IDENTIFYING TRAITS AND DEVELOPING GENETIC SOURCES FOR INCREASED LODGING RESISTANCE IN ELITE HIGH YIELDING WHEAT CULTIVARS

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Abstract

Lodging is a persistent phenomenon that reduces grain quality and grain yield of wheat. It is defined as the permanent displacement of the plant/shoots from their vertical position. During the Green revolution, wheat plant height was reduced to avoid lodging and allowed growers to increase nitrogen fertilization. This resulted in a considerable increase of grain yield. After the Green Revolution, plant growth regulators were used to further reduce plant height which continued increasing lodging resistance and grain yield. However, lodging susceptibility has not disappeared completely and as yields increase, there is evidence that growers cannot continue to rely on these strategies, raising the question of how to further improve lodging resistance of wheat. Collaborative studies by physiologists, biologists and engineers generated a deeper understanding of lodging (through stem and anchorage failure mechanisms) and the development of models of the lodging process, together with better crop husbandry or agronomic management strategies. Most of these studies were made on winter wheat under a reduced range of environmental conditions. This thesis attempted to further develop lodging mechanisms and models using spring wheat and a wider range of environmental and agronomic conditions and to investigate the genetic control of lodging-proof traits. Field experiments on spring wheat cultivars were carried out in irrigated conditions in NW Mexico and on a winter wheat Avalon x Cadenza doubled-haploid population in rainfed conditions in the UK. A lodging-proof crop was designed for spring wheat growing in NW Mexico that suggests the need for both increased structural stem biomass and a wider root plate spread (anchorage strength). The model also infers that trade-offs with grain yield will occur, mainly because

of overlapping of the development of lodging traits with grain yield formation processes. Rapid selection tools for lodging resistance will play a primary role if lodging resistance is to be improved concurrently with grain yield. Fine mapping and validation of QTLs related to lodging traits identified in this study can be used to develop reliable genetic markers that can accelerate selection for lodging resistance concurrently with improvement of genetic yield potential.

This thesis is dedicated to:

Mum, dad, brothers and sisters

Fernando and Maria Edith

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ºC	Degree centigrade
AD	Anthesis date
ADAS	Agricultural Development and Advisory Service
ADL	Acid detergent lignin
AFM/AS	Anchorage strength
CCRC	Complex Carbohydrate Research Center
CENEB	Campo Experimental Norman E. Borlaug
CG	Height at centre of gravity
CIMCOG	CIMMYT Mexico Core Germplasm Panel
CIMMYT	Centro Internacional de Mejoramiento de Maiz y Trigo
cM	centiMorgan
cm	centimetre
cm^2	squared centimetre
CV	coefficient of variation
d	days
DAE	Days after emergence
DArT	Diversity Arrays Technology
DEFRA	Department of Environment, Food and Rural Affairs
DH	Doubled haploid
DM	dry matter
EA	ear area
ENPP	Ear number per plant
FAO	Food and Agriculture Organization of the United Nations
g	gram
G×E	Genotype by environment interaction
GF	Grain filling
GS	Growth stage
GY	Grain yield
H^2	Broad sense heritability
HD	Heading date
HGCA	Home-Grown Cereal Authority
HI	Harvest index
Hz	Hertz
IBS	internode breaking strength
SD	Stem diameter
SS	Stem strength
SIL	Internode length
SMS	Stem material strength
SWW	Stem wall width
kg ha^{-1}	kilogram per hectare
kn	kilometer
kPa	kilopascal

LS	Lodging score
LTM	Long term mean
m	metre
m s^{-1}	metre per second
m^2	squared metre
MAS	Marker-assisted selection
masl	Metre above sea level
META	Multienvironment analysis
mg mm^{-1}	miligram per milimetre
MJ m^{-2}	Megajoule per squared meter
mm	milimetre
MPa	Megapascal
N	Nitrogen
N mm	Newton milimitre
NABIM	National Association of British and Irish Millers
NDVI	Normalized difference vegetation index
NF	Natural frequency
NW	North West
P	Phosporus
P value	Probability value
PA	Pre-anthesis
PH	Plant height
PVE	Phenotypic variation explained
QTL	Quantitative trait loci
r	coefficient of correlation
R^2	coefficient of determination
RDW	Root dry weight
REML	restricted maximum likelihood
RPS	Root plate spread
Rs	Random sample
RUE	radiation use efficiency
s	second
SED	standard error of differences of means
SNP	Single nucelotide polymorphism
SRD	structural rooting depth
SSR	Simple sequence repeat
t ha^{-1}	tonne per hectare
TGW	Thousand grain weight
UK	United Kingdom
UN	United Nations
WGIN	Wheat Genetic Improvement Network
WSC	Water soluble carbohydrate

Chapter 1 Introduction

1.1 Importance of wheat for the global food security and strategies to increase its production

Wheat (*Triticum aestivum* L.) is the largest primary commodity (FAO, 2014) grown more than any other crop in a wide range of latitudes, temperatures, water conditions and nutritional levels (Reynolds et al., 2012). It provides about 19 % of the total calories for the world's population (FAO, 2014). Considering the UN forecasts the world population will reach 9.4 billion by 2050 and factors such as climate change are reducing crop productivity in key regions due to increased heat and water stress (Lobell et al., 2008), a substantial increase in wheat yield production accounting for climate change scenario is required to ensure food security in future decades (Foulkes et al., 2011; Parry et al., 2011; Reynolds et al., 2011). Thus, this substantial increase of crop yield will require an increased crop stress resistance together with improved water and fertilizer practices (Reynolds et al., 2011). Raising yield potential through breeding is the primary objective of novel initiatives to meet this multiple challenge (Foulkes et al., 2011; Parry et al., 2011; Reynolds et al., 2012, 2011). Yield potential is directly linked to both attainable and on-farm yields (Fischer and Edmeades, 2010) and in fact it has been stated that an increase in yield potential is expressed over a wide range of conditions (stressful abiotic conditions included) (Lantican et al., 2003). An example of increased yield potential in wheat breeding was given with the increased partitioning and HI resulted from the introgression of semi-dwarf genes during the Green Revolution (Slafer and Araus, 2007). However, it is well known that in modern wheat cultivars new strategies are necessary to obtain further genetic gains. In addition, these novel initiatives have fully embraced the need to improve simultaneously lodging resistance if genetic gains are to be realized in the agronomic context (Reynolds et al., 2011).

1.2 Lodging phenomenon, its effect on wheat production and strategies to avoid it

Lodging is described as the permanent displacement of the stems/shoots from the vertical position of small grained cereals and normally occurs after ear emergence (Berry et al., 2004; Pinthus, 1974), although lodging in earlier stages such as elongation can also occur (Easson et al., 1993). By consensus, it has been recognized that two types of lodging occur in these crops: stem and root lodging. The first results from the buckling of the lowest basal internodes and the second as a consequence of the failure anchorage system. Additionally, buckling of the middle internodes or “brackling” is observed in barley and oats while buckling of the peduncle or “necking” is only observed in barley (Berry et al., 2004).

Abundant water and nitrogen supply, dense canopies and warm temperatures have been identified as the main lodging-promoting factors in small grained cereals (wheat, barley, rice and oats) (Berry et al., 2004; Pinthus, 1974). In wheat, root lodging risk can be increased by reducing soil strength due excess of water supply in both high rainfall (Berry et al., 2003b; Easson et al., 1993) and irrigated environments (Fischer and Stapper, 1987). An increased fertilization combined with high seed rates has also been found to increase lodging in both winter wheat (under rainfall conditions) (Easson et al., 1993) and spring wheat (under irrigated conditions) (Stapper and Fischer, 1990; Tripathi et al., 2003). Tripathi et al. (2003) indicated that the lodging area, angle of displacement and lodging score can increase with N applications up to 240 kg N ha^{-1} in irrigated spring wheat, although further increases in N did not increase further the level of lodging. Winter wheat dense canopies linked to high seed rates and excessive N applications have been found to be more prone lodging than less dense canopies (Berry et al., 2000).

Together with barley and maize, wheat is one of the crops that shows the highest incidence of lodging, with losses already averaging $60 million per year in the UK alone, and lodging can reduce yield significantly and can cause several knock-on effects including reduced grain quality, greater drying costs and slower harvest (Berry, 1998; Berry et al., 2004). Significant yield losses also occur wherever wheat is irrigated (e.g. Mexico, India and Australia) because applying water weakens plant anchorage by softening the soil. Widespread lodging affects from 15 to 20% of the UK wheat growing area once every three or four years (Berry, 1998), although, Griffin (1998) indicated a lodging incidence of 10% every year. For the Yaqui Valley, a survey conducted during 1981 to 1991 (80 farmers' fields each year) indicated occurrence of lodging from 18 to 40% of the growing area in several years (Tripathi et al., 2004). Lodging can reduce grain yield from 7 to 80% (Acreche and Slafer, 2011; Berry and Spink, 2012; Easson et al., 1993; Fischer and Stapper, 1987; Tripathi et al., 2005; Weibel and Pendleton, 1964). Lodging losses are greatest when lodging occurs soon after flowering and when the angle of stem displacement from the vertical is high (Berry and Spink, 2012; Fischer and Stapper, 1987), can be as great as those caused by pest and disease (Pinthus, 1974). Lodging also increases susceptibility to pests and diseases (Berry et al., 2004; Pinthus, 1974) and induce negative effects on crop development (decreasing grain per m^2 and average grain weight) (Acreche and Slafer, 2011; Fischer and Stapper, 1987). It has been reported that lodging with an angle of 45° would cause a grain yield loss of 18% (Berry and Spink, 2012). Meanwhile, an angle of 80° from the vertical at anthesis would cause a grain yield loss in the range of 7 – 35% (Fischer and Stapper, 1987), 43 – 61% (Acreche and Slafer, 2011), and 54% (Berry and Spink, 2012). Considering the lodging problem in the context of the 76 000 ha of wheat harvested area in the Yaqui Valley (in NWM) every year (SIAP, 2016) the economic loss in a severe lodging year would be around US$29 million. This is assuming 40% of the area affected with yield losses around 50% and US$215 per tonne of wheat grain (Lantican et al., 2016). Such a percentage of wheat area affected with lodging has been observed by CIMMYT researchers in NWM and reported by (Tripathi et al., 2005). The percentage of yield loss would be the average of the upper values reported by the aforementioned researchers when lodging angle is around 80°. It can be speculated that for a worldwide scale there would be a grain yield loss of around 22 million tonnes every year (equivalent to US$4.7 billion assuming a global wheat price of US$215). Considering 1.0 t ha^{-1} of yield loss due to lodging in 10% of the world wheat growing area, which was 222 million ha in 2014 (Lantican et al., 2016).

Strategies to avoid lodging include the use of better crop management practices (Berry et al., 2004, 2003b, 2000; Pinthus, 1974). With the introduction of dwarfing genes during the 1960's, plant height of wheat was reduced which in turn reduced the plant leverage and increased lodging tolerance in the semi-dwarf modern wheat varieties. Plant growth regulators have been used to further decrease plant height by reducing shoot length (Berry et al., 2004). Nevertheless, lodging is a persistent phenomenon that still occurs. Crop management to reduce lodging include better practices for sowing, plant establishment, fertiliser applications and soil conditions. Delaying sowing has been shown to reduce lodging considerably (Berry et al., 2004). Berry et al. (2000) stated that sowing winter wheat 1.5 month earlier significantly increased stem base bending moment. This can be a consequence of longer stems (greater number of extended internodes) (Stapper and Fischer, 1990). Also, early sowing increases number of tillers (Green et al., 1985). These two aspects can result higher tiller competition and weaker stems due to narrower internodes and thinner stem walls with less dry matter per unit length (Berry, 1998; Berry et al., 2000). A reduced anchorage strength can also be a consequence of higher establishment (denser canopies) and shading associated with the early sowing. Wheat has been shown to respond to shading partitioning more assimilates to the extending stems rather than the roots (Kasperbauer and Karlen, 1986). Delaying spring irrigation as long as possible can reduce root lodging susceptibility caused by an excess of moisture (Pinthus, 1974) or avoiding irrigation when expecting windy conditions (Stapper and Fischer, 1990). More recent studies indicated that, for winter wheat crops under high rainfall conditions, a soil with less residual nitrogen at sowing and reducing and delaying the application of fertiliser in spring would reduce stem lodging risk. Previous research had already indicated that nitrogen supply affected both directly and indirectly the stem base of wheat through increased shading due to larger canopies (Mulder, 1954). This author stated that thickness of sclerenchymatous ring and cell walls tended to decrease with high levels of *N*. This was probably the reason of reduced dry weight per unit length with increased levels of *N* identified by the same author. Root lodging risk can be reduced by establishing less plants per unit area and rolling the soil during spring (Berry et al., 2003b, 2000). Establishing less plants per unit area stimulates root growth and development (Easson et al., 1993; Easson et al., 1995) which results in a deeper and wider root plate with a better plant anchorage (Berry et al., 2000). Other researchers have also indicated that low plant densities (Stapper and Fischer, 1990; Webster and Jackson, 1993) or plant arrangement (different row spaces) (Brady, 1934) can reduce lodging risk. A crop management practices guideline was published by the HGCA (2005) with strategies to avoid lodging for UK winter wheat producers.

1.3 Lodging model: a novel approach to improve lodging resistance

A model of wheat able to indicate the level of susceptibility to lodging was developed by Baker et al. (1998) using crop and soil parameters. This model included stem base bending model estimation using Baker (1995) method and a simplified version of the root strength model of Crook and Ennos (1994). Baker et al (1998) model was further developed in an investigation to account for temporal and spatial non-uniformity of plant characters, and to make use of daily rainfall and wind run data (Berry et al., 2003c). Figure 1.1 describes the steps and parameters used by this model to calculate lodging risk (Berry et al., 2007). For this schematic of the lodging model, three main components are highlighted: a) the wind-induced leverage exerted on the stem base and plant anchorage system which is calculated from wind gust and plant characters such as height at centre of gravity, natural frequency, projected ear area and shoot number per plant which can be calculated from grain yield per unit area, shoot number per unit area and height to ear tip (Berry et al., 2004); b) the stem characters that enable the model to estimate the stem failure moment (stem strength at the point of failure) which is determined by the stem radius, stem wall width and stem material strength; and c) the anchorage characters that enable the model to estimate the anchorage failure moment (anchorage strength at the point of failure) which is calculated from soil shear strength and root plate dimensions. Equations for the calculation of plant leverage, stem strength and anchorage strength are given in Chapter 2. The model uses the leverage and stem and anchorage strength to estimate stem and anchorage failure wind speeds or stem and root lodging risk, respectively (Berry et al., 2003c).

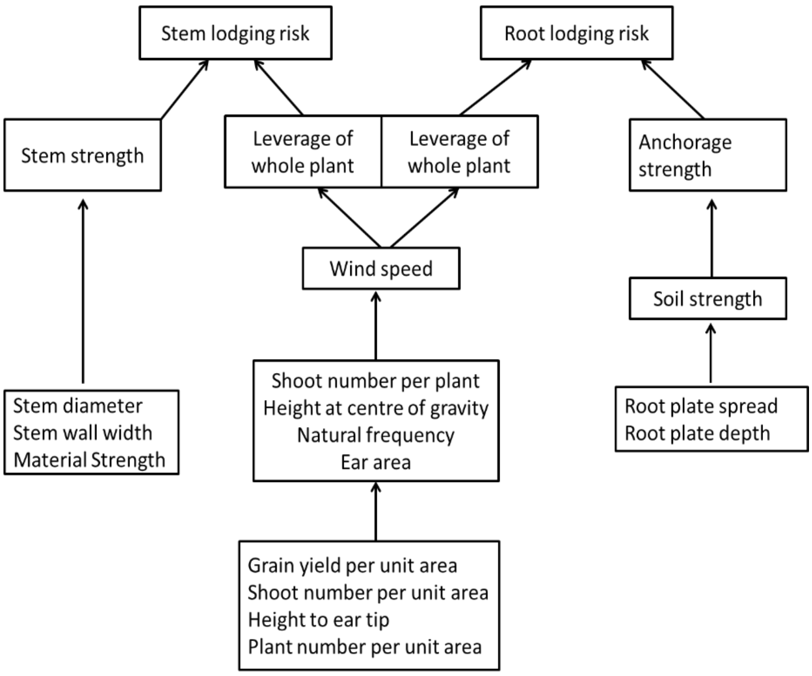

Fig. 1.1 Schematic of lodging model parameters and plant characters (Berry et al., 2007).

This novel approach has enabled researchers to estimate the dimensions of a lodging-proof winter wheat growing in the UK conditions (Berry et al., 2007) and in the present study for spring wheat growing in the North West (NW) Mexico conditions (see Chapter 2). Figure 1.2 shows the dimensions of a lodging-proof wheat plant for the UK environment (Reynolds et al., 2009).

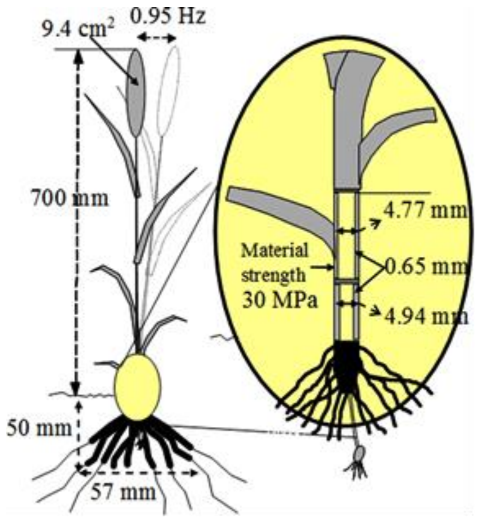

Fig. 1.2 Dimensions of a lodging proof plant (Reynolds et al., 2009)**.**

It is important to consider that the aforementioned novel approaches were preceded by seminal investigations in small grain cereals that established perhaps the foundation of the recent advances in understanding the lodging phenomenon. For instance, Brady (1934) investigated the differences in morphological characters associated with strength of straw of cultivars defined as resistant, intermediate and prone to lodging. Interestingly, this study identified that morphological characters related to straw strength were greater in the lodging resistant cultivar than the other two cultivars. Some of these key straw strength characters were stem diameter, stem wall width and from a cross sectional area from a stem the thickness of sclerenchyma cell walls and width of the lignified tissue.

Cultivar differences in lodging resistance were also associated with stem diameter and wall width in other studies (Hamilton, 1951; Hansel, 1957). Hamilton (1951) also indicated that a lodging resistance is related to a vigorous root plate (roots in the upper layer of soil). While (Hansel, 1957) stated that coronal root diameter (root plate spread) and number of coronal roots were associated with lodging resistance. (Neenan and Spencer-Smith, 1975) showed that a structural failure in small grain cereals occurred due to buckling of the stems and that resistance to failure was determined by stem diameter and Young's modulus (stem material strength). These researchers did an attempt to calculate the wind speed necessary to break the stems by implementing wind-tunnel experiments. On the other hand, (Crook and Ennos, 1993) indicated that in modern cultivars stem lodging is uncommon and the loss of anchorage is the predominant cause of structural failure. It has been reported that this anchorage is determined by the bending strength and the angle of spread of the basal coronal roots (Crook and Ennos, 1994, 1993) (soil properties, root plate spread and structural rooting depth).

1.4 Recent breeding efforts towards increased lodging resistance

Despite the increased lodging resistance achieved from the introduction of dwarfing genes in wheat (Berry et al., 2004; Pinthus, 1974) and the use of plant growth regulators to further reduce plant height, lodging has not been eradicated from wheat fields. In fact, it has been postulated that these tools will no longer be enough to counter the lodging risk associated with continued yield increases (Berry et al., 2004). Breeding efforts relying on information collected from wheat genotypes using lodging indices as selection criteria when artificial or random natural lodging occurs have been used to identify wheat lodging tolerant genotypes (Kelbert et al., 2004; Pinthus, 1974). Using this approach to select lodging resistance cultivars has been very useful, however, lodging-prone cultivars are not always identified (when screening is relying on random natural lodging events) and stem and lodging risks separately is difficult to achieve (Berry et al., 2003a).

In the last two decades, new strategies to address the lodging issue in winter wheat have made use of the improved understanding resulting from the mechanistic model of lodging described above (Berry and Berry, 2015; Berry et al., 2007, 2004; Foulkes et al., 2011; Reynolds et al., 2012, 2009). This new approach emphasizes the selection of key lodging-related traits stem and anchorage strength as well as continuing to shorten plants where possible. Nevertheless, little research has been done on spring wheat growing in irrigated environments (e.g. NW Mexico). In fact, all the relevant achievements were made in winter wheat under rainfed conditions in the UK. Additionally, NW Mexico wind characterization has shown significant differences (see Chapter 2). Thus, this lack of research for spring wheat and environmental differences together with the need for more suitable genetic ranges of key traits (e.g. stem strength, stem diameter) indicates the urgency to investigate these traits on spring wheat under irrigated environments.

1.5 Objectives

The research of this thesis has focussed on developing a greater understanding of the lodging process in spring wheat grown in irrigated environments. This has been achieved through the following objectives.

A. Investigating the relationship between stem strength and anchorage strength and the dry matter requirements of these structures for spring wheat in NW Mexico.

B. Estimate the structural dry matter requirements to enable spring wheat to avoid lodging in this particular environment.

C. Assessing to what extent the development of structural characteristics may compete with yield-forming processes and grain yield.

D. Understanding cell wall composition and its association with the stem strength.

E. Evaluate the genetic variation and heritability of the lodging-related traits, particularly, those strongly related to the stem and anchorage strength.

F. Assess the associations of stem and anchorage strength traits and other key physiological characters.

G. Evaluate the potential of achieving a lodging-proof ideotype defined for spring wheat grown in NW Mexico.

H. Developing methodologies enabling lodging traits to be rapidly assessed through optimization of phenotypic screening and the development of lodging-related genetic markers.

The findings obtained for each one of the objectives described above are presented in following chapters (Chapters 2 – 6). These results are discussed with a general perspective in Chapter 7 (General discussion) and final conclusions are given in Chapter 8 (Conclusions). Chapters 2 to 6 were prepared in the format of manuscripts to be submitted to peer-reviewed journals.

Chapter 2. Stem and root structural requirements of irrigated spring wheat to avoid lodging. (Manuscript submitted)

Chapter 3. Genetic variation of stem and root structural properties of irrigated spring wheat. (Manuscript submitted)

Chapter 4. Lignin and structural and non-structural carbohydrates and their association with stem strength components of irrigated spring wheat.

Chapter 5. Optimizing standard methods to evaluate lodging risk on irrigated spring wheat.

Chapter 6. Quantitative trait loci analysis for lodging-associated traits in wheat.

1.6 References

Acreche, M.M., Slafer, G.A., 2011. Lodging yield penalties as affected by breeding in Mediterranean wheats. F. Crop. Res. 122, 40–48. doi:10.1016/j.fcr.2011.02.004

Baker, C.J., 1995. The development of a theoretical model for the windthrow of plants. J. Theor. Biol. 175, 355–372. doi:10.1006/jtbi.1995.0147

Baker, C.J., Berry, P.M., Spink, J.H., Sylvester-Bradley, R., Griffin, J.M., Scott, R.K., Clare, R.W., 1998. A method for the assessment of the risk of wheat lodging. J. Theor. Biol. 194, 587–603. doi:10.1006/jtbi.1998.0778

Berry, P.M., 1998. Predicting lodging in wheat. Ph. D. Thesis. The University of Nottingham, UK, 210 pp.

Berry, P.M., Berry, S.T., 2015. Understanding the genetic control of lodging-associated plant characters in winter wheat (Triticum aestivum L.). Euphytica. doi:10.1007/s10681-015-1387-2

Berry, P.M., Griffin, J.M., Sylvester-bradley, R., Scott, R.K., Spink, J.H., Baker, C.J., Clare, R.W., 2000. Controlling plant form through husbandry to minimise lodging in wheat. F. Crop. Res. 67, 59–81.

Berry, P.M., Spink, J., Sterling, M., Pickett, A.A., 2003a. Methods for Rapidly Measuring the Lodging Resistance of Wheat Cultivars. J. Agron. Crop Sci. 189, 390–401. doi:10.1046/j.0931-2250.2003.00062.x

Berry, P.M., Spink, J.H., Gay, A.P., Craigon, J., 2003b. A comparison of root and stem lodging risks among winter wheat cultivars. J. Agric. Sci. 141, 191–202. doi:10.1017/S002185960300354X

Berry, P.M., Sterling, M., Baker, C.J., Spink, J., Sparkes, D.L., 2003c. A calibrated model of wheat lodging compared with field measurements. Agric. For. Meteorol. 119, 167–180. doi:10.1016/S0168-1923(03)00139-4

Berry, P.M., Sterling, M., Spink, J.H., Baker, C.J., Sylvester-Bradley, R., Mooney, S.J., Tams, A.R., Ennos, A.R., 2004. Understanding and reducing lodging in cereals. Adv. Agron. 84, 217–271. doi:10.1016/S0065-2113(04)84005-7

Berry, P.M., Sylvester-Bradley, R., Berry, S., 2007. Ideotype design for lodging-resistant wheat. Euphytica 154, 165–179. doi:10.1007/s10681-006-9284-3

Brady, J., 1934. Some factors influencing lodging in cereals. J. Agric. Sci. 24, 209–232.

Crook, M.J., Ennos, A.R., 1993. The Mechanics of Root Lodging in Winter Wheat, Triticum aestivum L. J. Exp. Bot. 44, 1219–1224.

Crook, M.J., Ennos, A.R., 1994. Stem and root characteristics associated with lodging resistance in four winter wheat cultivars. J. Agric. Sci. Agric. Sci. 123, 167–174. doi:10.1017/S0021859600068428

Easson, D.L., White, E.M., Pickles, S.J., 1993. The effects of weather, seed rate and cultivar on lodging and yield in winter wheat. J. Agric. Sci. 121, 145. doi:10.1017/S0021859600077005

FAO, 2014. FAO statistics [WWW Document]. URL http://www.fao.org/resources/infographics/infographics-details/en/c/240943/ (accessed 11.7.15).

Fischer, R.A., Edmeades, G.O., 2010. Breeding and Cereal Yield Progress. Crop Sci. 50, S–85–S–98. doi:10.2135/cropsci2009.10.0564

Fischer, R.A., Stapper, M., 1987. Lodging effects on high-yielding crops of irrigated semidwarf wheat. F. Crop. Res. 17, 245–258.

Foulkes, M.J., Slafer, G. a, Davies, W.J., Berry, P.M., Sylvester-Bradley, R., Martre, P., Calderini, D.F., Griffiths, S., Reynolds, M.P., 2011. Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. J. Exp. Bot. 62, 469–86. doi:10.1093/jxb/erq300

Green, C.F., Paulson, G.A., Ivins, J.D., 1985. Time of sowing and the development of winter wheat. J. agric. Sci., Camb. 105, 217–221. doi:10.1017/S0021859600055921

Griffin, J.M., 1998. Understanding and assessing lodging risk in winter wheat. Ph. D. Thesis. The University of Nottingham, UK, 171 pp.

Hamilton, D.G., 1951. Culm, crown and root development in oats as related to lodging. Sci. Agron. 31, 286–315.

Hansel, H., 1957. The influence of number of crown roots and the diameter and length of stalk on resistance to lodging in winter wheat (Triticum aestivum). F. Crop Abstr. 13.

HGCA, 2005. Avoiding lodging in winter wheat: practical guidelines.

Kasperbauer, M.J., Karlen, D.L., 1986. Light-mediated bioregulation of tillering and biosynthate partitioning in wheat. Physiol. Plant. 66, 159–163

Kelbert, A. J., Spaner, D., Briggs, K.G., King, J.R., 2004. Screening for lodging resistance in spring wheat breeding programmes. Plant Breed. 123, 349–354. doi:10.1111/j.1439-0523.2004.00976.x

Lantican, M.A., Pingali, P.L., Rajaram, S., 2003. Is research on marginal lands catching up? The case of unfavourable wheat growing environments. Agric. Econ. 29, 353–361.

