Chapter 6
Conclusion and future work

Abscission is an important process in the life cycle of a plant. In this project, an abscission-related gene *G2 (At1g64405)* was identified and characterized in order to understand more about the mechanism in regulating the process of organ shedding. Our reporter gene analysis has shown that the expression of *G2* takes place specifically in AZ cells and correlates with the organ separation. The expression pattern of *G2* is changed when introduced into genetic backgrounds such as *ida, 35S:IDA* and *bop1/bop2,* supporting an abscission-related role of *G2*. Reporter gene analysis of *G2* revealed that expression was also apparent in root cap, lateral root cap and cortical cells overlaying lateral root primordia, suggesting that *G2* may play a role in cell separation. *G2:GUS* expression was also detected at the sites of wounding, suggesting that *G2* could contribute to protecting plants against pathogenic attack after wounding or organ shedding.

G2 is auxin-inducible and expressed in AZ and cortical cells overlying lateral root primordia. These features are shared with *IDA.* Our reporter gene analysis showed an inverse correlation between *G2* and *IDA* as the wounding-induced expression of *G2* was enhanced in an *ida* background but reduced when introduced into a *35S:IDA* background. *In silico* analysis provided additional evidence of this inverse correlation between the expression of the two genes. A further study showed that the ectopic expression of *G2* could recover the abscission-related phenotype associated with the overexpression of *IDA.* RT-PCR analysis showed that *IDA* expression was down-regulated in a *35S:G2* background, and expression of both *IDA* and *G2* were down-regulated in *35S:G2 x 35S:IDA* homozygotes. The above data suggest that *G2* and *IDA* may contribute to a common pathway in regulating the abscission process.

Overexpression of *G2* causes root hairs to be extremely swollen (type I hairs), randomly forming bulges (type II hairs) and branched and crooked (type III hairs). Our data suggest that the initiation and elongation/tip growth process of root hair development are likely to be disrupted in *35S:G2* plants. The morphology of type I hairs was similar to that reported for the mutant *reb1-1 (rhd1)* (Schiefelbein and Somerville, 1990; Andeme-Onzighi *et al*., 2002). It has been shown that the bulging effect of root hairs in mutant *reb1-1* is caused by the absence of AGPs which leads to a disruption of cortical MTs (Andeme-Onzighi *et al*., 2002). Overexpression of *G2* has been shown to prevent AGPs from being secreted in floral organ AZ possibly through down-regulation of ectopically expressed *IDA.*

Therefore overexpression of *G2* could have a similar effect to *reb1-1* on root hair development and be caused by an absence of AGP. It has been suggested that overexpression of *G2* could down-regulate *IDA-LIKE* genes (most likely *AtIDL1*), which prevents AGP from being secreted by the root. Ectopic expression of *IDA* in a *35S:G2* background suppresses the formation of type I hairs. The reason for this effect could be that overexpression of *IDA* promotes the secretion of AGPs into root cortical cells. Approaches to test this hypothesis include that RT-PCR/QPCR analysis strategy could be carried out in root tissue to investigate the expression of *AtIDL* genes in a *35S:G2* background. Secondly, the null lines of the *AtIDLs* could be obtained and the phenotype characterized. In addition, an immunofluorescence labelling strategy could be applied to localize AGP and MT distribution in *35S:G2* root tissues.

Our data have also shown that overexpression of *G2* disrupts the normal tip growth process and morphology of root hairs (type II and type III hairs). Roots treated with MT disruption drug display similar phenotype with *35S:G2* plants as branched and crooked hairs (Bibikova *et al.,* 1999), therefore it is likely that overexpression of *G2* disrupts root hair MTs. In the future work, this hypothesis could be tested by an immunolocalization strategy carried out in the root. Similar phenotypes as type II and type III hairs were observed in several mutants of root hair development related genes, therefore it is worthwhile to investigate the gene expression in roots in a *35S:G2* background.

Our bioinformatics analysis revealed nineteen proteins from different plant species that shared four conserved motifs and might serve a similar function to G2. Our data showed that the four motifs might play an important role in G2 function. Further analysis revealed nine genes from *Arabidopsis thaliana* that contain two motifs conserved with G2 at N' and C' termini respectively. These proteins may form a new novel family in which the two conserved motifs play an important role in the protein function. *At1g10530,* one of the nine genes, has been shown to be specifically expressed in the AZ from Genevestigator_V3 results. Down-regulation of *G2* failed to lead to a detectable abscission-related phenotype. It is possible that *G2* is functional redundant with *At1g10530.* Future work could generate double KO of *G2* and *at1g10530* and study its abscission-relate phenotype.

This thesis has explored various aspects of *G2* including expression pattern, potential functions, correlation with *IDA* and protein structural analysis. Further work will be necessary to dissect the role of *G2* in additional in detail.

A promoter analysis was also carried out in this project. The Motif "**AATATACATT**" was identified from two abscission-related PGs and proposed to play a role in regulating gene expression in AZ. The motif was fused to a minimal promoter and the reporter gene *GUS* in order to test if it could promote GUS expression specifically in the AZ. The results showed that no GUS signal could be detected. One explanation for this is that

motif “**AATATACATT**” acts with other motifs to form a complex in order to regulate gene expression. To test this hypothesis, this motif could be removed from the 67 bp fragment and the resulting sequence used to test for abscission-related GUS expression.

In abscission related research, little is known about the mechanisms that regulate and co-ordinate gene expression, therefore it is important to carry out promoter analysis of abscission-related genes. Identification of new potential abscission-related motifs could be a fruitful area for future study.