.24	131.31	134.42	35.93			No annotation
Mtr.29414.1.S1_at	28.70	53.15	55.31	22.03			No annotation

Table S6 (continued)

	Raw expression values in shoots						
	Without caesium		With caesium				
***Medicago* probes**	**Non myc**	**Myc**	**Non myc**	**Myc**	**AGI ID**	**e-value**	**MtGEA Target Description / *TAIR Target Description***
Mtr.31523.1.S1_at	127.91	193.81	165.09	113.22			No annotation
Mtr.35562.1.S1_at	31.79	90.32	20.38	16.43			No annotation
Mtr.37091.1.S1_at	2760.26	1117.76	1457.40	1920.30			No annotation
Mtr.39777.1.S1_at	854.69	621.34	536.85	677.96			No annotation
Mtr.40613.1.S1_at	523.88	431.81	304.00	1150.12			No annotation
Mtr.43176.1.S1_s_at	33.03	69.56	40.13	50.56			No annotation
Mtr.43974.1.S1_at	39.14	86.67	62.78	39.17			No annotation
Mtr.44546.1.S1_at	171.09	268.26	230.11	83.92			No annotation
Mtr.44843.1.S1_at	94.83	119.58	124.45	92.78			No annotation
Mtr.4597.1.S1_at	651.51	1066.40	644.58	588.16			No annotation
Mtr.46651.1.S1_x_at	2213.46	829.52	1001.81	1574.76			No annotation
Mtr.47257.1.S1_at	5528.03	3991.30	3865.47	6277.23			No annotation
Mtr.7207.1.S1_at	608.68	1064.24	771.18	700.12			No annotation
Mtr.8309.1.S1_at	80.65	202.33	100.03	24.09			No annotation
Mtr.9377.1.S1_at	156.59	119.42	77.07	139.70			No annotation
Mtr.9483.1.S1_at	1927.79	1136.32	1293.89	1137.47			No annotation

Table S7: Gene ontology classification of *Medicago truncatula* genes in roots that where significantly differentially expressed due to the presence of Cs in the medium (Table S1). The classification was based on AmiGO using *Arabidopsis thaliana* orthologs with the whole genome of *Arabidopsis* as background.

GO Term (Biological Process)	**P-value**	**Sample frequency**	**Background frequency**	**Genes**
GO:0019748 secondary metabolic process	1.55E-03	11/145 (7.6%)	369/29974 (1.2%)	At1g15950; At1g58170; At2g29090; At2g29470; At3g04870; At4g25420; At4g32810; At4g36220; At5g17230; At5g23960; At5g25900
GO:0044255 cellular lipid metabolic process	8.58E-03	11/145 (7.6%)	442/29974 (1.5%)	At1g43800; At1g49430; At2g29090; At3g04870; At4g15560; At4g25420; At4g32810; At5g17230; At5g23960; At5g25900; At5g60600
GO:0006720 isoprenoid metabolic process	1.36E-05	9/145 (6.2%)	132/29974 (0.4%)	At2g29090; At3g04870; At4g15560; At4g25420; At4g32810; At5g17230; At5g23960; At5g25900; At5g60600
GO:0006721 terpenoid metabolic process	3.50E-04	7/145 (4.8%)	96/29974 (0.3%)	At2g29090; At3g04870; At4g25420; At4g32810; At5g17230; At5g23960; At5g25900
GO:0008299 isoprenoid biosynthetic process	8.32E-04	7/145 (4.8%)	109/29974 (0.4%)	At3g04870; At4g15560; At4g25420; At5g17230; At5g23960; At5g25900; At5g60600
GO Term (Molecular Function)	**P-value**	**Sample frequency**	**Background frequency**	**Genes**
GO:0019825 oxygen binding	1.15E-04	10/145 (6.9%)	223/29974 (0.7%)	At2g02580; At2g24180; At2g29090; At2g46950; At3g14680; At3g26290; At3g26300; At4g31950; At5g24910; At5g25900
GO:0016491 oxidoreductase activity	7.16E-06	25/145 (17.2%)	1363/29974 (4.5%)	At1g15950; At1g18270; At1g43800; At1g52820; At2g02580; At2g24180; At2g29090; At2g46950; At3g04870; At3g14680; At3g22400; At3g26290; At3g26300; At4g25420; At4g31950; At4g32810; At4g36220; At5g05340; At5g16970; At5g24910; At5g25900; At5g37980; At5g42180; At5g59540; At5g60600
GO:0015020 glucuronosyltransferase activity	7.17E-03	3/145 (2.1%)	9/29974 (0.0%)	At1g22360; At1g22370; At2g28110
GO:0009055 electron carrier activity	7.39E-03	11/145 (7.6%)	435/29974 (1.5%)	At2g02580; At2g24180; At2g44790; At2g46950; At3g14680; At3g22400; At3g26290; At3g26300; At4g31950; At5g24910; At5g42180
GO:0005506 iron ion binding	7.54E-03	10/145 (6.9%)	355/29974 (1.2%)	At2g02580; At2g24180; At2g46950; At3g14680; At3g22400; At3g26290; At3g26300; At4g31950; At5g24910; At5g42180

Table S8: Gene ontology classification of *Medicago truncatula* genes in shoots that where significantly differentially expressed due to the presence of Cs in the medium (Table S4). The classification was based on AmiGO using *Arabidopsis thaliana* orthologs with the whole genome of *Arabidopsis* as background.