Lantican, M.A., Braun, H.J., Payne, T.S., Singh, R.P., Sonder, K., Baum, M., van Ginkel, M., Erenstein, O., 2016. Impacts of international wheat improvement research, 1994-2014. Mexico, D. F.: CIMMYT

Lobell, D.B., Burke, M.B., Tebaldi, C., Mastrandrea, M.D., Falcon, W.P., Naylor, R.L., 2008. Prioritizing Climate Change Adaptation Need for Food Security in 2030. Science (80-.). 319, 607–610. doi:10.1126/science.1152339

Mulder, E.G., 1954. Effect of mineral nutrition on lodging of cereals. Plant Soil 5, 246–306. doi:10.1007/BF01395900

Neenan, M., Spencer-Smith, J.L., 1975. An analysis of the problem of lodging with particular reference to wheat and barley. J. Agric. Sci. 85, 495–507. doi:10.1017/S0021859600062377

Parry, M. a J., Reynolds, M., Salvucci, M.E., Raines, C., Andralojc, P.J., Zhu, X.-G., Price, G.D., Condon, A.G., Furbank, R.T., 2011. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. J. Exp. Bot. 62, 453–67. doi:10.1093/jxb/erq304

Pinthus, M.J., 1974. Lodging in wheat, barley, and oats: the phenomenon, its causes, and preventive measures. Adv. Agron. 25, 209–263.

Reynolds, M., Bonnett, D., Chapman, S.C., Furbank, R.T., Manès, Y., Mather, D.E., Parry, M. a J., 2011. Raising yield potential of wheat. I. Overview of a consortium approach and breeding strategies. J. Exp. Bot. 62, 439–52. doi:10.1093/jxb/erq311

Reynolds, M., Foulkes, J., Furbank, R., Griffiths, S., King, J., Murchie, E., Parry, M., Slafer, G., 2012. Achieving yield gains in wheat 1799–1823. doi:10.1111/j.1365-3040.2012.02588.x

Reynolds, M., Foulkes, M.J., Slafer, G. a, Berry, P., Parry, M. a J., Snape, J.W., Angus, W.J., 2009. Raising yield potential in wheat. J. Exp. Bot. 60, 1899–918. doi:10.1093/jxb/erp016

SIAP, 2016. Servicio de Informacion Agroalimentaria y Pesquera [WWW Document]. Wheat Harvest. area 2014. URL www.siap.gob.mx (accessed 5.8.16)

Slafer, G.A., Araus, J.L., 2007. Physiological traits for improving wheat yield under a wide range of conditions, in: J.H.J. Spiertz, P.C.S.& H.H. vanLaar (Ed.), Scale and Complexity in Plant Systems Research: Gene–Plant–Crop Relations. Springer, Dordrecht, the Netherlands, pp. 147–156.

Stapper, M., Fischer, R.A., 1990. Genotype, sowing date and plant spacing influence on high-yielding irrigated wheat in southern New South Wales. II. Growth, yield and nitrogen use. Aust. J. Agric. Res. 1990; 41, 1021–1041. doi:10.1071/AR9901043

Tripathi, S.C., Sayre, K.D., Kaul, J.N., Narang, R.S., 2003. Growth and morphology of spring wheat (Triticum aestivum L.) culms and their association with lodging: effects of genotypes, N levels and ethephon. F. Crop. Res. 84, 271–290. doi:10.1016/S0378-4290(03)00095-9

Tripathi, S.., Sayre, K.., Kaul, J.., Narang, R.., 2004. Lodging behavior and yield potential of spring wheat (Triticum aestivum L.): effects of ethephon and genotypes. F. Crop. Res. 87, 207–220. doi:10.1016/j.fcr.2003.11.003

Tripathi, S.C., Sayre, K.D., Kaul, J.N., 2005. Planting Systems on Lodging Behavior, Yield Components, and Yield of Irrigated Spring Bread Wheat. Crop Sci. 45, 1448–1455. doi:10.2135/cropsci2003-714

Weibel, R.O., Pendleton, J.W., 1964. Effect of artificial lodging on winter wheat grain yield and quality. Agron. J. 56, 487–488

Chapter 2 Stem and root structural requirements of irrigated spring wheat to avoid lodging

2.1 Abstract

A model of the lodging process has been successfully adapted for use on spring wheat grown in NW Mexico. The lodging model was used to estimate the lodging-associated traits required to enable spring wheat grown in NW Mexico with a typical yield of 6 t ha^{-1} and height of 0.7 m to achieve a lodging return period of 25 years. Target traits included a root plate spread of 51.1 mm and stem strength of the bottom internode of 268 N mm. These target traits increased to 54.5 mm and 325 N mm, respectively, for a crop yielding 10 t ha^{-1}. Analysis of multiple genotypes across three growing seasons enabled relationships between both stem strength and root plate spread with structural dry matter to be quantified. A NW Mexico lodging-resistant ideotype yielding 6 t ha^{-1} would require 3.93 t ha^{-1} of structural stem biomass and 1.10 t ha^{-1} of root biomass in the top 10 cm of soil, which would result in a harvest index (HI) of 0.46 after accounting for chaff and leaf biomass. A crop yielding 10 t ha^{-1} would achieve a HI of 0.54 for 0.7 m tall plants or 0.41 for more typical 1.0 m tall plants. This study indicates that for plant breeders to achieve both high yields and lodging-proofness then they must either breed for greater total biomass or develop high yielding germplasm from shorter crops.

2.2 Introduction

Lodging is defined as the permanent displacement of plant stems from their vertical position as a result of wind acting on the shoot and rain or irrigation weakening the soil and reducing anchorage strength (Berry et al. 2004). Lodging has been shown to reduce the yield of wheat by up to 80% (Easson et al. 1993; Berry and Spink 2012) and reduces bread-making quality (Berry et al. 2004). Lodging affects all cereal species and many other crops, such as oilseed rape and sunflowers, throughout the world.

Plant breeders have historically reduced lodging risk by introducing dwarfing genes to produce shorter varieties. Lodging resistance must be continually improved to counter the increasing lodging risk arising from continued yield potential increases. However, there may now be limited potential to continue improving lodging resistance through further decreasing plant height because the minimum height that is compatible with high yield (0.7 to 1.0 m (Allan 1986; Kertesz et al. 1991; Richards 1992; Balyan and Singh 1994; Miralles and Slafer 1995a; Flintham et al. 1997; Berry et al. 2014)) has now been reached in many environments. Miralles and Slafer (1995b) suggested that dwarfing genes may have a direct effect to reduce the final grain weight. Dwarfing genes have also been associated with a reduction of water soluble reserves storage capacity (Cossani and Reynolds, 2012) and leaf extension rate (Keyes et al. 1989) that might reduce grain weight. Thus, a reduction of the soluble reserves in the stem (particularly in dry environments) (Borrell et al. 1993) or reduction of final leaf area (McCaig and Morgan, 1993; Daniel J Miralles and Slafer, 1995) and radiation use efficiency at pre-anthesis (Miralles and Slafer, 1997) are possible reasons why extreme dwarfism could significantly reduce grain yield.

It therefore seems that reducing height to below 0.7 m might not be the best mechanism to improve lodging resistance in modern high yielding wheat. If we consider that the two types of lodging are due to the bending/buckling of the stem base (stem lodging) or the over-turning of the anchorage system (root lodging), then greater lodging resistance in wheat can be achieved by strengthening these structures (Berry et al. 2003b). Large genetic variation has been identified for the anchorage and stem strength of winter wheat in the UK (Berry et al. 2003a; Berry et al. 2007). However, breeding to improve these traits in high yielding wheat requires more understanding about how they develop and possible trade-offs with yield-forming processes in different environments.

Baker et al. (1998) developed a model of lodging that has been validated by Berry et al. (2003b) for winter wheat in the UK. The model was based on the interaction of plant, soil and wind characteristics and calculates the stem and root lodging risk according to the wind speed required to over-turn the root anchorage system or to buckle the stem base of a plant. A preliminary attempt to quantify the stem strength and anchorage strength required by winter wheat to withstand 1 in 25 year wind gusts of 18 m s^{-1} in the UK has been made by Berry et al. (2007) using this lodging model. This indicated that substantial amounts of dry matter may need to be invested in the stem and anchorage system to make plants lodging-proof, which were estimated at 7.9 t ha^{-1} of stem biomass and 1.0 t ha^{-1} surface root biomass (roots in the first top 10 cm of soil) (Foulkes et al. 2011). This would mean that the maximum harvest index (ratio of grain dry matter to above-ground dry matter) for a 0.7 m tall crop yielding 8 t ha^{-1} would only be 0.42, rising to 0.50 for a crop yielding 16 t ha^{-1}, which is significantly less than the theoretical maximum harvest index of 0.62 estimated by Austin (1980). Additionally it is possible that the investment in dry matter for the stem and anchorage system during stem elongation which is the critical phase for determination of grain number (Fischer, 1985) may compete for resources with grain yield determination. The implications of Berry et al. (2007) are that the dual requirements of breeding for greater yield and greater lodging resistance will be challenging. However, parts of the analysis were based on limited datasets for winter wheat and it was not possible to distinguish between the structural dry matter and water soluble carbohydrate in the stem, which may mean that the estimate of stem structural dry matter to avoid lodging was over-estimated.

The aims of this chapter were to 1) investigate the relationship between stem strength and anchorage strength with the dry matter requirements of these structures for spring wheat in NW Mexico, 2) adapt an existing model of lodging for winter wheat for spring wheat, 3) estimate the structural dry matter requirements to enable spring wheat to avoid lodging in this particular environment, and 4) consider to what extent the development of structural characteristics may compete with yield-forming processes and grain yield.

2.3 Materials and methods

2.3.1 Experiments and measurements

Four field experiments were established during the field seasons 2010-2011, 2011-2012, 2012-2013 and 2013-2014 (referred to hereafter as 2011, 2012, 2013 and 2014, respectively). Detailed information about experiments, cultivars, crop management and the main measurements will be described in the Materials and methods section of the following Chapter 2 (See also Appendix 1). Additionally, during 2013 and 2014 the main shoot measurements of internode length and breaking strength of the stem before and after removal of the leaf-sheath of the internodes 1 to 5 were determined for five cultivars with contrasting performances in stem strength, material strength and anchorage strength (Table 2.1). Internode 1 in the main shoot was identified, defined as the first internode of more than 10 mm, originating at or just below the ground surface and without crown roots emerging from its upper node. Subsequent internodes ascending the stem were numbered two, three, four etc., with the uppermost internode referred to as the peduncle. Also, dry weight and the determination of the water soluble carbohydrates content (WSC) was made on these stem internodes. WSC content was also determined for the whole main shoot in all cultivars used for experiments 2011, 2012 and 2013. These analyses were carried out in the Maize Nutrition Quality and Plant Tissue Analysis Laboratory from CIMMYT (El Batan, Mexico) using the Anthrone method (Galicia et al. 2008). Surface root dry weight (g) was determined by trimming the roots at the stem base (in the top 10 cm of soil), washing off any remaining soil and drying at 75°C until no further weight loss occurred.

Table 2.1 Cultivars from CIMCOG used for 2014 experiment

Cultivar	Character of interest
BACANORA T 88 [a]	Lowest stem and anchorage strength
CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/6/RIALTO [a]	Highest anchorage strength
CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2 [a]	Highest material strength
WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES [a]	Highest stem strength
YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA(498)/5/LINE1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ/7/KRONSTAD F2004/8/KAUZ/PASTOR//PBW343 [a]	Lowest material strength

2.3.2 Calculations

A validated model of lodging for winter wheat (Baker et al. 1998; Berry et al. 2003b) was used to calculate the stem failure moment (stem strength at the point of failure), anchorage failure moment (anchorage strength at the point of failure), the wind-induced base bending moment (leverage force) of the shoot and plant, and overall risk to stem and root lodging on spring wheat (stem and anchorage failure wind speed).

The stem failure moment (B_s) was calculated from the breaking strength (F_s) and length (h) of the internode (Eq. 2.1).

$$B_s = \frac{1}{4} F_s h \qquad (2.1)$$

Anchorage failure moment (B_R) was calculated from the root plate spread (d), the shear strength of the surrounding soil (s) and a constant of 0.43 (k_3) taken from Baker et al. (1998) (Eq. 2.2). The surrounding soil was assumed to be at field capacity with a shear strength of 6 kPa (Baker et al. 1998).

$$B_R = k_3 s d^3 \tag{2.2}$$

The shoot base bending moment (B) was obtained from the density of air (ρ = 1.2 kg m^{-3}), the projected ear area (A), the shoot's height at centre of gravity (X), the wind gust speed (V_g), the shoot's natural frequency (n), the acceleration due to gravity (g = 9.81 m s^{-2}), the shoot's damping ratio (ξ = 0.08) and the drag coefficient of the ear (C_d = 1.0). The base bending moment of the whole plant was calculated by multiplying B by the number of shoots per plant (Baker et al. 1998):

$$B = \frac{1}{2}\rho A C_d X V_g^2 \left(1 + \frac{g}{(2\pi n)^2\, X}\right)\left(1 + e^{-\pi\xi}\frac{\sin(\pi/4)}{\pi/4}\right) \tag{2.3}$$

Assuming the wind-induced leverage (bending moment) decreases linearly for progressively higher positions up the stem (Berry et al. 2006), bending moment at the base of subsequent internodes 2 to 5 was calculated by multiplying the leverage exerted at the base of internode 1 with the ratio of the distance between the base of internodes 2, 3, 4 or 5 and the mid-point of the ear with the total stem height at the mid-point of the ear (Berry et al. 2007). These ratios were measured in spring wheat at 0.84 for the base of internode 2, 0.70 for internode 3, 0.50 for internode 4 and 0.19 for internode 5. The ratio at the mid-point of the length of internode 5 (peduncle) was used to calculate the bending moment (19 %) due to non-uniform geometric properties of this internode (Berry et al. 2007). This means that leverage exerted at the base of internodes 2, 3, 4 and the peduncle should be 84, 70, 50 and 19 %, respectively, of the leverage exerted at the base of internode 1.

Stem material strength (σ) was calculated from the stem wall width (t) stem radius (ɑ) and stem strength (Bs).

$$Bs = \frac{\sigma\pi a^3}{4}\left(1 - \left(\frac{a-t}{a}\right)^4\right) \quad (2.4)$$

The stem failure wind speed (V_{gS}) (Eq. 2.5) and the anchorage failure wind speed (V_{gR}) (Eq. 2.6) were calculated by combining and re-arranging equations 2.1 and 2.2, with equation 2.3 (Berry et al., 2003c). Letter N in equation 2.6 indicates the number of shoots per plant.

$$V_{gS} = (2B_s)^{0.5} \times \left(\rho A C_d X \left(1 + \frac{g}{(2\pi n)^2 X}\right)\left(1 + K_\xi\right)\right)^{-0.5} \quad (2.5)$$

$$V_{gR} = (2NB_R)^{0.5} \times \left(\rho A C_d X \left(1 + \frac{g}{(2\pi n)^2 X}\right)\left(1 + K_\xi\right)\right)^{-0.5} \quad (2.6)$$

2.3.3 Statistical analysis

Simple linear and non-linear regression analysis and simple linear regression analysis with groups were used to investigate relationships between traits. Analysis of variance using a general linear model was used to test for differences between years, cultivars and leaf sheath removal treatments together with treatment interactions. All the analyses were carried out by GenStat 15th Edition (VSN International, 2012).

2.3.4 Wind speed characterisation

Daily wind run data were sourced from a local meteorological station within the wheat growing area of the Valle del Yaqui, Sonora, Mexico (grid reference 27.3°N and 109.1°W, 38 masl) spanning a 40-year period from 1973 to 2013. The daily wind run data were converted to the maximum hourly mean wind speed for each day by multiplying by a factor of 1.606

(Berry et al. 2003b). The hourly mean values were each converted to hourly gust values, for gusts of duration τ = 0.3 s, using the empirical equation described by Berry et al. (2003b):

$$U_{gust} = U_m\left(1 + 0.42\left(\frac{\sigma}{U_m}\right)\sin\left(\frac{3600}{\tau}\right)\right) \tag{2.7}$$

where σ/U_m is the turbulence intensity (TI). A value of σ/U_m = 0.5 was used, again following the work of Berry et al. (2003b) who used the value determined by Finnigan (1979) for wind over a wheat crop. These values were then corrected for differences in the roughness, z_0, at the airport weather stations and the crop locations, and also for the difference in height above ground, z, of the airport anemometers (10 m), the meteorological station anemometer (1.5 m) and the pertinent wind speed height for crop lodging of 2 m (Baker et al. 1998; Berry et al. 2003b). This correction takes the form:

$$U_c = U_w \frac{\ln\left(\frac{z-d}{z_0}\right)_c}{\ln\left(\frac{z-d}{z_0}\right)_w} \tag{2.8}$$

where subscripts c and w refer to the crop and weather station locations, respectively. z_0 over the crops has been estimated as $z_0 = \lambda(h - d)$, where λ = 1/3, h = 1 m and d = 0.75 h, giving a value of z_0 = 1/12 m, with z_0 = 0.01 m at the weather station (Berry et al. 2003b). Finally, an altitude correction has been applied to the gust wind speed using the V_{99} correction factor of (1 + 0.009h) specified in Baker et al. (1998). The correction is taken as the ratio (1 + 0.009h_c)/ (1 + 0.009h_w) where h_c and h_w are the crop and weather station altitudes respectively. As stated in Berry et al. (2003b), these methods were developed based on UK Meteorological Office data and should therefore be applied with care in other locations. In particular, these methods are only applicable where synoptic (non-convective) winds are expected.

In order to allow the analysis to concentrate on the period when lodging risk is possible, the hourly gust values were split by month (i.e. 12 groups of data were formed from the 40 years of data, each corresponding to a particular month). The probability of the gust speed exceeding a certain value was calculated for each month on a per day basis. At each scale, probabilities of the gust speed being within a certain range were calculated by sorting the values into 0.5 m s^{-1} wide "bins", with the probability of gusts within the range covered by each bin calculated simply from the number of values in that bin divided by the total number of values. Probabilities of exceeding a certain value were calculated as the sum of the probabilities for the bins whose ranges exceeded the required value. The probability of experiencing any particular wind gust during the lodging risk period was then calculated using daily gust speed probabilities for the months during which lodging is possible and assuming a stem lodging risk period of 50 days in March and April (assuming one wheat cycle per year). Root lodging risk increases when the soil surface is wet (Easson et al. 1995; Berry et al. 2003a), typically when the first 50 mm of soil is at field capacity (Baker et al. 1998). This can be attributed to the movement of the plant crown in a saturated soil surface after flood irrigation in irrigated environments (Fischer and Stapper, 1987) or after precipitation in rainfed environments (Crook and Ennos, 1994). Moreover, Sterling et al. (2003), using a portable wind tunnel in the field, found that root lodging occurred only when the soil was saturated. It therefore seems sensible to consider the root lodging risk when the soil surface is moist. For our purposes, we estimated the root lodging risk period for the NW Mexico environment to be 10 days, based on the number of days when the top 60 mm of soil depth (maximum root plate depth exceeded 50 mm in CIMCOG panel) was at 50% of plant available water during the 50-day lodging risk period. At lower soil moisture content, the soil is usually too strong to permit root lodging. Spring wheat in this region typically receives three flood irrigation events during the 50-day lodging risk period, each delivering approximately 76 mm water. Using internal records of field capacity and permanent wilting point from CENEB (Campo Experimental Norman E. Borlaug) and evapotranspiration data from nearest weather station it has been estimated that the top 150 mm of soil dries to 50 % of plant available water after 8 days of irrigation. The soil tends to dry from the top downwards which indicates that the top 60 mm of soil will be dried to 50% plant available water within about 3.2 days, giving about 10 days when the top 60 mm of soil may be moist and weak enough to permit root lodging. A review of rainfall data over the past 40 years showed that the chance of more than 10 mm of rain (enough to bring the top 60 mm of soil to field capacity) falling in one day during March or April was very small (See Table 3.1 in Chapter 3).

2.3.5 Lodging model further development

This section describes how for spring wheat the lodging model was further developed to estimate the key plant characteristics that determine base bending moment (shoot height at centre of gravity, shoot natural frequency and ear area) from plant characteristics that are more commonly measured by crop physiologists (grain yield, grain harvest index, plant height and shoots m^{-2}). This process was carried out for winter wheat by Berry et al. (2004), however spring wheat has fundamental differences which may affect how the plant characteristics described above are related. One key difference is the presence of awns on the spring wheat varieties. Theoretically, shoot height at centre of gravity (X) can be calculated from stem length (S_L), stem and leaf fresh weight (S_W), ear fresh weight (E_W) and ear length (E_L) following the Equation 2.9 which assumes uniform weight of shoot and ear (Berry et al. 2004).

$$X = \frac{(S_L S_w + 2S_L E_W + E_L E_W)}{2(S_W + E_W)} \tag{2.9}$$

The components of Equation 2.9 can be calculated from physiological crop traits measured commonly at harvest: grain yield (Y, g m^{-2}), the number of ears per metre square (E_n), the ratio of chaff dry weight to total ear dry weight (α), the harvest index (HI) and the crop height to the tip of the ear (h, m) (Equations 2.10 – 2.12).

$$E_W = \frac{{}^{Y}/_{(1-\alpha)}}{E_n} \tag{2.10}$$

$$S_W = \frac{E_W(1-\alpha)}{HI} - E_W \tag{2.11}$$

$$S_L = h - E_L \tag{2.12}$$

Equation 2.9 was tested using measurements of S_W and E_W at GS65 + 7 d in a random sample of 20 plants per plot, and X, S_L, E_n *and* E_L *at* GS65 + 20 d in 10 plants per plot in all the plots during 2012 and 2013 experiments. Figure 2.1 shows that Equation 2.9 accounted for a substantial proportion of the differences in height at centre of gravity, but over-predicted the measurement by about 6% on average. The most likely explanation for the overestimate is non-uniform distribution of the dry matter along the shoot, with relatively more at the base than the top (Berry et al. 2004). If Equation 2.9 is multiplied by a factor of 0.94 then the regression between the predicted and measured height at centre of gravity has an equation of y = 0.74x + 0.15 and R^2 of 0.64.

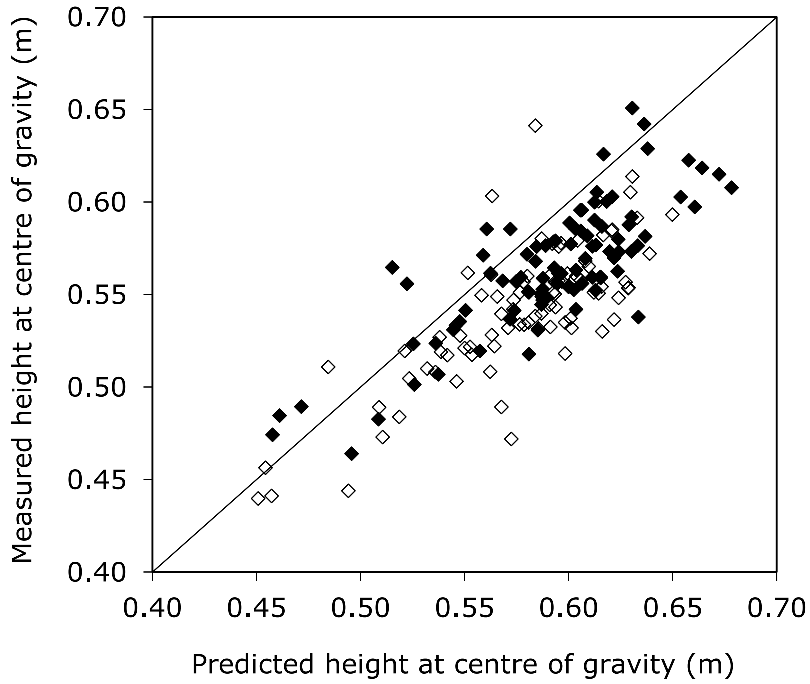

Fig. 2.1 Predicted and measured height at centre of gravity at GS65 + 7 days and GS65 + 20, respectively, for plot means of 2012 (closed squares) and 2013 (open squares). (—) 1:1 line. Best fit line, y = 0.70x + 0.15; R^2=0.64 (*P* < 0.001).

Regression analysis performed with data from all plots measured in 2012 and 2013 showed an inverse relationship between the natural frequency (n_n) of the main shoot with the measured height at centre of gravity (Figure 2.2a) resulting in Equation 2.13. The ear area (including awns) showed a positive relationship with the fresh weight of the ear resulting in Equation 2.14 (Figure 2.2b).

$$n_n = 0.7x^{-1.4} + 0.3 \qquad (2.13)$$

$$A = 9.95 + 1.02E_W \qquad (2.14)$$

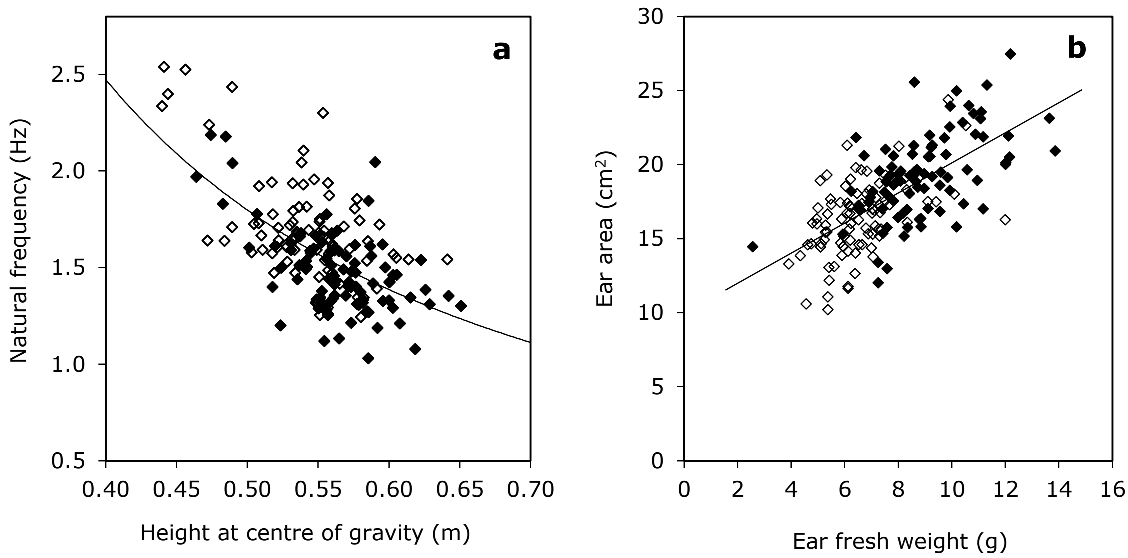

Fig. 2.2 (a) Height at centre of gravity at GS65 + 20 plotted against natural frequency for plot means of 2012 (closed squares) and 2013 (open squares). Regression line: y = $0.67x^{-1.4}$ (R^2=0.38; *P* < 0.001). (b) Ear fresh weight plotted against ear area at GS65 + 20, for plot means of 2012 (closed squares) and 2013 (open squares). Best fit line: y = 1.02x + 9.95 (R^2=0.44; *P* < 0.001)

Hence, the further developed Equations 2.9 to 2.14 for spring wheat have demonstrated how plant height, harvest index, shoots per square meter and yield can be used to calculate lodging model inputs; height at centre of gravity, natural frequency and ear area. Successively, these parameters can be used in Equation 2.3 to estimate the effect of changes to plant height, harvest index and yield on the base bending moment of a single shoot and the whole plant on spring wheat genotypes.

2.4 Results

2.4.1 Wind gust probabilities for North West Mexico

The probabilities for experiencing wind gust speeds at the height of the crop during stem and root lodging risk periods at NW Mexico are summarised in Figure 2.3.

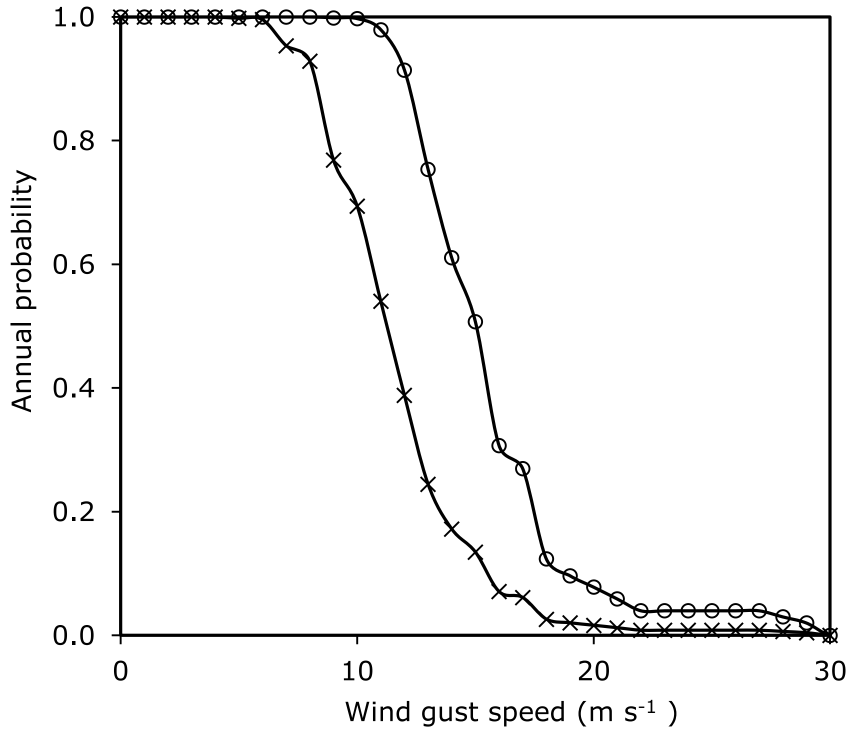

Fig. 2.3 NW Mexico seasonal maximum wind gust probabilities for 50 days of high stem lodging risk (○) and for 10 days of high root lodging risk (when surface soil horizon is moist) (×).

Annual wind gust speed return periods for the NW Mexico environment (Table 2.2) show that the plant must withstand a wind gust of 22 m s^{-1} to have a stem lodging return period of 25 years, and it must withstand a wind speed of 18 m s^{-1} to have a root lodging return period of 25 years. For a return period of 10 years the critical wind speeds that must be withstood fall to 19 m s^{-1} and 16 m s^{-1} for stem and root lodging, respectively.

Table 2.2 Seasonal wind gust speed return period for Obregon

Wind gust return period (years)	Wind gust speed (m s^{-1})	
	stem lodging risk	root lodging risk
5	18	14
10	19	16
15	21	17
25	22	18

2.4.2 Calculating the lodging-proof ideotype for spring wheat

The maximum wind-induced shoot and plant leverages for crops with a range of crop height and yield have been calculated for lodging return periods of 1 year in 5 to 1 year in 25 (Figure 2.4) using Equations 2.3 and 2.9 – 2.14. The wind-induced shoot base bending moment (leverage) (N mm) calculated for the maximum wind speed expected during the entire 50-day stem lodging risk period represents the minimum failure moment (strength) of the stem base (N mm) for supporting the shoot. The wind-induced plant leverage calculated for the wind speeds expected during the 10-day root lodging period represents the minimum strength of the anchorage for supporting all the shoots of a single plant. To carry out these calculations it was assumed that the crops had 500 shoots per metre squared and 200 plants m^{-2} which are typical for the Yaqui Valley near Ciudad Obregon. The ideotype crop with the current average on-farm yield of 6 t ha^{-1} (at 12 % moisture) for the NW Mexico environment (Fischer and Edmeades, 2010), and minimum crop height of 0.7 m that has been observed to be compatible with high yield, must have a stem strength equivalent to the 268 N mm of shoot leverage and anchorage strength equivalent to 448 N mm of plant leverage. If crop yield and plant height are increased to 10 t ha^{-1} and 1.0 m, respectively, then the stem strength required must be equivalent to 480 N mm of shoot leverage and anchorage strength equivalent to 803 N mm of plant leverage.

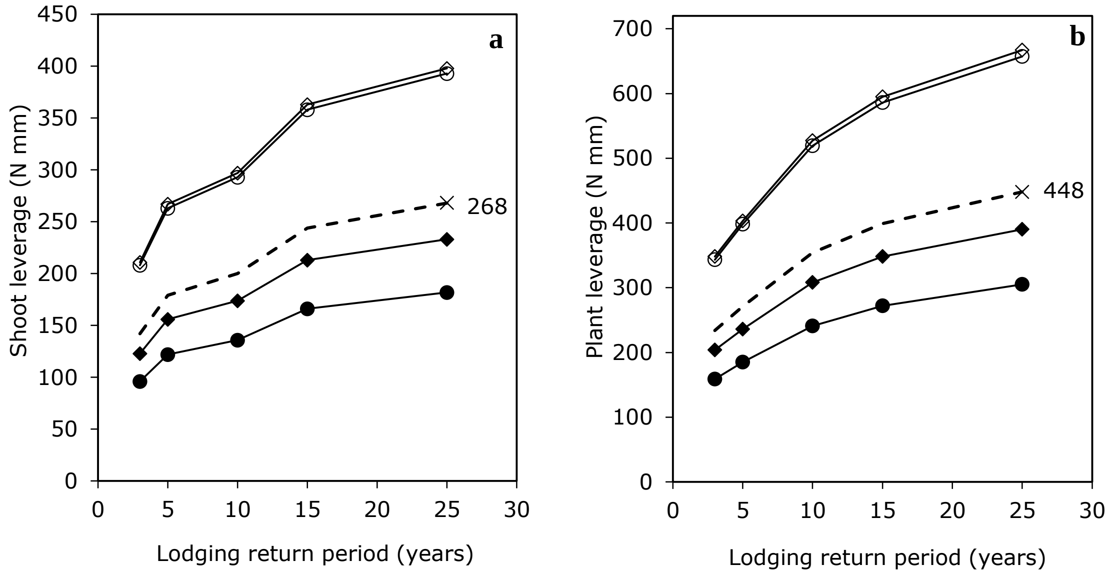

Fig. 2.4 Shoot leverage (a) and plant leverage (b) for a crop yielding 6 t ha^{-1} with a crop height of 1.0 m (○) and 0.5 m (•), and crop yielding 16 t ha^{-1} (◇) and 4 t ha^{-1} (♦) with a crop height of 0.7 m, for different lodging return periods in the NW Mexico environment. Dotted line indicates crop with current average yield of 6 t ha^{-1} and putative minimum crop height compatible with this yield of 0.7 m

The size of the root plate required to avoid lodging for a range of crop types and lodging return periods has been calculated using Equation 2.2. Stem radius (ɑ) was calculated to achieve the required stem strength (*Bs*) using Equation 2.4 where stem wall width (*t*) was constant (0.65 mm), a range of stem material strength (σ) values and stem strength (*Bs*) was defined by the maximum wind gust of each lodging return period (Table 2.2). A minimum stem wall width of 0.65 mm was assumed because it is understood that a thin-walled, but wide, cylinder is the best way of achieving strength for the minimum investment of dry matter (Berry et al.,

2007) and 0.65 mm was the thinnest wall width observed in the spring wheat experiments.

The target root plate spread ranged from 43.2 mm for a 0.7 m tall crop yielding 6 t ha^{-1} with a lodging return period of 5 years, to 62.1 mm for a 1.0 m tall crop yielding 10 t ha^{-1} with a lodging return period of 25 years. These calculations assume the soil was rolled after sowing to consolidate it. A 0.7 m tall crop yielding 6 t ha^{-1} with a lodging return period of 5 years would require a stem diameter of 4.04 mm with a material strength of 35 MPa or alternatively the required strength could be achieved with a stem diameter of 3.51 mm with a material strength of 50 MPa. A 1 m tall crop yielding 10 t ha^{-1} with a lodging return period of 25 years would require a stem diameter of 6.09 mm with a material strength of 35 MPa or 5.24 mm with a material strength of 50 MPa (Table 2.3).

Table 2.3 NW Mexico ideotype trait targets for different lodging return periods

Character	Lodging return period (years)			
	5	10	15	25
	0.7 m tall and 6 t ha^{-1}			
Root plate spread (mm)	43.2	47.3	49.2	51.1
[a]Internode diameter (mm)	4.04	4.23	4.58	4.76
[b]Internode diameter (mm)	3.51	3.67	3.97	4.12
	0.7 m tall and 10 t ha^{-1}			
Root plate spread (mm)	46.1	50.4	52.5	54.5
[a]Internode diameter (mm)	4.38	4.58	4.97	5.16
[b]Internode diameter (mm)	3.79	3.96	4.29	4.45
	1.0 m tall and 6 t ha^{-1}			
Root plate spread (mm)	49.1	53.7	55.9	58.1
[a]Internode diameter (mm)	4.73	4.94	5.38	5.59
[b]Internode diameter (mm)	4.09	4.27	4.63	4.82
	1.0 m tall and 10 t ha^{-1}			
Root plate spread (mm)	52.5	57.4	59.8	62.1
[a]Internode diameter (mm)	5.14	5.38	5.85	6.09
[b]Internode diameter (mm)	4.43	4.63	5.03	5.24

[a] Material strength of 35 Mpa; [b] material strength of 50 Mpa; * all crops assumed to have a stem wall width of 0.65 mm

2.4.3 Biomass and failure moment of stem and anchorage system

A positive regression ($R^2 = 0.63$; $P < 0.001$) was found between structural stem dry matter per unit length and internode failure moment for internodes 1 to 2 (27 cultivars, 2011, 2012, 2013 and 5 cultivars, 2014) and internodes 3 to 4 (5 cultivars, 2013 and 2014). According to this regression model where the response variable was the internode failure moment, a fitted value of 100 N mm in this parameter could be achieved with a structural stem dry weight per unit length of 1.13 mg mm^{-1} or with 1.53 mg mm^{-1} of structural plus WSC stem dry weight (Figure 2.5). There was no association between WSC content and internode failure moment for internodes 1 to 2 (2011, 2012, 2013 and 2014) and internodes 3 to 4 (2013 and 2014) ($R^2 = 0.009$, $P = 0.20$).

Regarding the anchorage system there was a positive relationship between root dry weight per plant and root plate spread among 27 genotypes which had a consistent slope across years 2012 and 2013 of 0.038 mm mg^{-1}, but different y axis intercepts of 24.7 and 35.6 mm, respectively, and an R^2 of 0.74 ($P < 0.001$) for the regression model (Figure 2.6). Regression analysis on this association for 2011 showed a fitted line with a slope of 0.011 and y-axis intercept of 28.8 mm and an R^2 of 0.18 ($P < 0.05$).

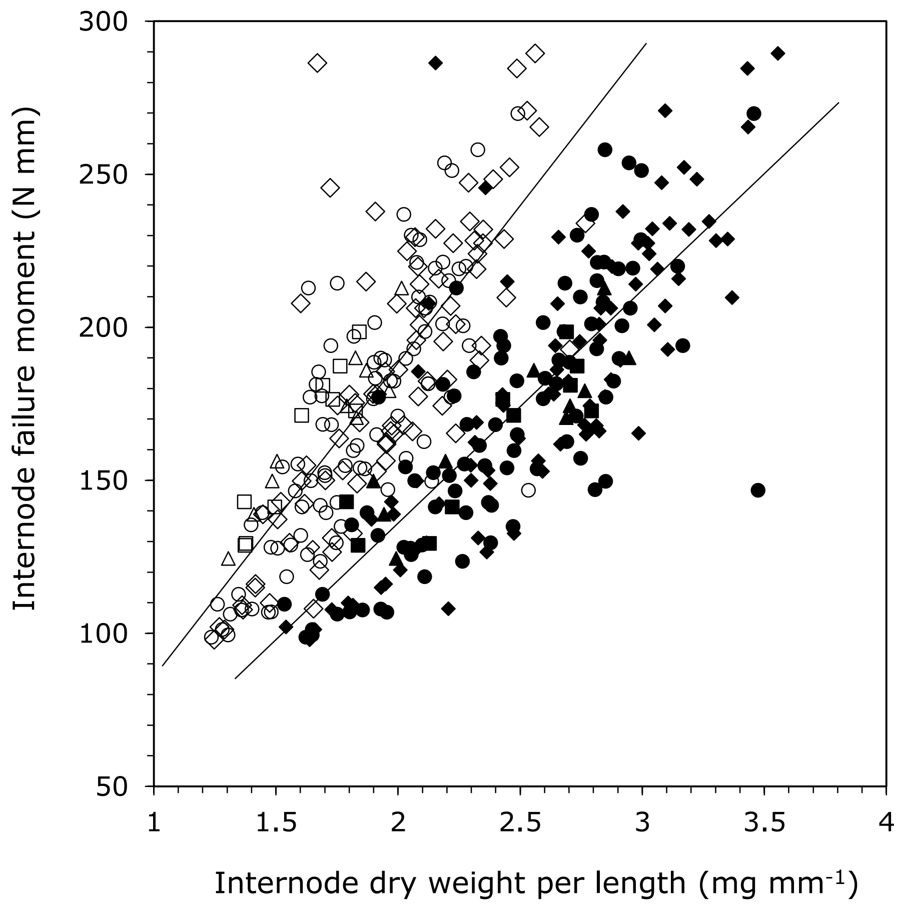

Fig. 2.5 Dry weight per unit length plotted against internode failure moment of internode 1 (diamonds), internode 2 (circles) for 27 genotypes (2011, 2012 and 2013) and of internodes 1, internode 2, internode 3 (triangles) and internode 4 (squares) for five genotypes (2013 and 2014). Open figures indicate structural dry weight ($y = 103x - 16.8$; $R^2 = 0.63$; *P* < 0.001) and closed figures indicate overall dry weight ($y = 76.2x - 16.3$; $R^2 = 0.64$; *P* < 0.001).

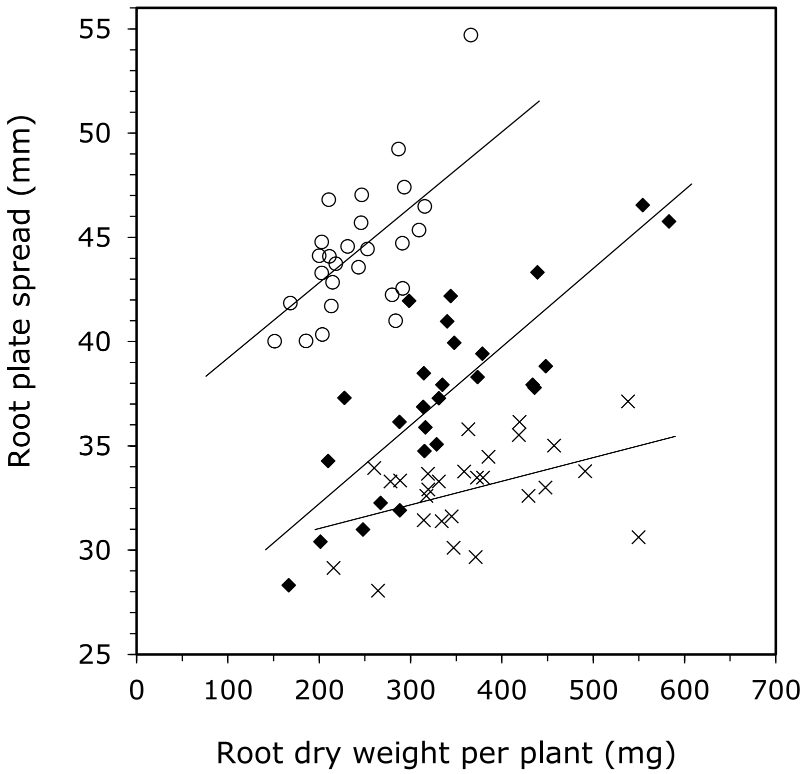

Fig. 2.6 Surface root dry weight per plant plotted against root plate spread of 27 spring wheat genotypes. Parallel model for (♦) 2012 (y = 0.038x + 24.7) and (○) 2013 (y = 0.038x + 35.6); R^2 = 0.74; P < 0.001). Experiment (×) 2011 showed a regression line of y = 0.011x + 28.8 with an R^2 of 0.18 (*P* < 0.05).

Experiments in 2013 and 2014 included a screening of five genotypes which were evaluated for internode failure moment with and without the leaf sheath. Analysis of variance showed that removing the leaf sheath significantly reduced the internode failure moment by 8 N mm, 23 N mm, 32 N mm, 31 N mm and 47 N mm for internodes 1, 2, 3, 4 and 5, respectively (4, 12, 19, 19 and 34 %, respectively). This variation was statistically significant (P < 0.05) for internodes 2 (SED = 5.55), 3 (SED = 4.97 N mm), 4 (SED = 4.81 N mm) and 5 (SED = 4.38 N mm). Differences between cultivars were found for all internodes (SED = 7.94, *P* < 0.001) and there were no significant interactions between cultivar and leaf sheath treatments.

2.4.4 Quantifying stem and root biomass requirements of a lodging-proof wheat crop

The amount of structural stem and surface root dry matter required to resist lodging for a range of crop types and lodging return periods are described in Figure 2.7. The structural stem biomass required to achieve specified lodging return periods was first estimated for each individual internode using the empirical equation y = 103x – 16.8 from Figure 2.5 for 2011-14 data, where the "y" value was the leverage exerted at the base of each internode and the "x" value was the structural dry weight per unit length. The structural dry weight of each internode was calculated by multiplying the dry weight per unit length by the internode length. The structural dry weight of the whole stem was calculated by summing the dry weights of all five individual internodes. The target structural stem biomass ranged from 2.78 t ha^{-1} for a 0.7 m tall crop yielding 6 t ha^{-1} with a lodging return period of 5 years to 10.1 t ha^{-1} for a 1.0 m tall crop yielding 10 t ha^{-1} with a lodging return period of 25 years. Root biomass was estimated by first calculating the root plate spread required to withstand the wind induced plant leverage, then using empirical equation y = 0.038x + 30.2 from Figure 2.6 for 2012-13 data, where the "y" value is the root plate spread and the "x" value is the surface root biomass per plant. The target root biomass ranged from 0.69 t ha^{-1} for a 0.7 m tall crop yielding 6 t ha^{-1} with a lodging return period of 5 years to 1.68 t ha^{-1} for a 1.0 m tall crop yielding 10 t ha^{-1} with a lodging return period of 25 years.

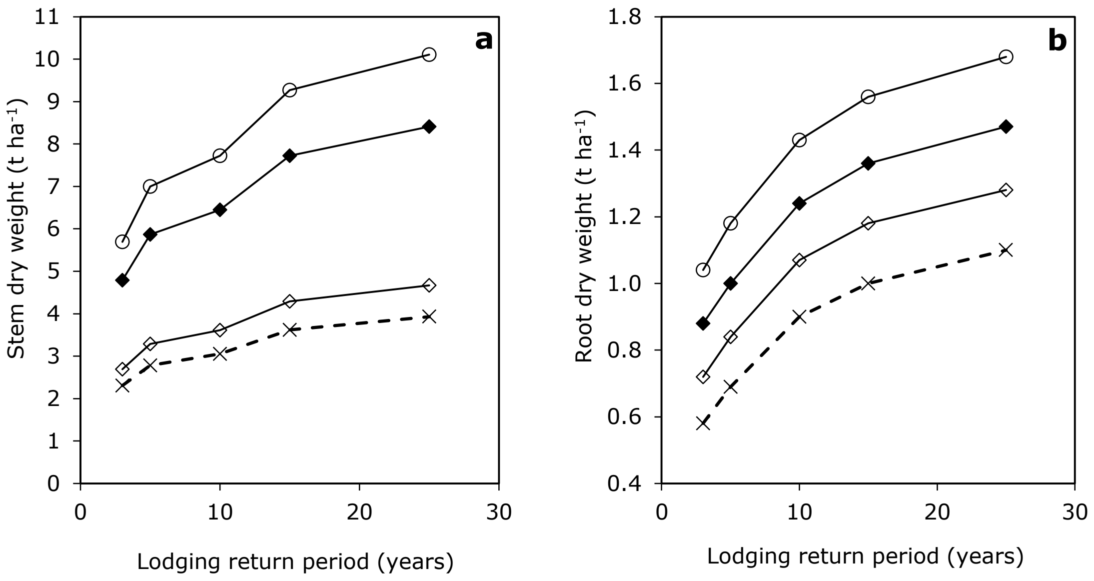

Fig. 2.7 Stem (a) and root dry weight (b) for a crop yielding 6 t ha^{-1} with a crop height of 1.0 m (♦) and 0.7 m (×), and crop yielding 10 t ha^{-1} with a crop height of 1.0 m (○) and 0.7 m (◇), for different lodging return periods in the NW Mexico environment. Dotted line indicates crop with current average yield of 6 t ha^{-1} and putative minimum crop height compatible with this yield of 0.7 m

2.5 Discussion

Firstly, in this section the applicability of the lodging model for spring wheat in the NW Mexico environment will be tested. Secondly, a comparison of the experimental results with published literature is made and, thirdly, implications of the results for plant breeders are considered.

2.5.1 Applicability of the lodging model

The winter wheat lodging model has demonstrated significant accuracy to predict timing and amount of lodging (Berry et al. 2003b). However, its applicability for spring wheat has not been tested. The experiment of 2011 experienced enough natural lodging to test the lodging model developed for spring wheat by comparing the severity of natural lodging against the predicted lodging risk calculated by the model. An index for natural lodging for each cultivar was calculated by summing the daily percentage of lodged area per plot between the first occurrence of lodging and harvest. The predicted lodging susceptibility was calculated by inputting the values of the lodging-associated characters into the model and calculating the mean value of the stem and root failure wind speed which ranged 7.6 to 11.7 m s^{-1}. Lodging occurred during early to mid-grain filling on 35 out of 60 cultivars of which 28 were predicted by the model; lodging was absent in 25 genotypes of which 12 were predicted by the model. Considering this, from the total of 60 cultivars the model correctly predicted 40 genotypes for either absence or presence of lodging giving a percentage of correct predictions of 67 %. Figure 2.8 shows a reasonable correlation between observed and predicted rankings for cultivar lodging resistance.

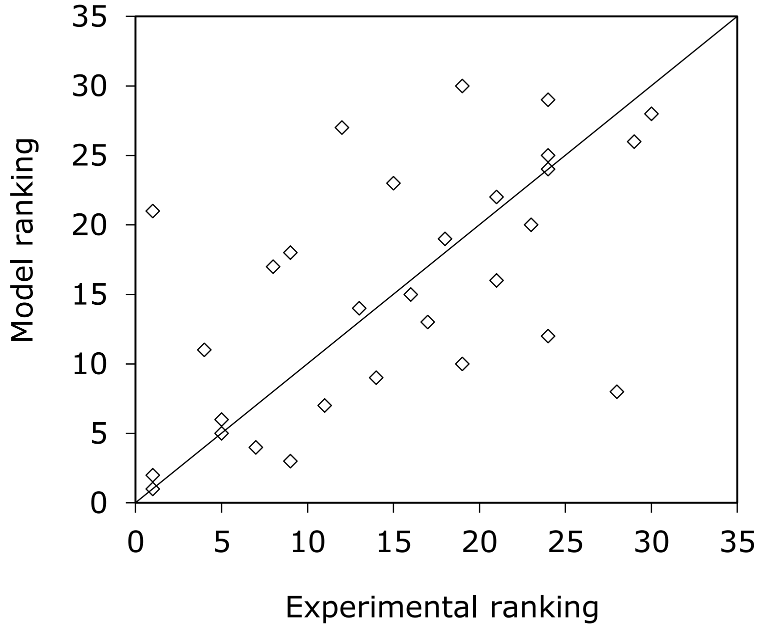

Fig. 2.8 Predicted and experimental ranking of spring wheat cultivars for lodging susceptibility under NW Mexico environment during 2011. Ranking is in ascending order. (—) 1:1 line. Best fit line, y = 0.60x + 6.52; R^2=0.35 (P < 0.001)

2.5.3 Comparison of results with published literature

Wind speed analysis for NW Mexico environment has demonstrated that spring wheat growing in these conditions must withstand 22 m s^{-1} and 18 m s^{-1} to resist stem and root lodging, respectively. This indicates that spring wheat will require stronger stems than UK winter wheat since this crop was estimated to be required to withstand 18 m s^{-1} for stem and root lodging risk) (Berry et al. 2007) and similar anchorage strength to support plants with the same height and yield and have lodging only once in 25 years. However, several differences between spring and winter wheat types must also be considered. It has been found that for a height at centre of gravity of 0.5 m spring wheat had a greater natural frequency of 1.5 Hz compared with about 1.0 Hz for winter wheat (Berry et al., 2004). Typical ear area for spring wheat averaged about 19 cm^2 compared with 12 cm^2 for winter wheat which had slightly greater yield per unit area and similar ears per square meter. It is likely that the absence of awns on UK winter wheat explains at least part of this difference. Overall these differences mean that spring wheat will have a greater leverage than winter wheat for crops with the same height, yield and ears m^{-2} (spring wheat = 383 N mm; winter wheat = 297 N mm).

Stem failure moment (stem strength) values for the bottom internode ranged from 134 to 252 N mm (See Chapter 3 for full description of analysis). These values are similar to the range of the stem strength measured in cultivar trials carried out on winter wheat in the UK which ranged from 122 to 230 N mm (Berry et al 2003b; Berry et al 2007). This study has shown a strong relationship between stem strength and structural stem biomass and between anchorage strength and surface root biomass. If there is limited scope to reduce lodging risk by further shortening crops then stem strength and anchorage strength will need to be increased, and this may have a substantial biomass cost that will compete against grain yield formation. A previous study on winter wheat (Berry et al. 2007) estimated that a stem dry weight per unit length of 1.65 mg mm^{-1} was required to achieve a stem strength of 100 N mm. However, the estimated biomass required for stem strength included both structural and water soluble carbohydrate (WSC) and it may therefore have over-estimated the amount of structural stem biomass required for stem strength. In the present study, WSC content was not related to the stem strength whereas structural stem biomass (including lignin) was strongly and positively associated with the stem strength. Knapp et al (1987) stated that lodging could not be related to fluctuations in WSC and structural carbohydrates content. On the other hand, Ma (2009) found the wheat gene *TaCM* (involved in lignin biosynthesis) was associated with stem strength and lodging index and Wiersma et al (2011) found a positive association between lodging resistance and acid detergent lignin (ADL) whilst Wang et al (2012) proposed that cellulose plays an important role in the ability of wheat stems to resist lodging. There is therefore little evidence to suggest that WSC contributes to stem strength. The present study estimated that 1.13 mg mm^{-1} of structural biomass is required to achieve a stem strength of 100 N mm. If it is assumed that spring and winter wheat have similar stem biomass/strength properties, then this indicates that the study of Berry et al (2007) may have over-estimated the stem biomass required to achieve specific strength targets by 40-50%.

Root biomass per plant in the top 10 cm of soil ranged amongst genotypes from about 200 to 500 mg per plant and a root plate spread of 30 to 55 mm. This is within a similar genetic range to a study carried out in the UK which observed a surface root biomass of 100 to 400 mg per plant and a root plate spread of 25 to 45 mm. Both studies had similar plant populations of close to 160 – 180 plants m^{-2}. This study has shown that breeding for a wider root plate will require greater investment in root biomass in the top 10 cm of soil. In order to increase root plate spread by 10 mm an additional 263 mg of surface root biomass per plant was required. For the average plant population (estimated at 163 plants m^{-2}), this equates to an additional surface root biomass of approximately 0.43 t ha^{-1} to increase root plate spread by 10 mm. This compares with a winter wheat study carried out in the UK which, for a single field experiment, estimated an additional 0.28 t ha^{-1} of surface root dry matter to increase the spread of the root plate by 10 mm (Berry et al., 2007). This comparison indicates that spring wheat grown in NW Mexico environment may require a greater investment in additional surface root biomass to widen its root plate than winter wheat grown in the UK.