GO Term (Molecular Function)	P-value	Sample frequency	Background frequency	Genes
GO:0003824 catalytic activity	6.73E-04	34/63 (54.0%)	7615/29974 (25.4%)	At1g06900; At1g07870; At1g11190; At1g32470; At1g35720; At1g59960; At1g65890; At1g66970; At1g71500; At1g76760; At2g26830; At2g27500; At2g31270; At2g35660; At2g41510; At2g41560; At2g41680; At2g46950; At2g46960; At3g15520; At3g16370; At3g26060; At3g30775; At3g63440; At4g12270; At4g28730; At4g33490; At4g34980; At4g37050; At5g05460; At5g18170; At5g18670; At5g23960; At5g38410
GO:0016491 oxidoreductase activity	8.22E-06	16/63 (25.4%)	1363/29974 (4.5%)	At1g32470; At1g35720; At1g59960; At1g71500; At1g76760; At5g18170; At2g35660; At2g41510; At2g41680; At2g46950; At2g46960; At3g26060; At3g30775; At3g63440; At4g12270; At4g28730

Table S9: Gene ontology classification of *Medicago truncatula* genes in shoots that where significantly differentially expressed due to arbuscular mycorrhiza (Table S5). The classification was based on AmiGO using *Arabidopsis thaliana* orthologs with the whole genome of *Arabidopsis* as background.

GO Term (Molecular Function)	P-value	Sample frequency	Background frequency	Genes
GO:0003824 catalytic activity	6.16E-05	108/268 (40.3%)	7615/29974 (25.4%)	At1g02930; At1g03220; At1g06410; At1g06620; At1g06900; At1g10550; At1g11190; At1g12410; At1g16670; At1g17020; At1g17650; At1g17710; At1g17840; At1g19920; At1g23740; At1g24170; At1g26160; At1g29810; At1g30040; At1g31860; At1g32470; At1g33290; At1g35720; At1g50700; At1g52700; At1g56190; At1g63180; At1g65890; At1g66180; At1g67280; At1g69740; At1g71500; At1g74070; At1g75280; At1g76760; At1g77420; At1g78440; At1g79870; At2g02500; At2g14750; At2g18950; At2g20830; At2g21280; At2g22680; At2g26540; At2g26640; At2g28110; At2g29990; At2g31270; At2g31970; At2g33170; At2g35450; At2g38270; At2g39770; At2g41510; At2g41680; At2g47590; At3g01180; At3g01480; At3g04220; At3g11630; At3g11950; At3g15090; At3g15520; At3g16370; At3g20630; At3g23840; At3g26060; At3g43270; At3g44620; At3g57240; At3g61680; At4g03390; At4g05090; At4g09010; At4g11980; At4g14350; At4g15440; At4g25080; At4g25310; At4g28706; At4g28730; At4g33490; At4g33510; At4g33950; At4g36810; At4g37070; At4g37890; At5g01210; At5g04610; At5g06290; At5g14470; At5g15870; At5g17230; At5g18660; At5g18670; At5g19370; At5g20250; At5g20550; At5g20870; At5g34930; At5g37600; At5g38410; At5g44380; At5g45930; At5g47910; At5g54160; At5g59810
GO:0051920 peroxiredoxin activity	2.97E-03	3/268 (1.1%)	4/29974 (0.0%)	At3g11630; At3g26060; At5g06290

GO Term (Cellular Component)	P-value	Sample frequency	Background frequency	Genes
GO:0009507 chloroplast	3.27E-21	74/268 (27.6%)	2179/29974 (7.3%)	At1g03600; At1g12250; At1g12410; At1g16080; At1g17650; At1g19150; At1g19920; At1g23740; At1g26160; At1g31860; At1g32500; At1g35720; At1g45474; At1g54500; At1g58280; At1g60000; At1g60950; At1g67280; At1g67740; At1g69740; At1g71500; At1g74070; At1g74970; At1g75350; At1g75690; At1g76760; At1g78620; At2g02500; At2g14750; At2g18950; At2g21280; At2g26540; At2g31270; At2g32590; At2g35450; At2g38270; At2g38550; At2g41680; At2g45180; At2g47450; At3g01180; At3g01480; At3g15520; At3g26060; At3g44620; At3g50700; At3g52380; At3g55330; At3g55560; At3g59980; At3g61680; At3g61870; At4g02530; At4g02770; At4g05090; At4g09010; At4g11980; At4g14890; At4g15440; At4g17560; At4g25080; At4g28660; At4g28730; At4g33510; At4g36810; At5g06290; At5g17230; At5g17520; At5g18660; At5g20250; At5g38410; At5g43745; At5g45930; At5g64290