2.5.4 Implications of achieving a lodging proof plant

It has been estimated that to achieve a 1 in 25 year lodging return period for a typical spring wheat crop grown in the NW Mexico environment will require approximately 3.93 t ha^{-1} of structural stem biomass. This assuming a crop yielding 6 t ha^{-1} (at 12 % moisture) with a height of 0.7 m. Unpublished data from experiments described in this study in 2011-13 showed that on average an additional 0.80 t ha^{-1} of biomass is required for the leaf lamina and sheath, and the chaff to grain dry weight ratio of 0.22 (see Chapter 3) gives a chaff dry weight of 1.16 t ha^{-1}. This gives a total non-grain biomass of 5.89 t ha^{-1}. Straw yields of up to 6 t ha^{-1} or just over 6 t ha^{-1} have been observed in NW Mexico (see Chapter 4). This ideotype would require a surface root biomass of approximately 1.10 t ha^{-1} which was not achieved by any cultivar in this study (estimated using equation from Fig. 2.6 for the targeted root plate spread of 51.1 mm). It therefore appears that for this ideotype it should be possible to achieve stem biomass requirements but not root biomass requirements with current germplasm. The NW Mexico environment can support greater yield than the average 6 t ha^{-1} currently achieved and could be up to 9 t ha^{-1} (Fischer and Edmeades, 2010). It is estimated that cultivars yielding 10 t ha^{-1} (with a height of 0.7 m) will require greater above-ground non-grain biomass equating to 4.67 t ha^{-1} (assuming no change in leaf and leaf sheath) and 1.28 t ha^{-1} of surface root biomass to achieve a lodging return period of 25 years. It should further be recognised that the breeding program at CIMMYT has increased the plant height of wheat to 1 m or above in the period of 1966 to 2009 (Aisawi et al. 2015), where the raised bed planting system may favour taller crops better at capturing the light in the gaps between the beds early in the season (Fischer et al. 2005). In the UK, Berry et al (2014) has shown that breeders have not shortened varieties since the 1990s. This indicates that achieving a high yield and a short (0.7 m tall) crop may be challenging. If a yield of 10 t ha^{-1} can only be achieved with a 1.0 m tall crop, then the above-ground non-grain biomass (stem, leaf and chaff) requirement increases to 10.1 t ha^{-1} and the surface root biomass to 1.68 t ha^{-1}. These biomass requirements will be very challenging to meet and illustrate that breeders must breed not only for greater total biomass, but also this biomass must be optimised carefully to maximise strength per unit of biomass. Certainly, it will be possible to increase total biomass as shown in the UK (Shearman et al. 2005) and in NW Mexico (Aisawi et al. 2015). Recently, several studies have identified QTLs that could be used to increase both yield and straw biomass. These QTLs were found in chromosome 7D of the Rialto x Savannah doubled haploid population (Berry et al. 2008), and chromosomes 4B and 4D of the Huapei 3 x Yumai 57 doubled haploid population (Li et al., 2014). Other cereals such as rice have shown QTLs related to both of these traits on chromosomes 1 and 6 of IR62266 x Norungan recombinant inbred line population (Suji et al., 2012). Additionally, QTL on chromosomes 2B, 6A and 6D were related to above-ground biomass and yield and QTL on chromosome 5A was related to above-ground biomass and straw yield in the wheat recombinant inbred line population Xiaoyan 54 x Jing 411 (Xu et al., 2014). Optimising how the additional dry matter is partitioned to maximise its usefulness will be very important. Targets for improving the efficiency with which non-grain biomass is used include; maximising stem strength per unit of stem biomass, maximising grain weight to ear weight ratio, minimising the production of infertile tillers and achieving high yields with shorter crops. Breeding for wider stems seems to be the most efficient way to increase the stem strength which together with a reduced leverage given by a shorter plant represents a strategic option to minimise structural biomass requirements. Additionally, breeding for more compact ears (unawned) in spring wheat could further reduce this leverage and consequently the biomass requirements. However, careful consideration must be taken because awned ears have been related to drought and heat resistance (Blum, 1986).

Dry matter harvest indices for these lodging-proof ideotypes equate to 0.46 for a 0.7 m tall crop yielding 6 t ha^{-1}, 0.54 for a 0.7 m tall crop yielding 10 t ha^{-1} and 0.41 for a 1.0 m tall crop yielding 10 t ha^{-1}. These figures are some way below the estimated potential harvest index for wheat of 0.62 (Austin, 1980). Winter wheat grown in the UK with a yield of 8 t ha^{-1} and height of 0.7 m was estimated to have a harvest index of 0.42 to be lodging-proof (Berry et al., 2007). However, it is likely that this study over-estimated the stem biomass requirement by including water soluble stem carbohydrate in the stem biomass measurements. If the same relationship between stem failure and structural stem weight observed for this present study for spring wheat is used for winter wheat, and a leaf and leaf sheath biomass of 1.0 t ha^{-1} is included, then this gives a harvest index of 0.49. The relatively high levels of non-grain biomass and low harvest indices that are estimated to result from breeding crops with a lodging return period of

25 years suggest that the high investment in non-grain biomass may compete with yield formation and limit the rate of breeding improvement in grain yield. This potential trade-off arises from the overlapping development periods of lodging traits and key yield-determining processes such as floret development and production of water soluble reserves. In fact, Slafer and Rawson (1994) stated that all the processes included from GS30 to GS60 (Zadoks et al., 1974) are considered of major importance for yield construction. Crook et al. (1994) described the development of the lodging characters (stem and root strength) from tillering (GS20) until maturity (GS87) and concluded that these traits stop developing soon after anthesis (GS65). There may be a net yield benefit from accepting a shorter lodging return period, since the advantage of lower non-grain biomass investment on yield potential may outweigh yield losses from more frequent lodging. If the lodging return period is reduced from 25 years to 10 years then the harvest index increases from 0.46 to 0.51 for a crop yielding 6 t ha^{-1} with height of 0.7 m, increases from 0.54 to 0.58 for a 0.7 m tall crop yielding 10 t ha^{-1}, and increases from 0.41 to 0.46 for a 1.0 m tall crop yielding 10 t ha^{-1}.

This chapter showed a significant effect of the leaf sheath on the stem strength measured 20 days after GS65 on internodes 2 to 5 (peduncle) where the presence of the leaf sheath increased stem strength by 12% for internode 2 to an increase of 34% on the peduncle. The effect on internode 1 was not significant because the leaf sheath was mostly senesced or not present at GS65 + 20 d. The leaf sheath has been reported to have an important mechanical role ensuring the plant standing ability in other species including; *Arundinaria tecta* (Poaceae) (Niklas, 1998), *Poa* *araratica*, *Bromus erectus*, *Arrhenatherum elatius* (Poacea), *Luzula nivea* (Juncaceae), *Carex arctata* (Cyperaceae) (Kempe et al. 2013) and *Triticale* (Zebrowski, 1992). These findings demonstrate that the leaf sheath is a mechanical component of the stem especially soon after flowering; however, its effects will diminish as the crop matures and the leaf sheath dries and eventually falls off. This study has estimated the structural requirements to avoid lodging for a plant at harvest without leaf-sheaths surrounding the internodes. This approach is appropriate for plants at harvest and is likely to be appropriate during a few weeks prior to harvest for the lower internodes, which most commonly buckle, and whose leaf sheaths senesce first. However, it will probably over-estimate the stem strength required to avoid lodging at earlier growth stages (e.g. at flowering) because the contribution of the leaf sheath is not included. Further work is required to quantify how the contribution of the leaf-sheath to the strength of each internode diminishes as the plant develops so that the minimum strength of the true stem required for various lodging return periods can be modelled more accurately.

NW Mexico spring wheat lodging ideotype trait values for a typical yield crop with a 25 year return period differ from the equivalent UK winter wheat lodging ideotype values as follows; spring wheat requires a 10% smaller root plate and a 7% stronger stem strength. Rainfall is practically absent during the lodging risk period in the NW Mexico environment and water supply has to be provided by periodic irrigation. This condition reduces the root lodging risk period to 10 days of grain filling period. Thus, the probability of a maximum wind gust speed during grain filling in 25 years decreases from 22 to 18 m s^{-1} which is a lower requirement to withstand root lodging. Drier, but windier, conditions in NW Mexico compared with the UK mean that both spring and winter wheat ideotypes must withstand the same maximum wind gust speed (18 m s^{-1}) for a 25-year root lodging return period; however, a yield of 8 t ha^{-1} for the UK ideotype compared with 6 t ha^{-1} for NW Mexico contributes to the greater root plate required by the UK ideotype. The greater stem strength requirement of spring wheat is mainly due to a higher maximum wind gust speed on the NW Mexico environment (22 m s^{-1}) and the greater ear area of spring wheat.

The genetic ranges for the key lodging traits are described in Chapter 3. This shows that it should be possible for plant breeders to achieve some of the ideotype dimensions for a spring wheat crop yielding 6 t ha^{-1} with height of 0.7 m. Nevertheless, if it is assumed that yield will increase in the following decades then the biophysical targets will increase. For example, if yield is increased to 10 t ha^{-1} then the stem diameter will need to increase by 8 %, root plate spread by 6 % and stem strength by 18 %. In this case it would be unlikely that plant breeders could achieve a lodging-proof plant with a lodging return period of 25 years with current germplasm. Our analysis also showed that the target dimensions will be further increased if yield improvements must also be accompanied by crop heights of more than 0.7 m.

2.6 Conclusion

Testing of an adapted lodging model for elite spring wheat lines showed reasonable cultivar ranking lodging susceptibility under crop, soil and weather conditions in NW Mexico. Also, the use of this model has enabled the calculation of the target lodging resistance traits of the lodging resistant ideotype for this particular environment. A positive stem and root biomass correlation with the stem strength (internode failure moment) and anchorage strength (root plate spread) was identified which enabled the structural dry matter requirements to be calculated for lodging proofness. It has been established that any improvement to achieve a lodging-proof crop that lodges only once in a period of 25 years would require an increase in the stem biomass which in turn could imply a trade-off with grain yield if improvement of the latter depends solely on increasing the HI. Alternatively, decreasing of the proportion of straw biomass to the total above-ground DM would increase the risk of lodging unless more total biomass is made available to straw through increasing RUE. This study therefore indicates that for plant breeders to achieve both high yields and lodging proofness they must either breed for greater total biomass or develop high yielding germplasm from shorter crops of 0.7 m or less.

2.7 References

Aisawi, K.A.B., Reynolds, M.P., Singh, R.P., Foulkes, M.J., 2015. The physiological basis of the genetic progress in yield potential of CIMMYT spring wheat cultivars from 1966 to 2009. Crop Sci. doi:10.2135/cropsci2014.09.0601

Allan, R.E., 1986. Agronomic comparisons among wheat lines nearly isogenic for three reduced-height genes. Crop Sci. 26, 707–710.

Austin, R.B., 1980. Physiological limitations to cereals yield and ways of reducing them by breeding, in: Hurd, R.G., Biscoe, P. V, Dennis, C. (Eds.), Opportunities for Increasing Crop Yield. Association of Applied Biology. Pitman, Boston, pp. 3–19.

Baker, C.J., Berry, P.M., Spink, J.H., Sylvester-Bradley, R., Griffin, J.M., Scott, R.K., Clare, R.W., 1998. A method for the assessment of the risk of wheat lodging. J. Theor. Biol. 194, 587–603. doi:10.1006/jtbi.1998.0778

Balyan, H.S., Singh, O., 1994. Pleiotropic effects of GA-insensitive Rht genes on grain yield and its component characters in wheat. Cereal Res. Commun. 22, 195–200.

Berry, P.M., Berry, S.T., Spink, J.H., 2008. Identification of genetic markers for lodging resistance in wheat. Home-Grown Cereal. Auth. Res. Proj. No. 441 HGCA 14 pp.

Berry, P.M., Kendall, S., Rutterford, Z., Orford, S., Griffiths, S., 2014. Historical analysis of the effects of breeding on the height of winter wheat (Triticum aestivum) and consequences for lodging. Euphytica.

doi:10.1007/s10681-014-1286-y

Berry, P.M., Spink, J., 2012. Predicting yield losses caused by lodging in wheat. F. Crop. Res. 137, 19–26. doi:10.1016/j.fcr.2012.07.019

Berry, P.M., Spink, J.H., Gay, A.P., Craigon, J., 2003a. A comparison of root and stem lodging risks among winter wheat cultivars. J. Agric. Sci. 141, 191–202. doi:10.1017/S002185960300354X

Berry, P.M., Sterling, M., Baker, C.J., Spink, J., Sparkes, D.L., 2003b. A calibrated model of wheat lodging compared with field measurements. Agric. For. Meteorol. 119, 167–180. doi:10.1016/S0168-1923(03)00139-4

Berry, P.M., Sterling, M., Mooney, S.J., 2006. Development of a Model of Lodging for Barley. J. Agron. Crop Sci. 192, 151–158.

Berry, P.M., Sterling, M., Spink, J.H., Baker, C.J., Sylvester-Bradley, R., Mooney, S.J., Tams, A.R., Ennos, A.R., 2004. Understanding and reducing lodging in cereals. Adv. Agron. 84, 217–271. doi:10.1016/S0065-2113(04)84005-7

Berry, P.M., Sylvester-Bradley, R., Berry, S., 2007. Ideotype design for lodging-resistant wheat. Euphytica 154, 165–179. doi:10.1007/s10681-006-9284-3

Blum, A., 1986. The efect on heat stress on wheat leaf and ear photosynthesis. J. Exp. Bot. 37, 111–118. doi:10.1093/jxb/37.1.111

Borrell, A.K., Incoll, L.D., Dalling, M.J., 1993. The influence of the Rht1 and Rht2 alleles on the deposition and use of stem reserves in wheat.pdf. Ann. Bot. 71, 317–326.

Cossani, C.M., Reynolds, M.P., 2012. Physiological traits for improving heat tolerance in wheat. Plant Physiol. 160, 1710–1718. doi:10.1104/pp.112.207753

Crook, M.J., Ennos, A.R., 1994. Stem and root characteristics associated with lodging resistance in four winter wheat cultivars. J. Agric. Sci. Agric. Sci. 123, 167–174. doi:10.1017/S0021859600068428

Crook, M.J., Ennos, A.R., Sellers, E.K., 1994. Structural development of the shoot and root systems of two winter wheat cultivars, Triticum aestivum L . J. Exp. Bot. 45, 857–863.

Easson, D.L., Pickles, S.J., White, E.M., 1995. A study of the tensile force required to pull wheat roots from soil. Ann. Appl. Biol. 127, 363–373.

Easson, D.L., White, E.M., Pickles, S.J., 1993. The effects of weather, seed rate and cultivar on lodging and yield in winter wheat. J. Agric. Sci. 121, 145. doi:10.1017/S0021859600077005

Finnigan, J.J., 1979. Turbulence in waving wheat. Boundary-Layer Meteorol. 16, 181–211.

Fischer, R.A., 1985. Number of kernels in wheat crops and the influence of solar radiation and temperature. J. Agric. Sci. 105, 447–461.

Fischer, R.A., Edmeades, G.O., 2010. Breeding and Cereal Yield Progress. Crop Sci. 50, S–85–S–98. doi:10.2135/cropsci2009.10.0564

Fischer, R.A., Sayre, K.D., Ortiz-Monasterio, J.I., 2005. The effect of raised bed planting on irrigated wheat yield as influenced by variety and row spacing, in: Roth, C.H., Fischer, R.A., Meisner, C. (Eds.), Evaluation and Performance of Permanent Raised Bed Cropping Systems in Asia, Australia and Mexico. ACIAR Proceedings No. 121, Griffith, Australia, pp. 1–11.

Fischer, R.A., Stapper, M., 1987. Lodging effects on high-yielding crops of irrigated semidwarf wheat. F. Crop. Res. 17, 245–258.

Flintham, J.E., Borner, A., Worland, A.J., Gale, M.D., 1997. Optimizing wheat grain yield: effects of Rht (gibberellin-insensitive) dwarfing genes. J. Agric. Sci. 128, 11–25.

Foulkes, M.J., Slafer, G. a, Davies, W.J., Berry, P.M., Sylvester-Bradley, R., Martre, P., Calderini, D.F., Griffiths, S., Reynolds, M.P., 2011. Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. J. Exp. Bot. 62, 469–86. doi:10.1093/jxb/erq300

Galicia, L., Nurit, E., Rosales, A., Palacios-Rojas, N., 2008. Laboratory protocols 2009: Maize nutrition quality and plant tissue analysis laboratory. CIMMYT, Mexico, D. F.

Kempe, A., Sommer, M., Neinhuis, C., 2013. A comparative analysis of the mechanical role of leaf sheaths of Poaceae, Juncaceae, and Cyperaceae. J. Bot. 2013, 1–6. doi:10.1155/2013/690549

Kertesz, Z., Flintham, J.E., Gale, M.D., 1991. Effects of Rht dwarfing genes on wheat grain yield and its components under eastern european conditions. Cereal Res. Commun. 19, 297–304.

Keyes, G.J., Paolillo, D.J., Sorrells, M.E., 1989. The effects of dwarfing genes Rht1 and Rht2 on cellular dimensions and rate of leaf elongation in wheat. Ann. Bot. 64, 683–690.

Knapp, J.S., Harms, C.L., Volenec, J.J., 1987. Growth regulator effects on wheat culm nonstructural and structural carbohydrates and lignin. Crop Sci. 27, 1201–1205.

Li, Z.K., Jiang, X.L., Peng, T., Shi, C.L., Han, S.X., Tian, B., Zhu, Z.L., 2014. Mapping quantitative trait loci with additive effects and additive x additive epistatic interactions for biomass yield, grain yield, and straw yield using a doubled haploid population of wheat (Triticum aestivum L.). Genet. Mol. Res. 13, 1412–1424.

Ma, Q.-H., 2009. The expression of caffeic acid 3-O-methyltransferase in two wheat genotypes differing in lodging resistance. J. Exp. Bot. 60, 2763–71. doi:10.1093/jxb/erp132

McCaig, T.N., Morgan, J.A., 1993. Root and shoot dry matter partitioning in near-isogenic wheat lines differing in height. Can. J. Plant Sci. 73, 679–689.

Miralles, D.J., Slafer, G.A., 1997. Radiation interception and radiation use efficiency of near-isogenic wheat lines with different height. Euphytica 97, 201–208.

Miralles, D.J., Slafer, G.A., 1995. Yield, biomass and yield components in dwarf, semi-dwarf and tall isogenic lines of spring wheat under recommended and late sowing dates. Plant Breed. 114, 392–396.

Miralles, D.J., Slafer, G.A., 1995. Individual grain weight responses to genetic reduction in culm length in wheat as affected by source-sink manipulations. F. Crop. Res. 43, 55–66.

Niklas, K.J., 1998. The Mechanical roles of clasping leaf sheaths: evidence from Arundinaria tecta (Poaceae) shoots subjected to bending and twisting forces. Ann. Bot. 81, 23–34.

Richards, R.A., 1992. The effect of dwarfing genes in spring wheat in dry environments. I. Agronomic characteristics. Aust J. Agric Res Res 43, 517–527.

Shearman, V.J., Sylvester-Bradley, R., Scott, R., Foulkes, R.K., 2005. Crop physiology and metabolism. Physiological processes associated with wheat yield progress in the UK. Crop Sci. 45, 175.

Slafer, G.A., Rawson, H.M., 1994. Sensitivity of wheat phasic development to major environmental factors: a re-examination of some assumptions made by physiologists and modellers. Aust. J. Plant Physiol. 21, 393. doi:10.1071/PP9940393

Sterling, M., Baker, C.J., Berry, P.M., Wade, A., 2003. An experimental investigation of the lodging of wheat. Agric. For. Meteorol. 119, 149–165. doi:10.1016/S0168-1923(03)00140-0

Suji, K.K., Biji, K.R., Poornima, R., Prince, K.S.J., Amudha, K., Kavitha, S., Mankar, S., Babu, R.C., 2012. Mapping QTLs for plant phenology and production traits using indica rice (Oryza sativa L.) lines adapted to rainfed environment. Mol. Biotechnol. 52, 151–60. doi:10.1007/s12033-011-9482-7

VSN International, 2012. GenStat for windows 15th edition.

Wang, J., Zhu, J., Huang, R., Yang, Y., 2012. Investigation of cell wall composition related to stem lodging resistance in wheat (Triticum aestivum L.) by FTIR spectroscopy. Plant Signal. Behav. 7, 856–863.

Wiersma, J.J., Dai, J., Durgan, B.R., 2011. Optimum timing and rate of trinexapac-ethyl to reduce lodging in spring wheat. Agron. J. 103, 864–870. doi:10.2134/agronj2010.0398

Xu, Y., Wang, R., Tong, Y., Zhao, H., Xie, Q., Liu, D., Zhang, A., Li, B., Xu, H., An, D., 2014. Mapping QTLs for yield and nitrogen-related traits in wheat: Influence of nitrogen and phosphorus fertilization on QTL expression. Theor. Appl. Genet. 127, 59–72. doi:10.1007/s00122-013-2201-y

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Reserach 14, 415–421.

Zebrowski, J., 1992. Planta complementary patterns of stiffness in stem and leaf sheaths of Triticale. Planta 187, 301–305.

Chapter 3 Genetic variation of stem and root structural properties of irrigated spring wheat

3.1 Abstract

Lodging-related traits were evaluated on the CIMMYT Core spring wheat Germplasm Panel (CIMCOG) in the Yaqui Valley of NW Mexico during three seasons (2010-11, 2011-12 and 2012-13). Genetic variation was significant for all the lodging-related traits in the cross-year analysis, however, significant G×E interactions due to rank changes or changes in the absolute differences between cultivars were identified. The inconsistences on cultivar performances across seasons particularly reduced the heritability of key characters related to root lodging resistance (anchorage strength). Target characters related to stem lodging resistance (stem strength) and shoot leverage (height) showed good heritability values equal or above 0.70. Positive correlations between stem strength and stem diameter and between root plate spread and root strength were found. Selecting for greater stem diameter and wall width, greater root plate spread and shorter plant height could enable breeders to increase lodging resistance by increasing stem strength, root strength and decreasing plant leverage, respectively. Achieving a lodging-proof crop will depend on finding a wider root plate spread than observed in current elite germplasm and implementing new crop management strategies. Genetic linkages between lodging traits will not constrain the achievement of a lodging-proof ideotype. However, strong association between stem strength and stem wall width will increase the biomass cost for stronger stems.

3.2 Introduction

The prediction of world population growth for the next decades entails an urgent need to adapt food crops to ensure global food supply demands in the future (Foulkes et al., 2011). Raising wheat productivity will be a fundamental strategy to achieve this (Reynolds et al., 2012). In recent years, breeding efforts have been focussed on increasing wheat production by raising yield potential (Acreche et al., 2008; Fischer and Edmeades, 2010; Slafer and Araus, 2007). Substantial yield increases will require a two-pronged approach; i) increasing photosynthetic capacity and above-ground biomass (Parry et al., 2011) and ii) optimizing DM partitioning to grain yield while maintaining lodging resistance (Foulkes et al., 2011). Lodging, the permanent displacement of stems from the vertical position, may limit yield improvement by two routes; i) directly by reducing photosynthetic capacity due to changes in canopy architecture (Berry and Spink, 2012) and ii) indirectly through breeding by increasing the amount of dry matter that must be partitioned to support structures at the expense of spike dry matter growth and yield when lodging resistance is increased (Berry et al., 2007). An improved lodging resistance achieved through careful optimisation of biomass partitioning will be required if genetic gains in yield potential are to be realized (Reynolds et al., 2011).

Lodging is a complex phenomenon influenced by many factors including wind, rain, topography, soil type, previous crop, husbandry and disease. There are two main types of lodging; root lodging caused by failure of the anchorage system and stem lodging caused by buckling of the stem. Conditions promoting plant growth, such as an abundant supply of nutrients, are also frequently associated with lodging (Berry et al., 2004).

Irrigation of wheat (e.g. in Mexico, India, and Australia) can cause significant lodging as the application of water reduces the soil strength, weakening plant anchorage. For instance, a survey conducted in a period of 10 years from 1981 to 1991 (80 commercial fields) lodging occurred in about 20 to 40% of a representative area in the Yaqui Valley, Obregon, Mexico (Tripathi et al., 2004). Lodging can reduce yield by up to 80%, and may result in reduced grain quality, greater drying costs, and slower harvest (Berry, 1998; Berry et al., 2004). Lodging losses are greatest when lodging occurs soon after flowering and when the angle of stem displacement from the vertical is high (Berry and Spink, 2012).

The introduction of dwarfing genes during the Green Revolution reduced the lodging susceptibility (Conway, 1997) by decreasing the leverage exerted on the stem base and anchorage system via reducing plant height, which allowed greater rates of fertilisation. However, this mechanism may no longer be the optimum approach for reducing lodging in modern wheat since recent studies have demonstrated that a putative minimum plant height of 0.7 m compatible with high yields (Allan, 1986; Balyan and Singh, 1994; Berry et al., 2015; Flintham et al., 1997; Kertesz et al., 1991; D J Miralles and Slafer, 1995; Richards, 1992) has been reached in many environments. On the other hand, important wheat breeding programs such as the one developed by CIMMYT have increased plant height of spring wheat cultivars released from 1966 to 2009 significantly above 0.7 m to in the region of 0.95 m (Aisawi et al., 2015).

If there is limited scope for plant breeders to counter the greater lodging risk caused by heavier yielding varieties by further shortening plants in some countries, then it follows that the biophysical components that support the plant (the stem and anchorage system) must be strengthened. The properties of the biophysical support structures have been quantified for winter wheat (Berry et al., 2007) and spring wheat (See Chapter 2) using a validated model of wheat lodging which evaluates the interaction of plant, soil (moisture) and wind characteristics (Berry et al., 2003c). Genetic variation of lodging-related traits, including stem and anchorage strength (stem and anchorage failure moment) found for winter wheat crops growing in UK conditions (Berry and Berry, 2015; Berry et al., 2007, 2003b), has demonstrated that breeding for these characters is feasible and will help towards the achievement of a lodging-proof plant. The biophysical structure dimensions required for a crop lodging return period of 25 years in spring wheat grown in NW Mexico have been quantified in this research (See Chapter 2). These requirements include a root plate spread of 51.1 mm and for the lowest basal internode, a stem strength of 268 N mm, diameter of 4.12 – 4.76 mm, material strength of 35 – 50 Mpa and wall width of 0.65 mm for a crop yielding 6 t ha^{-1}, with 500 shoots m^{-2}, 200 plants m^{-2} and crop height of 0.7 m. However, the potential for plant breeders to achieve these targets and whether this would incur any trade-offs with other traits affecting yield is unknown. Previous studies on spring wheat have reported genetic variation for length, diameter, wall width (A. J. Kelbert et al., 2004; Tripathi et al., 2003) and stem strength (Wiersma et al., 2011) of internodes and shoot height at centre of gravity (Tripathi et al., 2003). However, these efforts have not been enough to fully understand the lodging issue in spring wheat. For instance, Tripathi et al. (2003) and Kelbert et al. (2004) evaluated length, diameter and wall width of internodes in the NW Mexico environment but stem strength was not assessed; and Wiersma et al.

(2011) evaluated stem strength in a single cultivar. Dimensions for anchorage strength characters were only reported for a single cultivar growing under greenhouse conditions (Ennos, 1991a, 1991b). From the above it can be concluded that more research should be done on the full set of lodging-related traits on spring wheat.

The aim of this paper was to investigate the potential for plant breeders to improve lodging resistance in spring wheat grown in NW Mexico under high yield potential conditions by: a) evaluating the genetic variation and heritability of the lodging-related traits, particularly, those strongly related to the stem and anchorage strength; b) assessing the associations of stem and anchorage strength traits and other key physiological characters; and, c) evaluating the potential of achieving a lodging-proof ideotype defined for spring wheat grown in NW Mexico.

3.3 Materials and methods

3.3.1 Plant material and experimental conditions

The CIMMYT Mexico Core Germplasm Panel (CIMCOG), consisting of 58 *T. aestivum* and two *T. durum* cultivars was evaluated during 2010-11, and subsets of 30 cultivars during 2011-12 and 2012-13 and five cultivars during 2013-14 (Table S3-1) in four field experiments (referred to hereafter as 2011, 2012, 2013 and 2014 respectively) established at CENEB (Norman E. Borlaug Experiment Station) in the Valle del Yaqui, Sonora, Mexico (27° 24′ N, 109° 56′ W, 38 masl). The soil type at the experiment station is a coarse, sandy clay, mixed montmorillonitic typic caliciorthid, low in organic matter and slightly alkaline (pH 7.7) in nature (Sayre et al. 1997). For experiments 2011, 2012 and 2013 a typical raised bed planting system was used to arrange the cultivars (treatments) in a resolvable incomplete block design (Alpha Design). During 2011, each treatment was replicated twice in plots measuring 5 m x 3.2 m (each plot consisted of four raised beds each separated by a 0.56 m irrigation furrow and each bed had two rows with a row width of 0.24 m). Each replicate block contained 10 sub-blocks and each sub-block contained six treatment plots. During 2012 and 2013, each treatment was replicated three times in plots measuring 8.5 m x 2.4 m (3 raised beds each separated by a 0.8 m irrigation furrow, each bed had two rows with a 0.24 m row width). Each replicated block contained six sub-blocks and each sub-block contained five genotype treatment plots. The average seed rate for all plots in experiments 2011, 2012 and 2013 was 10.6 g m^{-2} which gave a range 213-292 seeds m^{-2}. For the experiment in 2014 a subset of five cultivars with contrasting values for stem strength, anchorage strength and stem wall material strength (cultivars 7, 19, 24, 57 and 60, see Table S3.1) was established using seed rates of 75, 125 and 175 seeds m^{-2} to evaluate the effect of low plant populations on lodging traits. A split plot design using the typical raised bed planting system was used. The seed rates were randomised on main plots and the five cultivars were randomised on sub-plots. Sub-plots were 8.5 m x 2.4 m (3 raised beds each separated by a 0.8 m irrigation furrow, each bed had two rows with a 0.24 m row width). Further plant population treatments were imposed after plant emergence on the lowest seed rate (75 seeds m^{-2}) treatment to increase the contrast between plant densities as: 1) six plants which were completely isolated within a 1.5 m x 2.4 m area of the plot; and 2) plant population on the rest of the plot was thinned to 25 plants m^{-2}. For all the experiments conventional agricultural management was used to ensure the crop was not deficient in water and nutrients or affected by weeds, pests or diseases. The irrigation schedule included five to six flood irrigation events (including one at sowing) during the growing season and the fertilization was 200 kg ha^{-1} of N (25% before sowing and 75% before first irrigation event) and 50 kg ha^{-1} of P (before sowing). Plant growth regulators were not applied in any of the experiments. Plant emergence dates (at 50 % of plants emerged) were recorded on 15 December 2010, 16 December 2011, 02 December 2012 and 01 December 2013 for experiments 2011, 2012, 2013 and 2014, respectively.

3.3.2 Weather conditions

Long-term mean weather conditions were obtained from two weather stations, the first was located at CENEB (1 – 2 km from the field trials) which collected all key weather parameters, but only spanned an 18-year period from 1997 to 2014, the second weather station was located 8 – 9 km from the field trials and collected only temperature data, but spanned a 40-year period from 1973-2014. Data were used to calculate the long-term mean and compared with data for each experimental year (weather station at CENEB) for the two major growth periods: pre-anthesis (December – February) and grain filling (March – April) (Table 3.1). Mean temperatures during the pre-anthesis period for 2011, 2012 and 2013 were 2 °C colder than the LTM at Obregon airport (1973-2014) and similar to LTM from CENEB. The mean temperature during 2014 was in between the LTMs at Obregon airport and CENEB. Solar radiation was similar in all cases for both growth periods. Accumulated rainfall was absent or very low during the whole 2011 growing season. Whilst rainfall was similar across 2012, 2013 and 2014 and LTM at CENEB for the pre-anthesis period. Rainfall for the grain filling period of the 2014 season was higher and significantly greater than the other seasons and the LTM at CENEB.

Table 3.1 Summary of weather conditions during pre-anthesis (PA) and grain filling (GF) periods for experiments 2011, 2012 and 2013 at the Yaqui Valley.

Parameter	Growth period	2011	2012	2013	2014	LTM[a]	LTM[b]
Min. temperature (°C)	PA	6.4	6.4	7.4	9.3	10.6	7.3
	GF	10.2	10.1	9.8	11.4	13.7	9.7
Max. temperature (°C)	PA	25.0	25.6	24.1	26.4	25.0	25.1
	GF	30.0	29.4	29.0	30.5	28.9	29.1
Mean temperature (°C)	PA	14.8	15.0	14.9	16.7	17.6	15.3
	GF	19.7	19.1	18.7	20.2	21.0	18.9
Mean solar radiation (MJ m^{-2})	PA	18.0	17.0	14.5	14.8	-	15.9
	GF	26.9	24.9	21.6	21.4	-	24.1
Mean accum. rainfall (mm)	PA	0.4	14.0	12.9	20.9	-	22.2
	GF	0.0	0.3	2.5	15.4	-	2.4

LTM[a], long term mean for 1973 – 2014 at Obregon Airport; LTM[b], long term mean for 1997-2014 at CENEB

3.3.3 Lodging traits measurements

Plant measurements were conducted 20 days after anthesis (GS65 + 20 days) (Zadoks et al. 1974) on 15 plants per plot during 2011 and 10 plants per plot during 2012 and 2013. These plants were randomly selected and extracted from each plot, avoiding the outer two rows in the plot and plot border. Plant extraction was achieved by pulling up the plants after excavating the surrounding ground with a hand fork to recover roots to a depth of 10 cm. After, the soil was removed from the roots by pressure washing. Measurements associated with lodging resistance included the root plate spread (mm) and structural rooting depth (mm) of the plant root system and fertile shoot number per plant. The remaining measurements were conducted on the main stem and included the height to the ear tip (mm), height at centre of gravity (mm) (with ears attached during 2012 and 2013 and without ears during 2011), natural frequency (Hz) for the whole shoot, and the diameter (mm), length (mm), wall width (mm), breaking strength (Newtons) and dry weight for internodes 1 and 2 (stem base) without leaf-sheath. Internode 1 was determined as the first internode of more than 10 mm originated just or below the soil surface (superior internodes in the stem were numbered in ascending order identifying the uppermost as the peduncle). The detailed methods for these measurements are described by Berry et al. (2000). Internode dry weight (g) was determined by drying the internodes until no further weight loss occurred at 75^{o}C. Surface root dry weight (g) was determined by trimming the roots at the stem base (in the top 10 cm of soil), washing off any remaining soil and drying at 75^{o}C until no further weight loss occurred.

Measurements taken on the 2014 experiment used in this chapter included only root plate spread at GS65 + 20 d.

3.3.4 Agronomic and physiological traits

Agronomic and physiological traits measured for this study included grain yield (t ha^{-1}), harvest index, thousand grain weight (g), straw yield (t ha^{-1}), chaff weight (t ha^{-1}), ears per square meter and heading and anthesis dates (GS55 and 65 in the Zadoks scale, respectively) (Zadoks et al., 1974). The methods used to collect the data for these traits were obtained from Pask et al. (2012). Measurements taken on the 2014 experiment included only grain yield. Heading and anthesis date were recorded when more than 50 % of the plot was at the respective growth stages. Grain yield was estimated from the harvested area which was determined avoiding border effects. The rest of the traits were estimated from a subsample of 100 ear-bearing shoots taken from the harvest area.

3.3.5 Calculated lodging parameters

A validated model of lodging for winter wheat (Baker et al., 1998; Berry et al., 2003c) was used to calculate the stem failure moment (stem strength), stem material strength, anchorage failure moment (anchorage strength), the wind-induced base bending moment (leverage) of the shoot and plant, and overall risk to stem and root lodging on spring wheat (stem and root failure wind speed). Details for these calculations are given in Chapter 2.

3.3.6 Statistical analysis

Individual analysis for each experiment was done using REML and performed with the MIXED procedure from SAS Institute Inc., 2004 considering the cultivar as a fixed effect and the replicate and blocks within replications as random effects. Combined analysis across experiments (cross-year) was also performed using REML considering the effects of experiments, replicates within experiments and genotype by environment (experiment) interaction (G×E) as random and genotype as fixed. Individual analysis was done according to the experimental design while cross-year analysis was done on 27 cultivars (consistent cultivars in each experiment, see Table S3.1) using a randomised complete block design. Adjusted means were estimated for each trait by experiment from individual analyses and combining data from 2011, 2012 and 2013 experiments (cross-year mean). Average standard error of difference (SED) between cultivar means by experiment and cross-year mean was calculated. Broad sense heritability (H^2) was calculated using Equation 3.1 for analyses for individual experiments and Equation 3.2 for analyses combined across experiments (Cooper et al., 1996).

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2/n_r} \tag{3.1}$$

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{n_e} + \frac{\sigma_e^2}{n_e n_r}} \tag{3.2}$$

where σ_g^2 and σ_e^2 are the genotypic (cultivar) and error variance, and σ_{ge}^2 is the G×E interaction. The number of environments and number of replicates are represented by n_e and n_r, respectively. Phenotypic correlations (r_p) between traits were simple Pearson correlations. Genetic correlations among traits (r_g) were calculated for cross-year means using the equation from Cooper et al (1996).

$$r_g = \frac{\overline{\sigma_{g(JJ')}}}{\sigma_{g(J)}\sigma_{g(J')}} \tag{3.3}$$

where $\overline{\sigma_{g(JJ')}}$ is the arithmetic mean of all pairwise genotypic covariances between trait j and j' and $\overline{\sigma_{g(j)}\sigma_{g(j')}}$ is the arithmetic average of all pairwise geometric means among the genotypic variance components of the traits. All the analyses, except for genetic correlations, were done using the suite META (Multi Environment Trial Analysis) which includes 33 SAS programs to analyse multi-environment trials (Vargas et al., 2013). Genetic correlations were done using a suite of R codes (META-R) for analysing multi-environment trials (Lopez-Cruz et al., 2015). SAS version 9.0 and R 3.2.1 were used to run the suites. Experiment 2014 was analysed separately using a PROC MIXED from SAS for a split plot design.

3.4 Results

3.4.1 Variation of lodging related traits due to genetic and environmental effects

Analysis of variance showed consistent differences ($P < 0.01 - 0.001$) between cultivars for most of the traits across all three experiments. Anchorage strength and root plate spread during 2011 and plant leverage during 2012 were the only cases were no statistically significant cultivar differences were detected. Reducing the number of cultivars from 60 in the 2011 experiment to 30 in 2012 and 2013 did not affect the statistical significance of the cultivar effects. The height at centre of gravity of the shoot measured in 2011 was less than in 2012 and 2013 because the ear was removed in the 2011 experiment before measuring this trait. This trait was, on average, 60 % of the plant height in 2012 and 2013, while it was 44 % of plant height in 2011. The cultivar range, expressed as the difference between the proportion of the grand mean of the smallest and largest cultivar, was greatest for anchorage strength, for which the values ranged from 1.39 in 2011 to 2.93 in 2012. For material strength this proportion ranged from 0.93 in 2012 to 1.29 in 2013 and for plant leverage from 0.93 in 2013 to 1.25 in 2011. The lowest proportion was found for internode diameter, root plate spread, structural rooting depth and plant height. These proportions ranged from 0.30 in 2011 to 0.35 in 2013 for internode diameter, 0.28 in 2011 to 0.47 in 2012 for root plate spread, 0.34 in 2011 to 0.45 in 2012 for structural rooting depth and 0.50 in 2012 to 0.39 in 2011 for plant height (Table 3.2).

Table 3.2 Summary of the individual analysis for stem (bottom internode 1), root and leverage characters associated to lodging resistance for spring wheat cultivars grown at NW Mexico during 2011, 2012 and 2013

Trait	2011[a]			2012[b]			2013[c]		
Stem	Mean	Range	SED	Mean	Range	SED	Mean	Range	SED
Diameter (mm)	4.21	3.60 – 4.87	0.157***	3.94	3.31 – 4.65	0.165***	3.74	3.07 – 4.37	0.150***
Wall width (mm)	0.80	0.67 – 1.25	0.059***	0.86	0.74 – 1.04	0.051***	0.72	0.53 – 0.96	0.065***
Strength (N mm)	134	97.9 – 189	20.3**	207	147 – 289	26.0***	206	137 – 295	20.5***
Material strength (MPa)	25.1	17.3 – 40.8	3.82***	39.0	28.9 – 65.2	3.77***	49.0	27.2 – 90.3	6.38***
Dry weight per length (DWL) (mg mm^{-1})	2.17	1.54 – 2.91	0.208***	2.88	2.38 – 3.56	0.244**	3.21	1.89 – 6.08	0.373***
DWL/strength (mg mm^{-1}/N mm)	0.015	0.010 – 0.021	0.0021***	0.014	0.012 – 0.020	0.0015***	0.016	0.010 – 0.034	0.002***
Stem failure wind speed (m s^{-1})	11.0	9.06 – 16.1	0.981***	13.7	11.6 – 17.9	0.879***	14.0	11.1 – 17.6	0.675***
Root strength									
Root plate spread (mm)	33.0	27.9 – 37.2	2.51^{ns}	37.0	28.5 – 45.8	3.77**	44.3	40.0 – 54.7	2.67**
Structural rooting depth (mm)	36.8	31.0 – 43.4	2.21***	38.6	32.6 – 50.0	2.55***	38.9	31.3 – 45.8	1.97***
Root dry weight (mg $plant^{-1}$)	372	215 – 550	78.3**	327	169 – 566	74.8***	244	151 – 366	42.0**
Anchorage strength (N mm)	232	78.0 – 400	80^{ns}	262	66.6 – 835	116***	397	177 – 1133	130***
Anchorage failure wind speed (m s^{-1})	8.67	4.63 – 12.6	1.47**	8.59	5.14 – 14.5	1.75***	11.5	7.98 – 19.6	1.39***
Leverage									
Plant height (mm)	1002	727 – 1120	25.0***	924	730 – 1049	21.5***	921	716 – 1047	19.5***
Height at centre of gravity (mm)	383	303 – 450	11.4***	565	483 – 640	12.2***	542	446 – 602	16.5***
Natural frequency (Hz)	1.28	0.97 – 2.21	0.115***	1.48	1.14 – 2.12	0.095***	1.68	1.28 – 2.45	0.130***
Ear area of main shoot (cm^2)	20.4	10.8 – 27.4	1.60***	19.3	12.8 – 25.3	1.24***	16.3	10.6 – 22.1	0.91***
Ear number per plant	2.73	1.60 – 3.50	0.360*	2.69	2.13 – 3.39	0.290**	2.63	1.97 – 3.07	0.250***
Shoot leverage (N mm)	192	66.0 – 283	20.8***	189	122 – 296	28.6***	179	89.1 – 279	13.4***
Plant leverage (N mm)	524	165 – 820	89.7***	506	359 – 845	113^{ns}	469	242 – 677	63.3***

[a] 60 cultivars; [b] 30 cultivars; [c] 30 cultivars; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Cross-year analysis of data from the 27 cultivars common across the experiments in 2011, 2012 and 2013 indicated large differences between the cultivars (G) ($P < 0.001$) (Table 3.3) consistent with the analyses of the individual experiments (Table 3.2). Environmental effects (E) were found ($P < 0.05 – 0.001$) for most of the traits, except for shoot and plant leverage and ear number per plant. A G×E interaction ($P < 0.05 – 0.001$) was observed for all traits (Table 4).

Table 3.3 Summary of the cross-year analysis for stem (bottom internode), root and leverage characters associated to lodging resistance for 27 spring wheat cultivars (G) grown at NW Mexico during 2011, 2012 and 2013 (E)

Trait	Mean	Range	SED (G)	*P* value (E)	*P* value (G×E)
Stem					
Diameter (mm)	3.96	3.35 – 4.47	0.120***	< 0.01	< 0.05
Wall width (mm)	0.80	0.64 – 0.92	0.054***	< 0.01	< 0.01
Strength (N mm)	184	134 – 252	20.5***	< 0.01	< 0.01
Material strength (MPa)	37.5	27.4 – 59.4	6.13***	< 0.001	< 0.001
DWL (mg mm^{-1})	2.78	1.95 – 3.85	0.525***	< 0.01	< 0.001
DWLSS (mg mm^{-1}/N mm)	0.016	0.013 – 0.023	0.0025***	< 0.05	< 0.001
SFWS (m s^{-1})	13.1	10.8 – 15.7	0.792***	< 0.01	< 0.001
Root					
Root plate spread (mm)	38.3	34.4 – 42.2	2.74***	< 0.01	< 0.01
Structural rooting depth (mm)	38.1	34.4 – 44.0	2.37***	< 0.05	< 0.001
Root dry weight (mg $plant^{-1}$)	315	213 – 437	55.9***	< 0.05	< 0.01
Anchorage strength (N mm)	302	169 – 585	0.120***	< 0.05	< 0.001
AFWS (m s^{-1})	9.84	7.12 – 12.8	1.68***	< 0.01	< 0.001
Leverage					
Plant height (mm)	938	726 – 1067	20.3***	< 0.01	< 0.01
Centre of gravity (mm)	492	410 – 543	10.9***	< 0.001	< 0.01
Natural frequency (Hz)	1.50	1.22 – 2.25	0.093***	< 0.01	< 0.01
Ear area of main shoot (cm^2)	18.4	11.5 – 24.8	1.05***	< 0.01	< 0.01
Ear number per plant	2.65	2.06 – 3.07	0.227***	ns	< 0.01
Shoot leverage (N mm)	183	103 – 283	17.8***	ns	< 0.05
Plan leverage (N mm)	483	263 – 643	69.1***	ns	< 0.05

DWL, dry weight per unit length; DWLSS, dry weight per unit length per unit strength; SFWS, stem failure wind speed; AFWS, anchorage failure wind speed; ***, $P < 0.001$; ns, not significant

3.4.2 Broad sense heritability of lodging related traits

Broad sense heritability for plant/shoot leverage and stem/root strength characters are illustrated in Table 3.4. Heritability values estimated for traits associated with stem strength in each experiment ranged 0.60 to 0.86 for 2011, 0.69 – 0.90 for 2012 and 0.74 to 0.94 for 2013. Despite the G×E interactions observed for all stem strength traits (Table 3.3) high values of heritability ranging from 0.70 to 0.94 were determined for the cross-year analysis, except for dry weight per unit of internode length (H^2 = 0.38) which in turn caused a low heritability for dry weight per unit length per unit strength (H^2 = 0.33). Stem diameter was the most consistent trait and showed the highest heritability (0.93). Root characters related to the anchorage strength had the lowest heritability values when compared with the rest of the characters (range 0.19 – 0.61 for 2011, 0.66 – 0.82 for 2012 and 0.62 – 0.82 for 2013) and for the cross-year values (range 0.11 – 0.48). Plant height had the highest and most consistent heritability ranging from 0.95 to 0.96 for the individual and cross-year analysis. Similarly, traits strongly related to plant height such as natural frequency (0.78 – 0.91) and height at centre of gravity (0.88 – 0.94) showed high consistent heritability values for the individual and cross-year analysis. Heritability for the area of the main shoot ear ranged from 0.85 – 0.93 between experiments and for the cross-year analysis. Ear number per plant had the lowest heritability ranging from 0.43 to 0.56. Shoot leverage heritability was consistent across years and for the combined analysis (H^2 = 0.72 – 0.92); plant leverage had lower heritability (0.36 – 0.74) which probably resulted from the low heritability of component trait ear number per plant.

Table 3.4 Broad sense heritability (*H^2*) for stem (bottom internode), root and leverage characters associated to lodging resistance for spring wheat cultivars (G) grown at NW Mexico during 2011, 2012 and 2013

Trait	2011[a]	2012[b]	2013[c]	Cross-year[*]
Stem				
Diameter	0.86	0.87	0.91	0.93
Wall width	0.84	0.82	0.74	0.76
Strength	0.60	0.71	0.88	0.78
Material strength	0.72	0.90	0.90	0.72
Dry weight per length	0.72	0.63	0.94	0.38
Dry weight per length per unit strength	0.68	0.69	0.92	0.33
Stem failure wind speed	0.71	0.80	0.91	0.82
Root				
Root plate spread	0.19	0.66	0.62	0.11
Structural rooting depth	0.61	0.82	0.74	0.40
Root dry weight	0.54	0.72	0.63	0.48
Anchorage strength	0.25	0.81	0.76	0.17
Anchorage failure wind speed	0.56	0.78	0.82	0.38
Plant and shoot				
Plant height	0.95	0.96	0.96	0.96
Height at centre of gravity	0.92	0.94	0.88	0.94
Natural frequency	0.78	0.89	0.88	0.91
Ear area of main shoot	0.85	0.89	0.93	0.92
Ear number per plant	0.43	0.53	0.56	0.46
Shoot leverage	0.85	0.72	0.92	0.87
Plant leverage	0.70	0.36	0.74	0.58

[a] 60 cultivars; [b] 30 cultivars; [c] 30 cultivars; [*] 27 cultivars

3.4.3 Agronomic and physiological traits

Cross-year analysis of variance has shown differences (*P* < 0.001) between cultivars for all the agronomic and physiological traits measured. Differences between environments (*P* < 0.05 – 0.01) were also found for all traits, except for ears per metre squared. Genotype by environment interaction (*P* < 0.05 – 0.001) affected all traits, except for thousand grain weight and chaff weight. High broad sense heritability was found for grain yield, yield components (TGW, ear m^{-2}), harvest index, non-grain biomass traits (straw yield, chaff) and key developmental stages (heading and anthesis date) (range 0.72 – 0.98) (Table 3.5).

Table 3.5 Summary of cross-year analysis of variance for agronomic and physiological traits of spring wheat cultivars (G) grown at NW Mexico during 2011, 2012 and 2013 experiments (E)

Trait	Mean	Range	SED (G)	***P* value (E)**	***P* value (G×E)**	***H*²**
Grain yield (t ha^{-1})	6.68	5.48 – 7.69	0.254***	< 0.05	< 0.01	0.82
Harvest index	0.47	0.43 – 0.53	0.0088***	< 0.01	ns	0.89
TGW (g)	42.8	31.2 – 51.6	1.12***	< 0.05	ns	0.98
Straw yield (t ha^{-1})	5.58	4.77 – 6.32	0.263***	< 0.01	< 0.05	0.86
Chaff (t ha^{-1})	1.88	1.52 – 2.24	0.111***	< 0.01	ns	0.72
Heading date (DAE)	83	73 – 93	1.61***	< 0.01	< 0.001	0.92
Anthesis date (DAE)	88	78 – 97	1.76***	< 0.01	< 0.001	0.91
Ear m^{-2}	303	224 – 375	15.5***	ns	< 0.05	0.93
Lodging score	2.28	0 – 15.7	–	–	–	–

H^2, broad sense heritability; TGW, thousand grain weight; DAE, days after emergence; ***, $P < 0.001$; ns, not significant

3.4.4 Association among traits

Associations between important agronomic, physiological and lodging traits for the 27 common cultivars in 2011-13 are described in Table 3.6. The correlations caused by both genetic and environmental factors combined are given by Pearson correlation coefficients (upper diagonal) (r_p) for the cross-year cultivar means and correlations caused by genetic factors alone are described by genetic correlation coefficients (lower diagonal) (r_g) obtained using the same cross-year cultivar means. Stem strength was positively correlated to stem diameter ($r_p = 0.44$, $r_g = 0.46$; $P < 0.05$), stem wall width ($r_p = 0.84$, $r_g = 0.97$; $P < 0.001$) and stem dry weight per unit length ($r_p = 0.61$, $r_g = 0.78$; $P < 0.001$). Stem dry weight per unit length per unit strength was negatively correlated to the stem strength ($r_p = -0.41$, $P < 0.05$; $r_g = -0.71$; $P < 0.001$). Material strength was not correlated with stem strength. Stem diameter, stem wall width and stem material strength are considered the major components of the stem strength. Within the stem strength components, the only significant negative association was found between stem diameter and stem material strength ($r_p = -0.75$, $r_g = -0.86$;

$P < 0.001$). Positive associations between stem dry weight per unit length and stem diameter ($r_p = 0.27$, not significant; $r_g = 0.48$; $P < 0.05$) and stem wall width ($r_p = 0.59$, $r_g = 0.90$; $P < 0.001$) were also found. Stem strength and its components were also related to grain yield and yield components and other physiological traits. For instance, stem wall width, stem diameter and stem strength were consistently and negatively associated with thousand grain weight and ears m^{-2} (r_p = -0.49 to -0.79, r_g = -0.53 to -0.78; P < 0.01 – 0.001). The strongest association with grain yield was found for stem diameter ($r_p = 0.37$, $P = 0.06$; $r_g = 0.41$, $P < 0.05$). There were no negative correlations between stem strength traits and grain yield. Material strength was correlated to the date of heading ($r_p = -0.53$, $r_g = -0.57$; $P < 0.01$) and anthesis ($r_p = -0.49$, $r_g = -0.52$; $P < 0.01$). This indicated that cultivars with later heading and anthesis developed smaller material strength.

Anchorage strength was positively correlated with its two components: root plate spread ($r_p = 0.88$, $r_g = 0.88$; $P < 0.001$) and structural rooting depth ($r_p = 0.89$, $r_g = 0.95$; $P < 0.001$), as expected given nature of the formula relating anchorage strength to its components. Surface root dry weight was positively related to anchorage strength ($r_p = 0.71$, $r_g = 0.91$; $P < 0.001$). Strong positive associations were also found between the anchorage strength components and root dry weight (r_p = 0.70 – 0.75, r_g = 0.77 – 0.94; $P < 0.001$). Root dry weight was positively correlated to internode diameter ($r_p = 0.48$, $P < 0.01$; $r_g = 0.71$, $P < 0.001$), stem strength ($r_p = 0.33$, ns; $r_g = 0.44$, $P < 0.05$) and dry weight per length ($r_p = 0.35$, ns; $r_g = 0.70$, $P < 0.001$) and negatively correlated to material strength ($r_p = -0.36$, ns; $r_g = -0.58$, $P < 0.001$). Additionally, a negative correlation between root plate spread and stem wall width (r_g = -0.44, P < 0.01) and positive association between anchorage strength and stem dry weight per unit length were found (r_p = 0.22, ns; r_g = 0.93, P < 0.001). Genetic correlation coefficients also indicated a positive association between root dry weight and grain yield, thousand grain weight and harvest index (r_g = 0.44 – 0.50; P < 0.01). In some cases, genetic correlation coefficients were not calculated or simply no association was identified due to genetic variance component values equal or close to zero (mainly when root traits were involved).

Plant height as a main component of the plant leverage was negatively correlated with the natural frequency (r_p = -0.65, r_g = -0.70; P < 0.001) and positively with the height at centre of gravity (r_p = 0.85, r_g = 0.87; P < 0.001). Plant height was also negatively correlated to stem dry weight per unit length per unit strength (r_p = -0.48, P < 0.05; r_g = -0.85, P < 0.001). Other agronomic traits related to plant height were thousand grain weight (r_p = 0.56, r_g = 0.58; P < 0.01), harvest index (r_p = -0.41, r_g = -0.44; P < 0.05) and lodging score (r_p = 0.30, not significant; r_g = 0.85; P < 0.001).

Grain yield was positively correlated with natural frequency (r_p = 0.41, P < 0.05; r_g = 0.50; P < 0.01) and harvest index (r_p = 0.58, P < 0.01 r_g = 0.62; P < 0.001). Also, a genetic correlation coefficient of 0.54 (P < 0.01) between grain yield and stem dry weight per unit length was found. No significant association was found for grain yield and lodging score. Harvest index was correlated positively to natural frequency (r_p = 0.36; P = 0.07, r_g = 0.42; P < 0.05), and negatively to the height at centre of gravity (r_p = -0.44, r_g = -0.47; P < 0.05) and lodging score (r_p = -0.15, not significant; r_g = 0.48; P < 0.05).

Table 3.6 Phenotypic (upper diagonal) and genetic (lower diagonal) correlations between cross-year means of lodging, agronomic and physiological traits of 27 spring wheat cultivars grown at NW Mexico during 2011, 2012 and 2013

Traits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1. GY		**0.41**	-0.12	0.15	-0.25	0.04	-0.09	-0.10	0.19	-0.19	0.37	0.24	0.18	0.11	-0.32	0.14	-0.22	0.02	0.05	0.33	**0.58**	0.01
2. NF	**0.50**		**-0.65**	0.23	**-0.81**	**-0.40**	-0.12	-0.17	0.14	-0.31	0.18	-0.06	0.20	**0.42**	-0.37	-0.11	-0.16	0.20	0.25	-0.07	0.36	0.27
3. PH	-0.16	**-0.70**		0.08	**0.85**	0.27	-0.06	-0.05	-0.09	0.30	0.33	0.12	-0.09	**-0.48**	0.00	0.33	-0.04	-0.29	-0.25	**0.56**	**-0.41**	-0.34
4. ENPP	0.21	**0.43**	0.11		-0.25	-0.35	-0.32	**-0.58**	**-0.39**	0.11	-0.09	-0.23	-0.25	-0.16	0.04	-0.16	**-0.46**	**-0.50**	**-0.45**	0.23	0.23	**0.40**
5. CG	-0.31	**-0.87**	**0.87**	**-0.42**		**0.40**	0.17	0.28	0.08	0.27	0.10	0.17	-0.06	**-0.38**	0.20	0.27	0.28	-0.09	-0.10	0.34	**-0.44**	**-0.42**
6. EA	0.02	**-0.42**	0.27	**-0.57**	**0.42**		**0.53**	**0.48**	**0.51**	0.04	0.37	**0.61**	0.28	-0.35	-0.07	**0.56**	**0.56**	0.01	-0.02	**0.54**	0.07	**-0.82**
7. RPS	0.04	-0.11	-0.12	na	**0.61**	na		**0.79**	**0.70**	-0.14	0.23	0.09	0.23	0.08	-0.20	0.14	**0.88**	0.23	0.19	0.07	0.15	**-0.49**
8. SRD	-0.06	-0.24	0.00	na	**0.55**	**0.76**	**0.90**		**0.75**	0.03	0.13	0.07	0.28	0.25	-0.14	0.09	**0.89**	0.36	0.32	0.00	0.07	**-0.48**
9. RDW	**0.50**	0.29	-0.07	na	0.13	**0.77**	**0.94**	**0.77**		-0.04	**0.48**	0.30	0.35	0.08	-0.36	0.33	**0.71**	0.32	0.31	0.31	0.28	**-0.65**
10. LS	0.00	**-0.79**	**0.85**	**0.41**	**0.90**	0.05	na	-0.09	-0.22		0.06	-0.08	-0.05	-0.13	0.04	0.07	-0.04	0.02	0.05	0.14	-0.15	-0.10
11. SD	**0.41**	0.21	0.34	-0.20	0.12	0.35	0.35	0.09	**0.71**	0.08		0.31	0.27	-0.14	**-0.75**	**0.44**	0.15	0.20	0.26	**0.59**	0.10	**-0.51**
12. SWW	0.37	-0.02	0.16	**-0.56**	0.21	**0.69**	**-0.44**	-0.21	0.23	**-0.43**	0.32		**0.59**	-0.28	0.19	**0.84**	0.14	-0.08	-0.08	**0.49**	-0.02	**-0.64**
13. SDWL	**0.54**	**0.51**	-0.17	**-0.48**	-0.12	**0.38**	Na	Na	**0.70**	**-0.54**	**0.48**	**0.90**		**0.45**	0.13	**0.61**	0.22	-0.21	-0.20	0.28	0.19	**-0.43**
14. SDWLSS	0.30	**0.89**	**-0.85**	-0.37	**-0.65**	**-0.65**	**0.89**	**0.95**	0.18	**-0.39**	-0.15	**-0.67**	-0.18		-0.13	**-0.41**	0.07	0.16	0.14	**-0.41**	0.26	0.32
15. SMS	-0.36	**-0.38**	-0.01	0.30	0.22	-0.08	-0.33	0.04	**-0.58**	0.11	**-0.86**	0.30	-0.11	-0.34		0.21	-0.12	**-0.49**	**-0.53**	-0.16	-0.16	0.08
16. SS	0.24	-0.10	0.37	-0.26	0.30	**0.59**	0.07	0.11	**0.44**	-0.21	**0.46**	**0.97**	**0.78**	**-0.71**	0.12		0.16	-0.29	-0.25	**0.65**	-0.04	**-0.72**
17. AS	-0.34	-0.18	-0.01	na	**0.72**	na	**0.88**	**0.95**	**0.91**	**-0.72**	0.09	-0.19	**0.93**	**0.55**	0.04	0.22		0.38	0.33	0.02	-0.03	**-0.52**
18. AD	0.00	0.20	-0.32	**-0.87**	-0.10	0.02	**0.69**	**0.56**	**0.56**	0.19	0.22	-0.09	-0.27	0.24	**-0.52**	-0.25	**0.80**		**0.99**	**-0.43**	-0.26	0.08
19. HD	0.02	0.25	-0.29	**-0.77**	-0.11	-0.02	**0.60**	**0.48**	**0.56**	0.22	0.28	-0.06	-0.25	0.20	**-0.57**	-0.22	**0.71**	**1.00**		**-0.38**	-0.28	0.08
20. TGW	0.37	-0.05	**0.58**	**0.39**	0.36	**0.57**	0.21	0.02	**0.48**	**0.40**	**0.62**	**0.56**	**0.49**	**-0.72**	-0.20	**0.76**	0.10	**-0.43**	**-0.39**		0.18	**-0.67**
21. HI	**0.62**	**0.42**	**-0.44**	0.35	**-0.47**	0.05	0.35	0.07	**0.44**	**-0.48**	0.08	-0.03	0.30	**0.38**	-0.10	-0.01	-0.19	-0.32	-0.33	0.19		-0.10
22. Ear m^{-2}	-0.06	0.31	-0.36	**0.57**	**-0.46**	**-0.88**	na	**-0.70**	**-0.97**	0.01	**-0.53**	**-0.73**	**-0.74**	**0.46**	0.14	**-0.79**	na	0.05	0.06	**-0.70**	-0.12	

GY, grain yield; NF, natural frequency; PH, plant height; ENPP, ear number per plant; CG, height at centre of gravity; EA, ear area; RPS, root plate spread; SRD, structural rooting depth; RDW, root dry weight; LS, lodging score; SD, stem diameter; SWW, stem wall width; SDWL, stem dry weight per unit length; SDWLSS, stem dry weight per unit length per unit strength; SMS, stem material strength; SS, stem strength; AS, anchorage strength; AD, anthesis date; HD, heading date; TGW, thousand grain weight; HI, harvest index; coefficients in bold indicate significant correlations ($P < 0.05 – 0.001$); na, not available

3.4.5 Seed rate effect on root plate spread and grain yield

A summary of the statistical analysis performed on root plate spread and grain yield data from the 2014 experiment indicated genetic differences for both traits. Also root plate spread differed between seed rates and there was an interaction between seed rate and cultivar ($P < 0.001$). These results showed that root plate spread could be increased from 49.2 mm to 68.7 mm by reducing seed rate from 175 to 25 m^{-2} without reducing grain yield. (Table 3.7).

Table 3.7 Analysis of variance for root plate spread and grain yield under different seed rates (SD) for a subset of five cultivars (G) from CIMCOG panel grown at NW Mexico during 2014

Seed rate	**Root plate spread (mm)**	**Grain yield (t ha^{-1})**
Isolated plants	102.3	–
25 seed m^{-2}	68.7	6.33
125 seed m^{-2}	48.4	6.21
175 seed m^{-2}	49.2	6.40
SED (SD)	2.65***	0.173ns
SED (G)	2.20***	0.135***
SED (interaction)	4.74***	0.297ns

***, $P < 0.001$; ns, not significant

3.5 Discussion

3.5.1 Breeding a lodging-proof plant

It was estimated in Chapter 2 that a spring wheat yielding 6 t ha^{-1} with 500 shoots m^{-2} and 200 plants m^{-2} grown in the NW Mexico environment with a lodging return period of 25 years will require a maximum height of 0.7 m, a minimum root plate spread of 51.1 mm and a minimum stem strength for the bottom internode of 268 N mm. The target stem strength could be achieved with a diameter of 4.76 mm, and a material strength of 35 MPa, or with a diameter of 4.12 mm and a material strength of 50 MPa, each with a maximum wall width of 0.65 mm. The rationale for having a thin internode wall width was to minimise biomass cost to increase stem strength based on engineering principles. The cross-year cultivar analysis demonstrated that not all of the target traits could presently be achieved by a single cultivar. Individual target traits that could be achieved included stem diameter and material strength. Minimum wall width observed in spring wheat was 0.64 mm which was just below the maximum requirement of 0.65 mm. Maximum stem strength found in the CIMCOG panel (252 N mm) was 6% below the target requirement (268 N mm). Root plate spread was not achieved by any of the CIMCOG panel cultivars investigated, with a maximum value observed of 42.2 mm which was 17% less than the requirement of 51.1 mm. A minimum plant height of 0.73 m was observed in the CIMCOG panel which was 4% above the maximum requirement (0.70 m) (Table 3.8). Coincidentally the best values observed in winter wheat cultivars were also lower than the target stem strength and root plate spread and with greater plant height than the estimated lodging-proof winter wheat in a UK environment (Berry et al., 2007).

Table 3.8 Ideotype targets, genetic range and best cultivar for the key lodging traits of spring wheat (CIMCOG panel) for 27 wheat cultivars. Values represent means of 2011-13.

Trait	Ideotype target	Genetic range	Best observed value
Stem diameter (mm)	4.12 – 6.03	3.35 – 4.47	4.47 (cv. 60)
Stem wall width (mm)	0.65	0.64 – 0.92	0.64 (cv. 37)
Stem strength (N mm)	268	134 – 252	252 (cv. 23)
Stem material strength (MPa)	20 – 50	27.4 – 59.4	59.4 (cv. 45)
Root plate spread (mm)	51.1	33.7 – 42.2	42.2 (cv. 16)
Height (m)	0.70	0.73 – 1.07	0.73 (cv. 9)

Correlations between lodging and agronomic traits are relevant to inform breeders on strategies to improve resistance to both stem and root lodging, whilst also increasing grain yield. For instance, spring wheat has shown significant associations between stem strength and stem wall width and diameter and a positive weak but not significant association between diameter and wall width (Table 3.6). No significant relationship was found for stem strength and stem material strength. Maximising stem diameter and minimizing stem wall width will result in less investment of biomass to increase stem strength (Berry et al., 2007). However, our results have demonstrated a strong linkage between stem wall width and stem strength which will be difficult to break. It appears then that thicker walled stems should be considered for spring wheat. For example, to obtain the targeted stem strength (268 N mm) with the maximum stem wall width dimension observed in spring wheat of 0.92 mm, a stem diameter of only 3.89 mm with a material strength of 50 MPa will be required. A thicker stem wall will increase the biomass per unit strength (Berry et al., 2007) which in turn will demand more biomass investment for support structures. This additional biomass investment in the stem may compete for resources with spike dry matter growth and yield determination. However, this maybe an acceptable compromise if it makes breeding for strong stems easier and quicker.

This study has indicated that genetic linkages between stem strength and its components, root strength and its components, plant height and yield and its components do not represent a significant limitation to achieve the dimensions of a desired lodging-resistant ideotype. Similar investigations on winter wheat reached the same conclusions (Berry et al., 2007, 2003c) and in fact several of these inter-relationships can help breeders to improve stem and root strength (Berry and Berry, 2015). Additionally, traits with positive effects on stem and root strength such as natural frequency, stem diameter, stem dry weight per unit length and root dry weight showed positive associations with grain yield.

3.5.2 Heritability

This study has observed large genetic variation in spring wheat for the majority of the traits involved in the stem strength, anchorage strength, leverage of the stem base and structural biomass for plant support. Apart from root plate spread, anchorage strength and plant leverage this large genetic variation has been found for the individual and cross-experiment analysis of variance. Experimental means differed in all cases, except for ear number per plant and shoot and plant leverage. Lodging-traits have been reported to show G×E interactions in winter wheat (Berry and Berry, 2015; Berry et al., 2003b) and our study has confirmed the same finding for spring wheat. Breeding for these traits may therefore need selection in multiple environments. Additionally, repeatability of cultivar performance across experiments will be an interesting parameter to check since G×E decreases both heritability and response to selection (Cooper et al., 1996). The key traits for stem strength (stem diameter, wall width and material strength) and plant leverage (plant height) have heritability values equal or above 0.70, which makes them useful for breeding selection. However, for anchorage strength characters such as root plate spread and structural rooting depth had heritability values of 0.11 and 0.40, respectively. Thus, response to selection will be more difficult for anchorage characters.

High yielding spring wheat (yield potential: 6.12 to 8.40 t ha^{-1} at 12% moisture) used in this study has been demonstrated to have promising performances for yield and yield components and harvest index, all with high heritability.

3.5.3 Implications for selection of improved lodging resistant cultivars

The investment of additional structural biomass in roots and stems required for greater lodging resistance has been fully explained in Chapter 2 and is one of the challenges of improved lodging resistance in wheat. The strong positive correlations between stem dry weight per unit length and stem strength, and root plate spread with root surface biomass indicated in Table 3.6 supports this premise. However, even with greater biomass, careful optimization of yield and lodging traits will be required to minimize the inevitable trade-offs between grain yield-formation traits and lodging-related traits that develop at the same time. The strong genetic linkage found between stem strength and stem wall width is likely to compromise the strategy of minimising the biomass invested in the stem by breeding for wide thin walled stems, as was done in Berry et al. (2007). A comparison between thin walled stems with a dimension of 0.65 mm and thick walled stems with 0.92 mm has indicated that stem biomass investment in support structures would increase by approximately 1.0 t ha^{-1} with thicker walled stems.

Reducing plant height to 0.7 m might decrease overall stem biomass and the storage capacity of stems. This will represent a trade-off for yield if lodging-resistant ideotype is achieved. Nevertheless, to counteract this situation other traits such as fruiting efficiency (i.e. the number of grains set per unit of spike dry weight at anthesis) can be improved. Improving fruiting efficiency has been proposed as one of the strategies to raise grain yield through increasing grain number per unit area in cultivars that already reached the optimum height (0.7 – 1.0 m) (Slafer et al., 2015).

Finding germplasm with the root plate spread target dimension will be difficult based on the known genetic ranges of winter and spring wheat. Low repeatability will limit the response for selection of improved lodging resistance of this trait. Root dry weight may play an important role for anchorage strength improvements since it was found to be more heritable. Possible strategies to find material with the target dimension will include screening new germplasm or even wild relatives, and improving this trait by crop management. For instance, reducing the plant population density increases root lodging resistance (Berry et al., 2007, 2004) by increasing anchorage strength (Berry et al., 2000). Present results from the 2014 experiment have also indicated that root plate spread dimensions identified at 213-292 seeds m^{-2} (experiments 2011, 2012 and 2013) could be enhanced by reducing seed rate while maintaining high grain yields (Table 3.7). This demonstrates the great potential for improving anchorage strength by establishing lower plant populations. However, using lower seed rates does run the risk of establishing a sub-optimal plant population for yield when plant establishment conditions are poor and may increase weed growth between widely spaced plants.

Optimisation of sample size and number of traits to evaluate lodging must be done in order to reduce the time-consuming nature of the methods used in this paper. However, precision and power to detect genetic differences among cultivars must be maintained. The time-consuming nature of measuring these traits represents a disadvantage and restricts the capacity to evaluate a large number of cultivars. In Chapter 5 using data from this study both the minimum plant sample size while maintaining reliability in identifying genetic differences and a reduced number of traits that enables the lodging model to estimate cultivar lodging susceptibility will be estimated.

The absence of rapid lodging screening methodologies could also be counteracted by the implementation of molecular tools to improve lodging resistance of wheat. QTLs (quantitative trait loci) or other trait-marker associations related to lodging resistance traits must be identified to develop genetic markers for marker-assisted selection that can accelerate breeding for these characters. Several studies have identified QTLs linked to stem diameter (Berry and Berry, 2015; Hai et al., 2005; Keller et al., 1999), stem wall width (Berry and Berry, 2015; Hai et al., 2005), stem strength, stem material strength, root plate spread and root plate depth (Berry and Berry, 2015). Gene TaCM, involved in the biosynthesis of lignin, was associated with the stem strength and lodging index (Ma, 2009). Moreover, it has been found that several QTL in the wheat genome are linked to both yield and straw biomass (Berry et al., 2008; Li et al., 2014) which might be useful in increasing yield and lodging resistance simultaneously.

3.6 Conclusion

The present study has identified genetic variation in spring wheat elite germplasm for lodging related traits. However, significant G×E interactions have also been found affecting consistency across experiments and resulting in low heritability for several characters. Target stem strength traits had good heritability values equal or above 0.70. The major anchorage strength trait (root plate spread) was strongly affected by G×E interaction and this had a low heritability of 0.11. This study has indicated that lodging-proof ideotype targets for diameter, material strength and wall width of the stems can be achieved in spring wheat, but not yet in the same cultivar. Stem strength and plant height were 6 and 4 percent below the required dimensions and do not represent a difficult challenge. However, new germplasm and management strategies should be implemented to achieve the target root plate spread since this was significantly below the ideotype target. Linkages between lodging traits do not represent a significant challenge to achieve the lodging-proof ideotype through breeding, although strong association between stem strength and stem wall width will increase the biomass cost for stronger stems. These results will help breeders to (i) select potential parents for strategic crosses, ii) focus on the most important traits that determine genetic variation in lodging risk and (iii) indirectly select for stem lodging related traits during the early segregating generations when yield tests are not yet being conducted.

3.7 References

Acreche, M.M., Briceño-Félix, G., Martín Sanchez, J.A., Slafer, G.A., 2008. Physiological bases of genetic gains in Mediterranean bread wheat yield in Spain. Eur. J. Agron. 28, 162–170. doi:10.1016/j.eja.2007.07.001

Aisawi, K.A.B., Reynolds, M.P., Singh, R.P., Foulkes, M.J., 2015. The physiological basis of the genetic progress in yield potential of CIMMYT spring wheat cultivars from 1966 to 2009. Crop Sci. doi:10.2135/cropsci2014.09.0601

Allan, R.E., 1986. Agronomic comparisons among wheat lines nearly isogenic for three reduced-height genes. Crop Sci. 26, 707–710.

Baker, C.J., Berry, P.M., Spink, J.H., Sylvester-Bradley, R., Griffin, J.M., Scott, R.K., Clare, R.W., 1998. A method for the assessment of the risk of wheat lodging. J. Theor. Biol. 194, 587–603. doi:10.1006/jtbi.1998.0778

Balyan, H.S., Singh, O., 1994. Pleiotropic effects of GA-insensitive Rht genes on grain yield and its component characters in wheat. Cereal Res. Commun. 22, 195–200.

Berry, P.M., 1998. Predicting lodging in wheat. Ph. D. Thesis. The University of Nottingham, UK, 210 pp.

Berry, P.M., Berry, S.T., 2015. Understanding the genetic control of lodging-associated plant characters in winter wheat (Triticum aestivum L.). Euphytica. doi:10.1007/s10681-015-1387-2

Berry, P.M., Berry, S.T., Spink, J.H., 2008. Identification of genetic markers for lodging resistance in wheat. Home-Grown Cereal. Auth. Res. Proj.

No. 441 HGCA 14 pp.

Berry, P.M., Griffin, J.M., Sylvester-bradley, R., Scott, R.K., Spink, J.H., Baker, C.J., Clare, R.W., 2000. Controlling plant form through husbandry to minimise lodging in wheat. F. Crop. Res. 67, 59–81.

Berry, P.M., Kendall, S., Rutterford, Z., Orford, S., Griffiths, S., 2015. Historical analysis of the effects of breeding on the height of winter wheat (Triticum aestivum) and consequences for lodging. Euphytica 203, 375–383. doi:10.1007/s10681-014-1286-y

Berry, P.M., Spink, J., 2012. Predicting yield losses caused by lodging in wheat. F. Crop. Res. 137, 19–26. doi:10.1016/j.fcr.2012.07.019

Berry, P.M., Spink, J.H., Gay, A.P., Craigon, J., 2003a. A comparison of root and stem lodging risks among winter wheat cultivars. J. Agric. Sci. 141, 191–202. doi:10.1017/S002185960300354X

Berry, P.M., Sterling, M., Baker, C.J., Spink, J., Sparkes, D.L., 2003b. A calibrated model of wheat lodging compared with field measurements. Agric. For. Meteorol. 119, 167–180. doi:10.1016/S0168-1923(03)00139-4

Berry, P.M., Sterling, M., Spink, J.H., Baker, C.J., Sylvester-Bradley, R., Mooney, S.J., Tams, A.R., Ennos, A.R., 2004. Understanding and reducing lodging in cereals. Adv. Agron. 84, 217–271. doi:10.1016/S0065-2113(04)84005-7

Berry, P.M., Sylvester-Bradley, R., Berry, S., 2007. Ideotype design for lodging-resistant wheat. Euphytica 154, 165–179. doi:10.1007/s10681-006-9284-3

Conway, G., 1997. The Doubly Green Revolution. Food for all in the 21st century. Penguin Group, London, England.

Cooper, M., DeLacy, I.H.H., Basford, K.E.E., 1996. Relationshis among analytical methods used to analyse genotypic adaptation in multi-environment trials, in: Cooper, M., Hammer, G.L. (Eds.), Plant Adaptation and Crop Improvement. CAB International, Wallingford, UK, pp. 193–224.

Ennos, A.R., 1991a. The Mechanics of Anchorage in Wheat Triticum aestivum L.: II. Anchorage of mature wheat against lodging. J. Exp. Bot. 42, 1607–1613.

Ennos, A.R., 1991b. The Mechanics of Anchorage in Wheat Triticum aestivum L.: I. The anchorage of wheat seedlings. J. Exp. Bot. 42, 1601–1606. doi:10.1093/jxb/42.12.1601

Fischer, R.A., Edmeades, G.O., 2010. Breeding and Cereal Yield Progress. Crop Sci. 50, S–85–S–98. doi:10.2135/cropsci2009.10.0564

Flintham, J.E., Borner, A., Worland, A.J., Gale, M.D., 1997. Optimizing wheat grain yield: effects of Rht (gibberellin-insensitive) dwarfing genes. J. Agric. Sci. 128, 11–25.

Foulkes, M.J., Slafer, G. a, Davies, W.J., Berry, P.M., Sylvester-Bradley, R., Martre, P., Calderini, D.F., Griffiths, S., Reynolds, M.P., 2011. Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. J. Exp. Bot. 62, 469–86. doi:10.1093/jxb/erq300

Hai, L., Guo, H., Xiao, S., Jiang, G., Zhang, X., Yan, C., Xin, Z., Jia, J., 2005.

Quantitative trait loci (QTL) of stem strength and related traits in a doubled-haploid population of wheat (Triticum aestivum L.). Euphytica 141, 1–9. doi:10.1007/s10681-005-4713-2

Kelbert, A.J., Spaner, D., Briggs, K.G., King, J.R., 2004. The association of culm anatomy with lodging susceptibility in modern spring wheat genotypes. Euphytica 136, 211–221. doi:10.1023/B:EUPH.0000030668.62653.0d

Keller, M., Karutz, C., Schmid, J.E., Stamp, P., Winzeler, M., Keller, B., Messmer, M.M., 1999. Quantitative trait loci for lodging resistance in a segregating wheat x spelt population. TAG 98, 1171–1182.

Kertesz, Z., Flintham, J.E., Gale, M.D., 1991. Effects of Rht dwarfing genes on wheat grain yield and its components under eastern european conditions. Cereal Res. Commun. 19, 297–304.

Li, Z.K., Jiang, X.L., Peng, T., Shi, C.L., Han, S.X., Tian, B., Zhu, Z.L., 2014. Mapping quantitative trait loci with additive effects and additive x additive epistatic interactions for biomass yield, grain yield, and straw yield using a doubled haploid population of wheat (Triticum aestivum L.). Genet. Mol. Res. 13, 1412–1424.

Lopez-Cruz, M.A., Alvarado, G., Vargas, M., Pacheco, A., Rodriguez, M., Crossa, J., 2015. Analyses of multi-environment trials in R. Submited 1–26.

Ma, Q.-H., 2009. The expression of caffeic acid 3-O-methyltransferase in two wheat genotypes differing in lodging resistance. J. Exp. Bot. 60, 2763–71. doi:10.1093/jxb/erp132

Miralles, D.J., Slafer, G.A., 1995. Yield, biomass and yield components in dwarf, semi-dwarf and tall isogenic lines of spring wheat under recommended and late sowing dates. Plant Breed. 114, 392–396.

Parry, M. a J., Reynolds, M., Salvucci, M.E., Raines, C., Andralojc, P.J., Zhu, X.-G., Price, G.D., Condon, A.G., Furbank, R.T., 2011. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. J. Exp. Bot. 62, 453–67. doi:10.1093/jxb/erq304

Pask, A., Pietragalla, J., Mullan, D., Reynolds, M.P., 2012. Physiological breeding II: a field guide to wheat phenotyping. CIMMYT, Mexico, D. F.

Reynolds, M., Bonnett, D., Chapman, S.C., Furbank, R.T., Manès, Y., Mather, D.E., Parry, M. a J., 2011. Raising yield potential of wheat. I. Overview of a consortium approach and breeding strategies. J. Exp. Bot. 62, 439–52. doi:10.1093/jxb/erq311

Reynolds, M., Foulkes, J., Furbank, R., Griffiths, S., King, J., Murchie, E., Parry, M., Slafer, G., 2012. Achieving yield gains in wheat 1799–1823. doi:10.1111/j.1365-3040.2012.02588.x

Richards, R.A., 1992. The effect of dwarfing genes in spring wheat in dry environments. I. Agronomic characteristics. Aust J. Agric Res Res 43, 517–527.

SAS Institute Inc., 2004. SAS System for Windows. Version 9.1. SAS Institute, Inc., Cary, NC, USA.

Sayre, K.D., Rajaram, S., Fischer, R.A., 1997. Yield potential progress in short bread wheats in Northwest Mexico. Crop Sci. 37, 37–36.

Slafer, G.A., Araus, J.L., 2007. Physiological traits for improving wheat yield under a wide range of conditions, in: J.H.J. Spiertz, P.C.S.& H.H. vanLaar (Ed.), Scale and Complexity in Plant Systems Research: Gene–Plant–Crop Relations. Springer, Dordrecht, the Netherlands, pp. 147–156.

Slafer, G.A., Elia, M., Savin, R., García, G.A., Terrile, I.I., Ferrante, A., Miralles, D.J., González, F.G., 2015. Fruiting efficiency: an alternative trait to further rise wheat yield. Food Energy Secur. 4, 92–109. doi:10.1002/fes3.59

Tripathi, S.., Sayre, K.., Kaul, J.., Narang, R.., 2004. Lodging behavior and yield potential of spring wheat (Triticum aestivum L.): effects of ethephon and genotypes. F. Crop. Res. 87, 207–220. doi:10.1016/j.fcr.2003.11.003

Tripathi, S.C., Sayre, K.D., Kaul, J.N., Narang, R.S., 2003. Growth and morphology of spring wheat (Triticum aestivum L.) culms and their association with lodging: effects of genotypes, N levels and ethephon. F. Crop. Res. 84, 271–290. doi:10.1016/S0378-4290(03)00095-9

Vargas, M., Combs, E., Alvarado, G., Atlin, G., Mathews, K., Crossa, J., 2013. Meta: A suite of sas programs to analyze multienvironment breeding trials. Agron. J. 105, 11–19. doi:10.2134/agronj2012.0016

Wiersma, J.J., Dai, J., Durgan, B.R., 2011. Optimum timing and rate of trinexapac-ethyl to reduce lodging in spring wheat. Agron. J. 103, 864–870. doi:10.2134/agronj2010.0398

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Reserach 14, 415–421.

3.8 Supplementary material

Table S3.1 Cultivars of the CIMMYT Mexico Core Germplasm Panel

No.	NAME	Experiment
1	ATTILA (PBW 343)	2011
2	ATTILA*2/PBW65	2011
3	ATTILA*2/PBW65*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	2011
4	ATTILA//PGO/SERI/3/PASTOR	2011, 12
5	BABAX/LR42//BABAX/3/ER2000	2011
6	BABAX/LR42//BABAX/3/VORB	2011, 12, 13
7	BACANORA T 88	2011, 12, 13, 14
8	BAVIACORA M 92	2011
9	BCN/RIALTO	2011, 12, 13
10	BCN/WBLL1	2011
11	BECARD (Wbll 1*2/Kiritati)	2011, 13
12	BECARD/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	2011, 12, 13
13	BRBT1*2/KIRITATI (HIST 14)	2011, 12, 13
14	C80.1/3*BATAVIA//2*WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES	2011
15	CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR/7/CROC_1/AE.SQUARROSA (205)//KAUZ/3/ATTILA/8/CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR	2011, 12, 13
16	CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR/7/WHEAR/8/CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR	2011, 12, 13
17	CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/5/PFAU/WEAVER	2011
18	CHWL86/6/FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER	2011
19	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/6/RIALTO	2011, 12, 13, 14
20	CIRNO (SOOTY_9/RASCON_37//CAMAYO)	2011, 12, 13
21	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/OCI/5/PASTOR/6/TEMPORALERAM 87/ROMO96	2011
22	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ	2011
23	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7	2011, 12, 13
24	CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2	2011, 12, 13, 14
25	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/BAV92	2011
26	KBIRD//INQALAB 91*2/TUKURU	2011, 12, 13
27	KFA/3/PFAU/WEAVER//BRAMBLING/4/PFAU/WEAVER*2//BRAMBLING	2011, 13
28	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN	2011
29	MILAN/KAUZ//PRINIA/3/BAV92	2011, 12, 13
30	MUNAL #1	2011
31	Navojoa M2007 (=ATTILA/PASTOR)(Hist 10)	2011
32	OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI	2011, 12
33	OASIS/SKAUZ//4*BCN/3/2*PASTOR (= Misr#1 in Egypt)	2011
34	OASIS/SKAUZ//4*BCN/3/2*PASTOR/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/7/CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR	2011
35	PANDORA//WBLL1*2/BRAMBLING	2011
36	PASTOR/3/URES/JUN//KAUZ/4/WBLL1	2011
37	PAVON F 76	2011, 12, 13
38	PBW343*2/KUKUNA*2//FRTL/PIFED	2011, 12, 13
39	PFAU/SERI.1B//AMAD/3/WAXWING (=Super 152)	2011, 12, 13
40	ARMENT//2*SOOTY_9/RASCON_37/4/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN	2011
41	QUAIU #3//MILAN/AMSEL	2011
42	RL6043/4*NAC//2*PASTOR (WAMI 249)	2011
43	ROLF07*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	2011
44	SERI M 82 (Hist 3)	2011, 12, 13
45	SIETE CERROS T66 (Hist 1)	2011, 12, 13
46	SOKOLL*2/3/BABAX/LR42//BABAX	2011
47	SOKOLL//PBW343*2/KUKUNA/3/ATTILA/PASTOR	2011, 12, 13
48	TACUPETOF2001/7/CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR/8/BABAX/LR42//BABAX*2/3/KURUKU	2011, 12, 13
49	TACUPETO F2001/BRAMBLING*2/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	2011, 12, 13
50	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1	2011, 12, 13
51	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	2011, 12, 13
52	TRCH/SRTU/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	2011
53	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	2011, 12, 13
54	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1	2011
55	WBLL1*2/KIRITATI (BECARD)	2011, 12, 13
56	WBLL1*2/4/BABAX/LR42//BABAX/3/BABAX/LR42//BABAX	2011, 13
57	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	2011, 12, 13, 14
58	WBLL1*2/TUKURU*2/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN	2011
59	WHEAR/SOKOLL	2011, 12
60	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA(498)/5/LINE1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ/7/KRONSTAD F2004/8/KAUZ/PASTOR//PBW343	2011, 12, 13, 14

Chapter 4 Lignin, structural and non-structural carbohydrates and their association with stem strength components of irrigated spring wheat

4.1 Abstract

Wheat structural stem biomass is composed of three major components: lignin, cellulose and hemicellulose. Previous studies have shown each of these components to confer mechanical strength to the stems. However, assumptions about which is the key component for increasing this mechanical strength and in consequence, lodging resistance might be complex. Most of the literature indicates that lignin is more related, although, cellulose and hemicellulose are also mentioned in some studies. In this study, the objective was to compare cultivars with low, intermediate and high stem strength. Fractions of biomass components (lignin, cellulose, hemicellulose) and water soluble carbohydrates (WSC) as well as key stem strength traits were estimated for each cultivar across 2012, 2013 and 2014 experiments. Significant differences were identified between experiments and cultivars for cellulose, hemicellulose, lignin and WSC. G×E interaction was significant ($P < 0.01 - 0.001$) for lignin and cellulose but not for WSC and hemicellulose. Analysis of variance indicated no significant differences between means of low, intermediate and high stem strength cultivar. Additionally, regression analysis suggested that there was no individual effect of the three major components of the structural biomass (lignin, cellulose and hemicellulose) on the stem strength and stem material strength. Although, some associations should be further investigated (e.g. association between hemicellulose and stem material strength).

4.2 Introduction

Previous chapters have described the complexity of lodging in wheat and discussed several strategies that can help plant breeders to overcome this issue by improving lodging resistance. One of these strategies was the importance of strengthening the support plant structures (stem base and anchorage system) to overcome the lodging problem since there appears to be limited scope to further reduce plant height to reduce lodging risk while increasing grain yield. Chapter 2 has discussed the stem biomass requirements for a lodging-proof wheat plant and used the novel approach of excluding non-structural carbohydrates from these dry matter requirements. This helped to provide a more precise estimate of the stem strength and structural biomass since no contribution of non-structural carbohydrates for the stem strength was found. However, structural biomass can be broken down into lignin, cellulose and hemicellulose and several studies have described different associations of these components with lodging. For instance, Knapp et al. (1987) concluded that the arrangement and inter-relationships of the lignin and structural carbohydrates in wheat cell walls may be more important than their simple concentrations. Most recent investigations have reported findings where lignin or cellulose contents were more related to the stem strength and lodging resistance. For instance, Ma (2009) found that the wheat gene TaCM (involved in lignin biosynthesis) is associated with stem strength and lodging index. It has also been reported that growth regulators alter the lignin accumulation and strength of basal internodes (Peng et al., 2014). Wiersma et al. (2011) found a positive association between lodging resistance and acid detergent lignin (ADL); On the other hand, Wang et al.

(2012) proposed that cellulose plays a more important role in the ability of wheat stems to resist lodging. None of these studies indicated any contribution of hemicelluloses to stem strength.

For this study, were selected cultivars with contrasting overall stem strength from the CIMCOG panel (Table 4.1) in order to understand the role of the cell wall composition on conferring strength to the stem base.

4.3 Materials and methods

4.3.1 Plant materials and experiments

Three field experiments established during the field seasons 2011-2012, 2012-2013 and 2013-2014 (referred to hereafter as 2012, 2013 and 2014, respectively) were used for this study. A subgroup of 10 cultivars from the CIMCOG panel were pre-selected to integrate a wide range of stem strength dimensions on internode 2 and were grouped into high, intermediate and low stem strength cultivars (Table 4.1). Detailed information about experiments, cultivars and crop management were described in the materials and methods section of Chapter 3.

Table 4.1 Cultivars from CIMCOG with contrasting stem strength performances

No.†	Name	Stem strength score	Stem strength (N mm)‡
7	BACANORA T 88	Low	127
9	BCN/RIALTO	Low	146
39	PFAU/SERI.1B//AMAD/3/WAXWING (=Super 152)	Low	154
19	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/6/RIALTO	Intermediate	196
24	CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2	Intermediate	162
45	SIETE CERROS T66 (Hist 1)	Intermediate	186
49	TACUPETOF2001/BRAMBLING*2/5/KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	Intermediate	183
57	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES	High	208
60	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA(498)/5/LINE1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ/7/KRONSTAD F2004/8/KAUZ/PASTOR//PBW343	High	204
23	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7	High	229

†According to Table S3.1 from Chapter 3; ‡Performance from cross-year analysis for genetic differences in stem strength of internode 2 from Chapter 3.

4.3.2 Measurements

Lodging measurements used for this study included overall stem strength (SS) and stem material strength (SMS) which was measured at GS65 + 20 d on the basal internode 2 from the main shoot of 10 plants selected randomly in the plot. Internode 1 was determined as the first internode of more than 10 mm originated at or below the soil surface (superior internodes in the stem were numbered in ascending order and identifying the uppermost as the peduncle). The detailed methods for the measurement of this trait were given in Chapter 3. Dry weight of internode 2 (mg) was determined by drying the internodes until no further weight loss occurred at 75^{o}C. Water-soluble carbohydrate content (dry weight basis) was also determined on milled samples of the true stem of 10 main shoots per plot. These analyses were carried out in the Maize Nutrition Quality and Plant Tissue Analysis Laboratory of CIMMYT (El Batan, Mexico) using the Anthrone method (Galicia et al. 2008). Lignin, cellulose and hemicellulose concentrations (dry weight basis) were estimated for milled samples of internode 2 of 10 main shoots per plot. Lignin concentration was estimated by using the Acetyl Bromide Spectrophotometric Method (Fukushima and Hatfield, 2001). Cellulose and hemicellulose fractions were estimated according to the method described by Fang et al. (1999). These procedures were carried out at the Bioenergy and Brewing Science Building laboratories of the University of Nottingham.

4.3.3 Statistical analysis

Combined analysis of variance across experiments 2012, 2013 and 2014 was carried out by using an unbalanced treatment structure for lignin concentration and WSC. Combined analysis of variance for cellulose and hemicellulose concentrations was carried out by using a general treatments structure with data of two replicates from experiments 2012 and 2013. These analyses were implemented on GENSTAT 16th Edition.

4.4 Results

4.4.1 Lignin, cellulose, hemicellulose and water-soluble carbohydrate content

Individual cultivar means for each experiment, standard error of differences (SED) and significance for main effects (experiment and cultivar) and the interaction are given in Table 4.2. Significant differences ($P < 0.001$) were identified between experiments and cultivars for all traits. Genotype by environment interaction was significant ($P < 0.01 – 0.001$) for lignin and cellulose but not for WSC and hemicellulose. It is important to mention that cultivars selected for these analyses were chosen with a wide range of stem strength, providing enough of a testbed for testing of relationships between stem strength and stem biomass components. These analyses have shown that the simple proportion of WSC, lignin, cellulose and hemicellulose to the overall stem dry weight, showed significant differences among cultivars. However, cellulose and lignin cultivar ranks from one experiment were different when compared with the rest of the experiments meaning that environment or genotype are not just simple main effects but depend on each other. This indicated that the best cultivar would not be stable across environments.

Table 4.2 Mean values of stem strength (SS) of internode 2, overall stem water-soluble carbohydrate content (WSC) and internode 2 lignin, cellulose and hemicellulose fractions for 10 cultivars from CIMCOG panel across experiments 2012, 2013 and 2014. All the percentages are on a dry weight basis

Group	Cultivar	WSC (%)			Lignin (%)			Cellulose† (%)		Hemicellulose† (%)	
		2012	2013	2014	2012	2013	2014	2012	2013	2012	2013
Low	7	23.0	25.7	19.6	22.3	34.7	33.3	38.2	26.8	24.9	21.5
	9	26.8	26.1	–	26.1	38.4	–	41.5	23.1	27.2	23.1
	39	25.7	29.5	22.4	26.8	34.5	31.0	42.9	32.8	32.2	24.0
	Mean	25.2	27.1	21.0	25.1	35.9	32.2	40.9	27.6	28.1	22.9
Intermediate	19	18.3	22.4	–	27.4	33.8	–	46.7	22.5	32.1	24.9
	24	23.6	24.2	15.2	25.2	34.3	32.5	46.2	24.0	33.1	24.7
	45	28.3	34.8	–	29.1	37.7	–	41.2	20.9	37.5	24.4
	49	22.2	27.2	–	24.4	33.1	–	43.0	20.8	28.8	25.6
	Mean	23.1	27.2	15.2	26.5	34.7	32.5	44.3	22.1	32.9	24.9
High	23	30.0	29.1	–	29.2	37.4	–	34.0	16.5	27.3	25.5
	57	27.9	28.9	20.3	30.0	35.6	30.4	36.0	27.5	27.5	20.5
	60	25.7	26.1	22.0	27.4	32.6	27.3	35.6	19.4	33.8	25.7
	Mean	27.9	28.0	21.2	28.9	35.2	28.9	35.2	21.1	29.5	23.9
	Grand Mean	25.1	27.4	19.9	26.8	35.2	30.9	40.5	23.4	30.4	24.0
	Experiment (SED)	0.87***(2)			0.62***(2)			1.24***(1)		1.40***(1)	
	Cultivar (SED)	1.56***(9)			1.11***(9)			1.96***(9)		2.21*(9)	
	Interaction (SED)	2.19ns(13)			1.55**(13)			2.90**(9)		3.28ns(9)	

***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant

General analysis of variance indicated no significant differences between means of low, intermediate and high stem strength grouped cultivars (Figure 4.1). This output was found across experiments and was indicative of no individual effect of lignin, cellulose, hemicellulose or WSC content on stem strength.

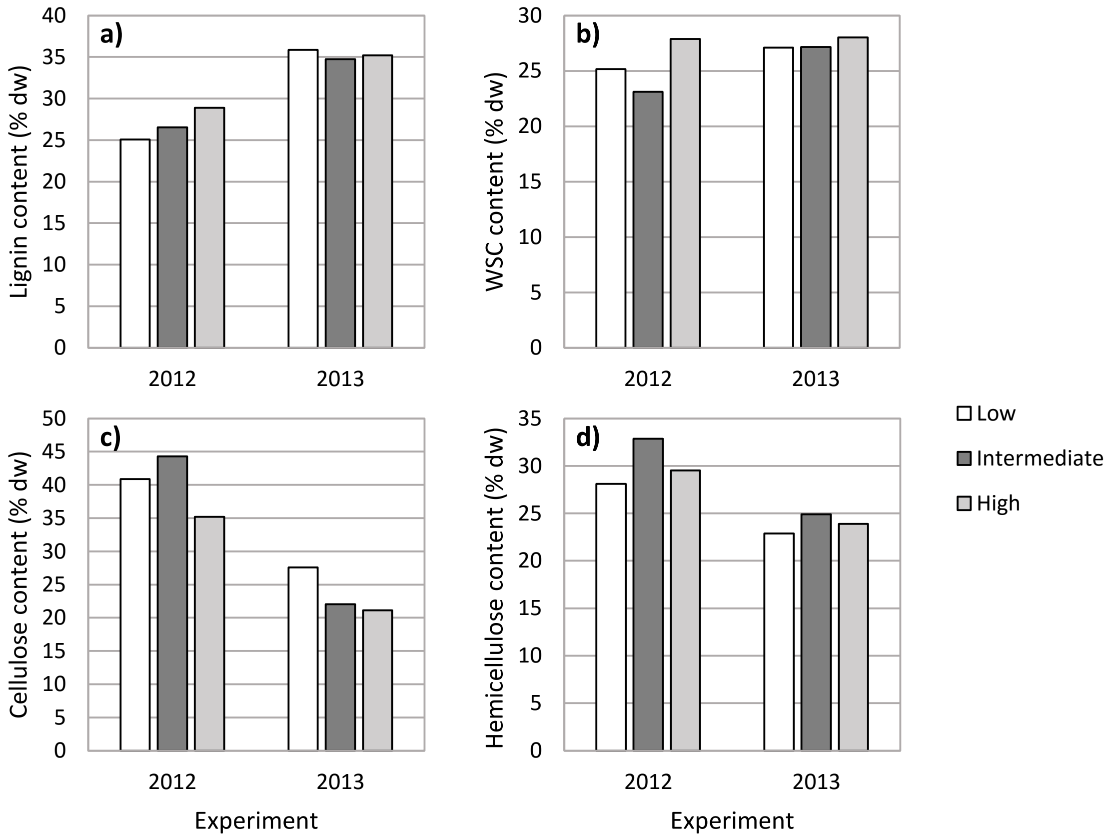

Fig. 4.1 Mean values for lignin (a), water-soluble carbohydrate (WSC) (b), cellulose (c) and hemicellulose (d) content of cultivars with low, intermediate and high stem strength scores from experiments in 2012 and 2013.

4.4.2 Associations among stem strength and structural biomass components

The summary of the regression analysis for each individual year and for the cross-experiment means indicated that there was no clear or no relationship between stem strength and stem material strength with the dry weight components (lignin, cellulose, hemicellulose and water-soluble carbohydrates) (Table 4.3). For instance, stem strength was negative and significantly related with lignin only for the experiment in 2014 with a similar trend in the experiment in 2012 but there was no relationship for the experiment in 2013 or the cross-year mean. Cellulose content also showed a strong positive association with stem material strength during 2012 but no relationship for 2013. Data for the regression lines are shown graphically in Figures 4.2 and 4.3.

Table 4.3 Linear regression equations for relationships between stem strength (SS) and stem material strength with lignin, cellulose, hemicellulose and water-soluble carbohydrate content from each individual experiment. Significance indicated in brackets when needed.

		Stem strength		Stem material strength	
		Equation	R^2	Equation	R^2
Lignin					
	2012	y = 9.21x-36.1	0.21	y = -1.08x+58.7	0.11
	2013	y = -3.45x+315	0.05	y = -1.64x+99.9	0.06
	2014	y = -9.78x+486	0.86 $(P < 0.05)$	y = 2.45x-44.6	0.33
	Mean	**y = -2.99x+292**	**0.02**	**y = -1.40x+79.0**	**0.05**
WSC					
	2012	y = -1.15x+240	0.007	y = -1.07x+56.6	0.22
	2013	y = 0.87x+1.68	0.008	y = 0.82x+19.9	0.04
	2014	y = 2.96x+125	0.12	y = -2.38x+78.3	0.46
	Mean	**y = -1.61x+241**	**0.03**	**y = -0.36x+44.7**	**0.02**
Cellulose					
	2012	y = -1.98x+289	0.03	y = 1.49x-28.9	0.71 $(P < 0.01)$
	2013	y = -1.89x+239	0.07	y = 0.009x+42.1	0.00
	Mean	**y = -0.71x+222**	**0.006**	**y = 1.54x-13.7**	**0.30** $(P = 0.10)$
Hemicellulose					
	2012	y = -0.68x+233	0.03	y = 0.72x+6.93	0.13
	2013	y = 8.14x+9.21	0.19	y = 2.99x-24.9	0.15
	Mean	**y = 3.53x+103**	**0.08**	**y = 1.84x-14.6**	**0.21**

2012, N = 10; 2013, N = 10; 2014, N = 5; Mean, N = 10

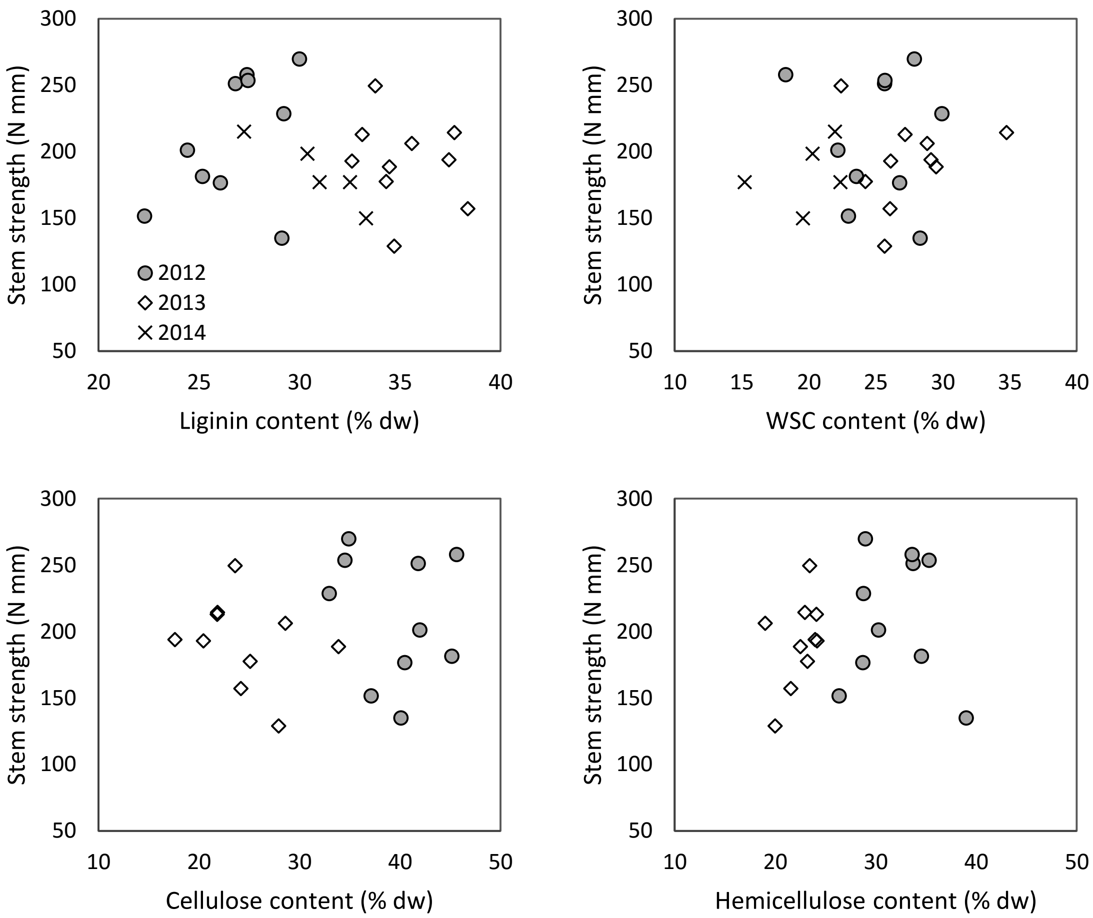

Fig. 4.2 Stem strength plotted against water-soluble carbohydrate (WSC), lignin, cellulose and hemicellulose. All the values were in terms of percentage of dry weight of internode 2, except for WSC (whole stem).

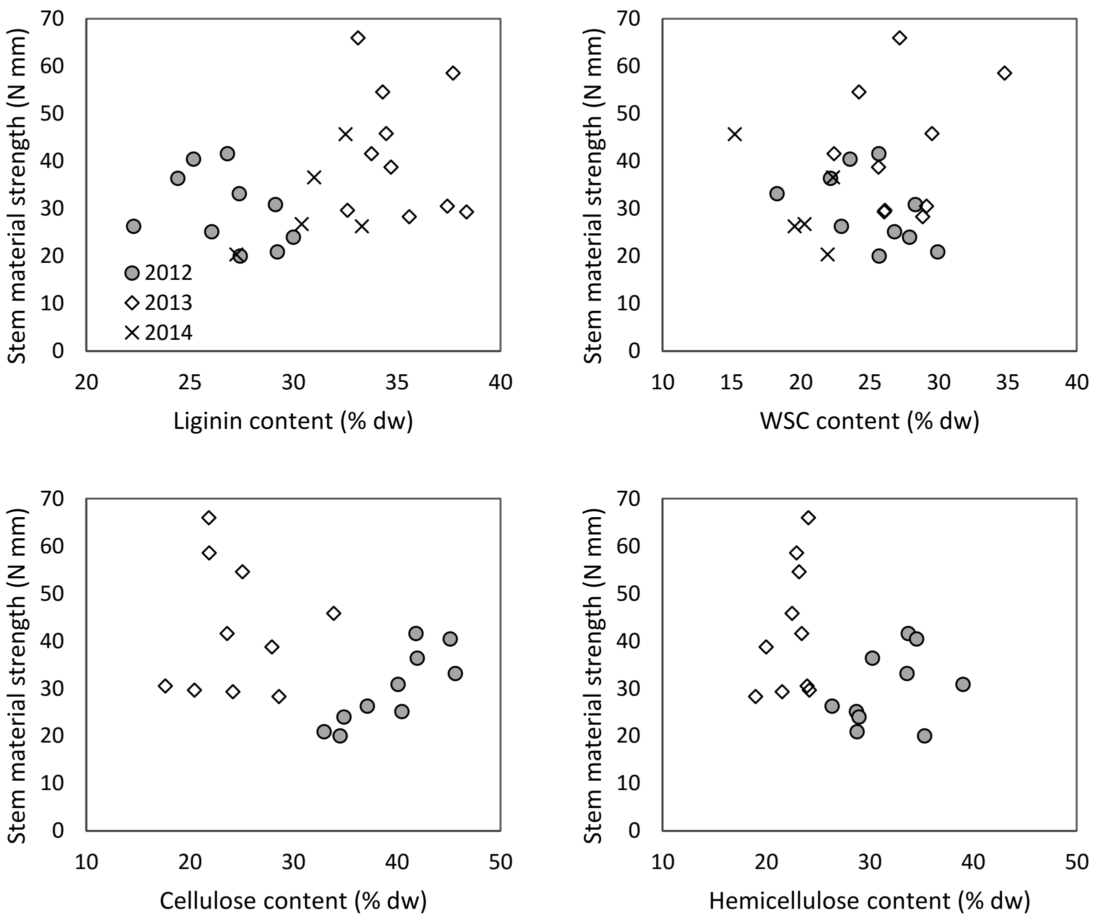

Fig. 4.3 Stem material strength plotted against water-soluble carbohydrate (WSC), lignin, cellulose and hemicellulose. All the values were in terms of percentage of dry weight of internode 2, except for WSC (whole stem).

4.5 Discussion

The secondary plant cell wall is the main component of the structural biomass and is composed mainly of cellulose, hemicellulose and lignin. However, the primary plant cell wall is also predominantly composed by cellulose and hemicellulose (Figure 4.4).

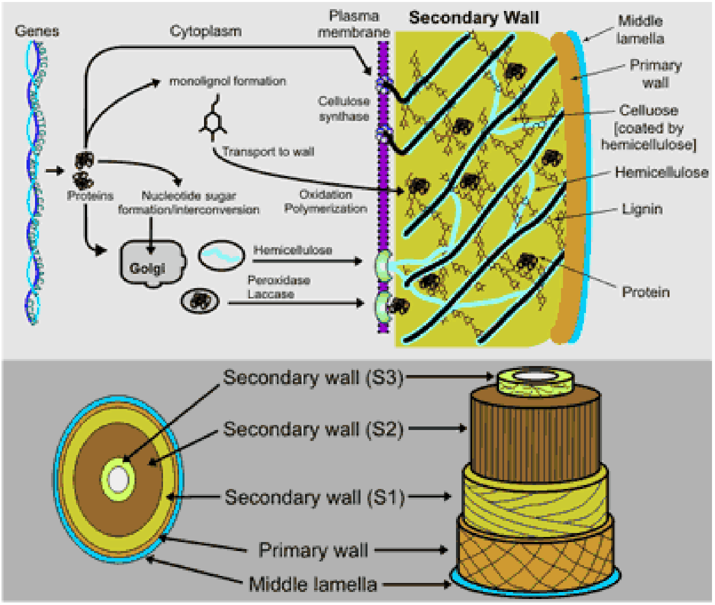

Fig. 4.4 Model of plant cell wall (CCRC, 2007)

Within the many functions of both primary and secondary plant cell walls are the mechanical properties which were our interest for this paper (CCRC, 2007; Taiz, 1984). If we consider that, the structural and chemical composition of the plant cell wall is extremely complex it seems logical that this mechanical support would not be explained by a single component. It has been suggested that the mechanical strength of the stem base of wheat plants is conferred by biophysical properties such as diameter, wall width and material strength (Berry et al., 2004). However, it is obvious that the components of the structural biomass (lignin, cellulose and hemicellulose) and their interactions combined with the biophysical dimensions of stems will have an influence on the stem strength properties.

The structural biomass composition of wheat cell walls has been described previously. For instance, Collins et al. (2014) and Ibbett et al. (2014) indicated lignin content ranged 13 – 23 % on a dry weight basis. Whilst cellulose and hemicellulose have been estimated from 39 – 40 % and 28 – 29 %, respectively (Ibbett et al., 2014, 2011). For wheat stems percentages of lignin were estimated in the range of 9 – 14 % (Fukushima and Hatfield, 2004; Qingxiang, 2002), cellulose at 46 % and hemicellulose at 32 % (Qingxiang, 2002). In the specific case of the lowest basal internodes (affected by lodging) lignin has been quantified from 10 -30 % (Knapp et al., 1987; Kong et al., 2013; Ma, 2009), cellulose from 36 – 50 % (Knapp et al., 1987; Kong et al., 2013) and hemicellulose from 35 – 36 % (Knapp et al., 1987). The CIMCOG panel subgroup used in this study has shown particularly high lignin (28 – 33 %) concentrations if compared with the ranges mentioned before. Whilst cellulose (28 – 35 %) and hemicellulose (23 – 31 %) concentrations were lower than these ranges. These variations can be due to differences in methods used to quantify the component content or that in some cases this content was estimated on a whole stem dry weight basis instead of a particular internode. Moreover, some ranges were done on genetic variation basis and others included environment effects.

A review of several studies suggested that there is no consensus on the key component of the cell wall conferring strength to the stems of wheat (Knapp et al., 1987; Kong et al., 2013; Ma, 2009; Peng et al., 2014; J. Wang et al., 2012; Wiersma et al., 2011). Lignin has been long proposed to be associated to the stem strength. Greater *TaCM* protein levels and *TaCM* activity in a lodging-resistant wheat cultivar than a lodging-susceptible cultivar and association with stem strength inferred that lignin synthesis plays an important role in lodging resistance (Ma, 2009). Interestingly, Peng et al. (2014) indicated that growth regulators paclobutrazol and gibberellic acid not only reduced lodging risk by reducing plant height but also increasing physical strength of basal internodes, particularly, by increasing lignin accumulation and lignin related enzymatic activity. While, Wiersma et al. (2011) identified an increment of acid detergent lignin (ADL) when growth regulator trinexapac-ethyl rates increased. This last study also identified positive correlations between lodging resistance and acid detergent lignin. Nevertheless, a histochemical evaluation of different wheat stems differing in lodging resistance performance identified the cellulose component as the main factor conferring these differences in lodging resistance (J. Wang et al., 2012). Our study has indicated that individually none of the cell wall components (% of stem dry weight) affects the stem strength or stem material strength of spring wheat. However, some individual associations by experiment suggested that there could be interesting interrelationships to follow up in further investigations. For instance, there was a tendency between hemicellulose and stem material strength that was consistent among experiments. These results should be considered together with the strong relationship identified between stem dry weight per unit length with the stem strength (See chapter 2) and between stem material strength and stem wall density (Berry et al., 2007). Thus, it can be inferred that all three major components of the plant cell walls of spring wheat have positive effects on strengthening the base of the stem, with special importance of the interactions between them (Knapp et al., 1987). Pinthus (1973) stated that plants with high cellulose, hemicellulose and lignin were more resistant to lodging. If these findings are considered in the context of the discussion in Chapter 2, then the biomass as a complex has major effects on stem strength. Interestingly, when WSC content was discarded from this biomass, the relationship with stem strength improved. This was consistent with Knapp et al. (1987) who also identified that lodging was not associated with WSC content in wheat.

4.6 Conclusions

The complexity of the plant cell wall architecture should be considered when assumptions about what is the key component of the cell wall conferring the mechanical strength of the stem base of wheat plants. Our results suggested that there is no individual effect of the three major components of the structural biomass (lignin, cellulose and hemicellulose) on the stem strength and stem material strength. Although, some association should be further investigated (e.g. association between hemicellulose and stem material strength).

4.7 References

Berry, P.M., Sterling, M., Spink, J.H., Baker, C.J., Sylvester-Bradley, R., Mooney, S.J., Tams, A.R., Ennos, A.R., 2004. Understanding and reducing lodging in cereals. Adv. Agron. 84, 217–271. doi:10.1016/S0065-2113(04)84005-7

Berry, P.M., Sylvester-Bradley, R., Berry, S., 2007. Ideotype design for lodging-resistant wheat. Euphytica 154, 165–179. doi:10.1007/s10681-006-9284-3

CCRC, 2007. Plant cell walls. Complex Carbohydrate Research Center, BioEnergy Science Center, University of Georgia, Athens GA, USA [WWW Document]. URL http://www.ccrc.uga.edu/~mao/intro/ouline.htm (accessed 12.7.15).

Collins, S.R., Wellner, N., Martinez Bordonado, I., Harper, A.L., Miller, C.N., Bancroft, I., Waldron, K.W., 2014. Variation in the chemical composition of wheat straw: the role of tissue ratio and composition. Biotechnol. Biofuels 7, 121. doi:10.1186/s13068-014-0121-y

Fang, J.M., Sun, R., Fowler, P., Tomkinson, J., Hill, C.A.S., 1999. Esterification of wheat straw hemicelluloses in theN,N-dimethylformamide/lithium chloride homogeneous system. J. Appl. Polym. Sci. 74, 2301–2311. doi:10.1002/(SICI)1097-4628(19991128)74:9<2301::AID-APP20>3.0.CO;2-7

Fukushima, R.S., Hatfield, R.D., 2004. Comparison of the acetyl bromide spectrophotometric method with other analytical lignin methods for determining lignin concentration in forage samples. J. Agric. Food Chem. 52, 3713–3720. doi:10.1021/jf035497l

Fukushima, R.S., Hatfield, R.D., 2001. Extraction and isolation of lignin for utilization as a standard to determine lignin concentration using the acetyl bromide spectrophotometric method. J. Agric. Food Chem. 49, 3133–3139. doi:10.1021/jf010449r

Galicia, L., Nurit, E., Rosales, A., Palacios-Rojas, N., 2008. Laboratory protocols 2009: Maize nutrition quality and plant tissue analysis laboratory. CIMMYT, Mexico, D. F.

Ibbett, R., Gaddipati, S., Davies, S., Hill, S., Tucker, G., 2011. The mechanisms of hydrothermal deconstruction of lignocellulose: New insights from thermal–analytical and complementary studies. Bioresour. Technol. 102, 9272–9278. doi:10.1016/j.biortech.2011.06.044

Ibbett, R., Gaddipati, S., Greetham, D., Hill, S., Tucker, G., 2014. The kinetics of inhibitor production resulting from hydrothermal deconstruction of wheat straw studied using a pressurised microwave reactor. Biotechnol. Biofuels 7, 45. doi:10.1186/1754-6834-7-45

Knapp, J.S., Harms, C.L., Volenec, J.J., 1987. Growth regulator effects on wheat culm nonstructural and structural carbohydrates and lignin. Crop Sci. 27, 1201–1205.

Kong, E., Liu, D., Guo, X., Yang, W., Sun, J., Li, X., Zhan, K., Cui, D., Lin, J., Zhang, A., 2013. Anatomical and chemical characteristics associated with lodging resistance in wheat. Crop J. 1, 43–49. doi:10.1016/j.cj.2013.07.012

Ma, Q.-H., 2009. The expression of caffeic acid 3-O-methyltransferase in two wheat genotypes differing in lodging resistance. J. Exp. Bot. 60, 2763–71. doi:10.1093/jxb/erp132

Peng, D., Chen, X., Yin, Y., Lu, K., Yang, W., Tang, Y., Wang, Z., 2014. Lodging resistance of winter wheat (Triticum aestivum L.): Lignin accumulation and its related enzymes activities due to the application of paclobutrazol or gibberellin acid. F. Crop. Res. 157, 1–7. doi:10.1016/j.fcr.2013.11.015

Pinthus, M.J., 1973. Lodging in wheat, barley, and oats: the phenomenon, its causes, and preventive measures. Adv. Agron. 25, 209–263.

Qingxiang, M., 2002. Composition , nutritive value and upgrading of crop residues, in: Tingshuang, G., Sanchez, M., Pei Yu, G. (Eds.), Animal Production Based on Crop Residues - Chinese Experiences. FAO, Rome, Italy, pp. 24–52.

Taiz, L., 1984. Plant cell wall expansion: Regulation of Cell Wall Mechanical Properties. Annu. Rev. Plant Physiol. 35, 585–657.

Wang, J., Zhu, J., Huang, R., Yang, Y., 2012. Investigation of cell wall composition related to stem lodging resistance in wheat (Triticum aestivum L.) by FTIR spectroscopy. Plant Signal. Behav. 7, 856–863.

Wiersma, J.J., Dai, J., Durgan, B.R., 2011. Optimum timing and rate of trinexapac-ethyl to reduce lodging in spring wheat. Agron. J. 103, 864–870. doi:10.2134/agronj2010.0398

Chapter 5 Optimizing standard methods to evaluate lodging risk on irrigated spring wheat

5.1 Abstract

Lodging is a very complex phenomenon affecting wheat production worldwide and is a consequence of the interaction of wheat plants with abiotic and biotic factors. In the last two decades a novel methodology for estimating the lodging susceptibility of plants to lodging has been developed which involves measuring the plant characteristics that influence lodging, then using this information with a model of the lodging process to estimate lodging risk. The main shortcoming of this methodology for plant breeders is the large amount of time required to measure the lodging-related traits. This study investigated three strategies to optimise these measurements: reducing plant sample number, reducing number of traits to measure and finding rapid indirect methods associated with the lodging traits. This investigation was done on spring wheat growing under North West Mexico environmental conditions during seasons 2010-11, 2011-12, 2012-13 and 2013-14. Results indicated that the time of 100 to 150 minutes per experimental plot to measure lodging traits using the standard methods (Berry et al., 2003c, 2000) can be optimised to 20 minutes per plot by reducing the number of plants per plot from 10-15 to 7 while maintaining power and precision to detect genetic differences, and reducing the number of lodging characters from 18 to 8. Further investigation of the associations between NDVI and lodging characters will be necessary to comprehend to what extent NDVI can be used to infer on lodging resistance.

5.2 Introduction

Lodging is a persistent phenomenon affecting wheat that involves the interaction of plants with abiotic (wind, rain, soil) and biotic (e.g. diseases) factors and the crop management (e.g. fertilisers, irrigation systems, plant growth regulators). Reductions of grain yield have been estimated at between 61 – 80% in severe lodging occurrence (Berry and Spink, 2012; Berry et al., 2004). Reduced grain quality (Berry, 1998; Berry et al., 2004) is also commonly associated with lodging. Plant breeders often use observations of lodging events under natural conditions in the field to rank cultivars for lodging resistance. However, this phenomenon does not occur during every crop cycle which limits the capacity to identify the lodging susceptibility of cultivars before they are introduced to a large scale production scheme (Berry et al., 2003a). In recent years a new model has been developed that evaluates lodging risk based on the failure of root anchorage (root lodging) or the stem base (stem lodging) (Berry et al., 2003c). This model indicated that increasing the stem strength and the anchorage strength would be necessary to improve stem/root lodging resistance in winter wheat (Berry et al., 2007) and spring wheat (See Chapter 2) under rainfed or irrigated areas. A set of stem/root biophysical properties related to the stem and anchorage strength (lodging-related traits) now can be used to calculate stem/root lodging resistance enabling breeders to rank cultivars by type of lodging and importantly in the absence of natural lodging. Wide genetic variation has been found for these lodging-related traits (Berry et al. 2003b; Berry et al. 2007; See also Chapter 2). Nevertheless, measuring these traits requires a great amount of time in the field and laboratory. This time-consuming nature of lodging trait measurement represents a disadvantage that restricts the capacity to evaluate a large number of genotypes. Thus, optimizing the time for measuring these traits will be of great importance if improved lodging resistance is to be obtained by these methods. Berry et al (2003a) tested a lodging instrument capable of assessing lodging directly in the field, detecting significant genetic variation between cultivars and predicting lodging with moderate precision. This instrument used a lodging model where stem and root lodging occur when the wind induced base bending moment of a shoot/shoots (shoot/plant leverage) was higher than the stem/anchorage failure moment (stem/anchorage strength). They concluded that automation would be necessary to overcome time implications and more importantly it was necessary to use the instrument when the soil moisture content was at field capacity to estimate the risk of root lodging. Other studies have also designed devices to measure crop lodging resistance in the field and concluded that a rapid assessment of lodging resistance of wheat can be possible (Niu et al., 2012a). A study carried on in rice used a prostrate tester to evaluate the stem base resistance identified a quantitative trait loci (QTL) related to this trait that could be used to improve lodging resistance in this crop (Kashiwagi and Ishimaru, 2004). The development of genetic markers related to lodging resistance is another promising strategy to reduce time and increase capacity for selection in wheat. Recent studies have reported the identification of QTL related to stem strength (Berry and Berry, 2015; Hai et al., 2005; Keller et al., 1999) and anchorage strength (Berry and Berry, 2015). It should be recognised that the problem already identified of the time-consuming nature of measuring lodging-associated traits also applies to the identification of QTL due to the large number of genotypes that must be measured to find reliable genetic markers.

The aims of this chapter were to optimize the lodging-related trait measurement methods (Berry et al., 2003c, 2000) used to assess genetic differences in lodging resistance by: a) determining the minimum number of plants measured per experimental plot to provide enough precision and power to identify genetic differences, b) minimizing the number of traits required to assess lodging susceptibility, and c) finding rapid indirect methods associated with these lodging traits.

5.3 Experimental methods

5.3.1 Field experiments

The full details about plant materials, experimental design, field sites, crop management and weather conditions were given previously in Chapter 3. Data from all experiments was included in the present chapter.

5.3.2 Plant characters

Data collected on 15 plants per plot for experiment 2011 and 10 plants per plot for experiments 2012 and 2013 were used for this chapter. These data included measurements of the root plate spread (mm) (RPS), structural rooting depth (mm) (SRD) and root dry weight (mg per plant) (RDW) of the plant crown root system. Ear number per plant (ENPP), ear area (cm^2) (EA), height to ear tip (mm) (PH), height at centre of gravity (mm) (CG) and natural frequency (Hz) (NF) of the main shoot of each plant. Measurements on the stem base (lowest basal internodes 1 and 2) of the main shoot included diameter (mm) (ID), length (mm) (IL), wall width (mm) (SWW), breaking strength (g) (IBS), dry weight (IDW), material strength (SMS) and failure moment or stem strength (SS). Anchorage and stem failure wind speed (AFWS and SFWS, respectively) are also calculated as indicators of lodging risk. For methods of calculations or measurements of the variables above see Chapters 2 and 3, respectively.

This chapter also included data for RPS, SRD, soil root plate spread (SRPS) and soil structural rooting depth (SSRD) for the experiment in 2014. RPS and SRD were measured as indicated above and SRPS and SSRD were measured by pulling up the plants after excavating from the surrounding ground with a hand fork to recover roots to a depth of 10 cm. Any loose soil attached to the soil root ball was gently shaken off. The average of the widest and narrowest diameter of the soil bulk adhered to the roots was considered as the SRPS and the distance from the base to the surface of that soil bulk was considered as SSRD.

Normalized difference vegetation index (NDVI) was measured at ground level with a Trimble® GreenSeeker® NDVI portable sensor in each experimental plot at 42 days after emergence for 2011 and 47 days after emergence for 2012 and 2013. The developmental period that corresponded to these dates was between GS30 – 39 (Zadoks et al., 1974). The detailed methods for these measurements were described in Pask et al. (2012).

5.3.3 Statistical analysis

All the statistical analyses included in this study were done in each experimental data set separately. Using the RAND function in excel random numbers were generated for each one of the 15 plants per plot (sample size) measured in 2011 and from the 10 plants per plot in the 2012 and 2013 experiments. Then, a random sample of 1 to 14 plants per plot for 2011 and 1 to 9 plants per plot for 2012 and 2013 was obtained from the initial sample size. This random sample (*Rs*) was produced using the INDEX and RANK functions from Excel and measurement value (*Mv*) and random numbers (*Rn*) for each plant as follows:

$$Rs = INDEX(Mv1{:}Mv15, RANK(Rn_{Pv1,2\dots14}, Rn_{Pv1}{:}Rn_{Pv15})$$

A mean value was calculated for each random plant sample size as follows; mean values for 1 – 15 plant numbers per plot for the 2011 experiment and mean values for 1 – 10 plant numbers for the 2012 and 2013 experiments. Probability values for differences between cultivars and coefficients of variation (CV) were estimated for each plant sample size by using a mixed model where the fixed effect was the cultivar and the random effect was the replicate and sub-block. This mixed model was run by using a PROC MIXED in SAS version 9. Simple linear regression analysis was used to determine equations for the association between traits of internodes 1 and 2 and between RPS and SRPS and between SRD and SSRD. A general linear model by using PROC GLM in SAS version 9 was used to determine whether there were genetic differences for cultivar means of RPS, SRPS, SRD and SSRD.

5.4 Results

5.4.1 Identifying genetic differences

A summary of the statistical parameters conferring power and precision to identify genetic differences between cultivar means estimated with 1, 3, 5, 7, 10 and 15 plants per plot is given in Table 5.1 for all lodging-related direct measurements. Six of these measurements or traits (CG, NF, PH, IL, ID, SWW) showed a coefficient of variation (CV) below or slightly above 10% and cultivar differences with probability values (*P*) below 0.001 whether five or the largest number of plants per plot were used. Similarly, SRD had a CV below 10% and *P* values below 0.001 when five or the largest number of plants were used in 2012 and 2013, except for 2011 where $P < 0.01$ was estimated. ENPP showed consistently a CV below 12% with $P < 0.05$ with a minimum of five plants per plot for experiments 2012 and 2013 but no significance and a considerably higher CV were found for experiment 2011. Coefficient of variation below 11% with $P < 0.05 – 0.001$ were found for genetic differences of RPS when five or maximum number of plants were used to calculate plot means for experiments 2012 and 2013 and below 10% with no significant differences for cultivar from five to the maximum number of plants per plot for experiment 2011. The highest coefficients of variation were found for IBS with values between 15 to 18% and $P < 0.05 – 0.001$ from five to the maximum number of plants per plot in all experiments. Supplementary data showing the full comparison of statistical parameters for genetic differences between cultivar means estimated with 1 – 15 plants per plot for 2011 and with 1 – 10 plants per plot for 2012 and 2013 is given in Supplementary Table S5.1.

Table 5.1 Standard error differences (SED) and coefficient of variation percentages (CV) of lodging-related characters' means estimated with different plot sample size for spring wheat growing at NWM during 2011, 2012 and 2013 experiments

Exp.	Plant no.	CG		ENPP		NF		PH		SIL		SD		SWW		IBS		RPS		SRD	
		SED	CV	SED	CV	SED	CV	SED	CV	SED	CV	SED	CV	SED	CV	SED	CV	SED	CV	SED	CV
2011	1	3.09**	8.06	0.89ns	33.6	0.18***	14.1	6.54***	7.47	13.0**	14.6	0.42ns	9.95	0.10***	13.0	219*	30.0	5.38ns	16.7	6.13ns	16.5
	3	1.74***	4.51	0.51*	18.8	0.15***	11.7	3.99***	4.53	9.10***	10.3	0.28***	6.66	0.10***	11.9	147*	21.1	3.40ns	10.3	3.33ns	9.05
	5	1.33***	3.48	0.55ns	19.6	0.14***	11.1	2.98***	3.41	8.18***	9.04	0.23***	5.45	0.07***	8.68	124**	17.5	3.05ns	9.14	2.74**	7.39
	7	1.41***	3.69	0.46ns	16.8	0.13***	10.2	2.78***	3.17	6.47***	7.24	0.20***	4.81	0.08***	9.93	120**	17.0	2.87ns	8.68	2.75*	7.45
	10	1.27***	3.32	0.38*	13.8	0.13***	10.1	2.56***	2.93	6.41***	7.14	0.17***	3.95	0.06***	7.84	112**	18.9	2.70ns	8.16	2.48**	6.73
	15	1.14***	2.97	0.36*	13.2	0.12***	9.22	2.41***	2.75	5.64***	6.29	0.16***	3.73	0.06***	7.43	106**	15.1	2.51ns	7.62	2.21***	6.01
2012	1	3.47***	6.16	0.72ns	27.3	0.19***	12.1	4.36***	4.71	13.5ns	23.6	0.31***	7.80	0.13ns	15.6	558ns	36.8	5.25*	14.3	4.63**	12.0
	3	2.14***	3.79	0.39ns	14.7	0.12***	7.75	3.59***	3.90	6.56***	11.4	0.23***	5.72	0.06***	6.92	250***	16.9	4.49**	12.3	3.13***	8.15
	5	1.38***	2.45	0.30***	11.2	0.11***	7.36	2.75***	2.97	5.64***	10.1	0.18***	4.45	0.06***	7.09	226***	14.7	3.86**	10.5	2.74***	7.11
	7	1.27***	2.24	0.32*	11.9	0.11***	7.04	2.10***	2.28	5.29***	9.33	0.18***	4.46	0.06***	6.88	231***	15.3	3.80***	10.3	2.56***	6.65
	10	1.22***	2.17	0.29**	10.8	0.10***	6.37	2.15***	2.33	4.63***	8.15	0.17***	4.18	0.05***	5.97	192***	12.7	3.77**	10.2	2.55***	6.62
2013	1	2.88***	5.34	0.67ns	26.2	0.18***	10.5	3.85***	4.19	14.7ns	24.3	0.36**	9.55	0.11ns	15.4	456*	31.4	5.12ns	11.5	3.72*	9.48
	3	1.96***	3.63	0.35*	13.3	0.15***	8.74	2.41***	2.62	8.11***	13.2	0.19***	5.09	0.08***	10.7	209***	15.1	3.43**	7.77	2.73**	7.04
	5	1.99***	3.66	0.31**	11.6	0.14***	8.03	2.45***	2.66	7.73***	12.3	0.20***	5.25	0.07***	9.30	200***	14.6	3.21*	7.24	2.32***	5.98
	7	1.84***	3.40	0.29*	11.1	0.13***	7.89	2.30***	2.50	6.26***	9.96	0.17***	4.48	0.07***	9.54	170***	12.7	2.63***	5.93	2.05***	5.28
	10	1.65***	3.05	0.25**	9.31	0.13***	7.56	1.95***	2.12	5.50***	8.68	0.15***	4.03	0.07***	9.00	163***	12.2	2.69**	6.03	1.97***	5.07

CG, height at centre of gravity; ENPP, ear number per plant; NF, natural frequency; PH, plant height; SIL, internode length; SD, internode diameter; SWW, internode wall width; IBS, internode breaking strength; RPS, root plate spread; SRD, structural rooting depth: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant

Root and stem lodging risks can be calculated in terms of AFWS and SFWS, respectively, these two indicators of lodging were calculated from a combination of the direct lodging-related measurements from Table 4.1 using equations given in Chapter 2. A CV of between 10 – 20% with a $P <$ 0.001 was found for AFWS using 5 to 10 plants for experiments 2012 and 2013, while for experiment 2011 a cv of 17 – 19% with a $P < 0.05$ was found using five to fifteen plants per plot. SWFS showed CV below 7% with $P < 0.001$ for genetic differences when cultivar means were estimated with five to ten plants per plot for experiments 2012 and 2013 and equal or below 10% with $P < 0.01$ with five to 15 plants per plot for experiment 2011 (Table 5.2). Supplementary Table S5.2 shows the full comparison of statistical parameters for genetic differences between cultivar means estimated with 1 – 15 plants per plot for 2011 and with 1 – 10 plants per plot for 2012 and 2013.

Table 5.2 Standard error differences (SED) and coefficient of variation percentages (CV) of stem and anchorage failure wind speed for spring wheat growing at NWM during 2011, 2012 and 2013.

Experiment	Plant no.	Anchorage failure wind speed		Stem failure wind speed	
		SED	CV	SED	CV
2011	1	3.61ns	35.6	2.03ns	17.4
	3	2.16ns	21.6	1.50*	13.0
	5	1.91*	18.7	1.19***	10.1
	7	1.90*	18.8	1.20**	10.2
	10	1.87*	18.5	1.13***	9.54
	15	1.70**	17.0	1.05***	8.94
2012	1	2.90***	26.6	1.40**	11.7
	3	2.28***	21.6	0.92*	7.48
	5	2.12***	19.9	0.78***	6.33
	7	2.05***	19.3	0.71***	5.68
	10	1.95***	18.2	0.63***	5.06
2013	1	3.60*	21.6	1.47**	10.9
	3	2.12***	13.6	0.91***	6.59
	5	1.98***	12.6	0.83***	5.93
	7	1.61***	10.2	0.70***	5.00
	10	1.50***	9.53	0.65***	4.67

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant

In addition to the results above, a simple linear regression analysis was carried out to compare cultivar means and ranks estimated by using the maximum plant number per plot (10 or 15) for all measurements in each experiment with means and ranks calculated with 1 – 14 plants per plot for 2011 and 1 – 9 plants per plot for 2012 and 2013 (Table S5.3 – S5.5). The slope of the regression analysis equation and the coefficient of determination (R^2) between means and ranks estimated with 5 plants per plot compared with the maximum plant sample number of 10 or 15 are summarized in Table 5.3. Regression equations indicating best fit lines with a slope differing by less than 5% from the 1:1-line slope with an R^2 above 90% were identified for CG, NF and PH. While, SFWS and ID also had R^2 above 90% but regression lines with slopes differing between 5 – 10% from the 1:1-line slope. Regression lines with slopes differing by between 6 – 17% from the 1:1-line slope and with R^2 ranging from 0.82 – 0.94 were identified for IL, SWW, IBS, SRD and AFWS. RPS had regression line slopes differing by 13 – 14% from the 1:1-line slope with R^2 from 0.76 to 0.82. Lowest average slopes and R^2 between estimated means and ranks with 5 and 10 or 15 plants were identified for ENPP. Single linear regression analysis between means and ranks estimated with seven plants per plot plotted against means and ranks estimated with 10 or 15 plants per plot showed averaged line slopes across experiments differing by about 5% from the 1:1-line slope and with an R^2 above 95% for PH, NF, ID and AFWS, while for CG, IL, SWW, IBS RPS, SRD and SFWS also had slopes within 5% and with R^2 between 90 – 98%. For 7 plants per plot, ENPP had slopes differing by 7 – 14% from 1:1-line slope with R^2 between 0.87 – 0.89. (Table S5.3 – 5.5).

Table 5.3 Regression coefficient and slope of the relationship between cultivar means and ranks calculated with 5 and 10 plants per plot for lodging traits during 2011, 2012 and 2013. For 2011 15 plants were used as maximum plot sample.

Trait	Experiment	R^2		Slope	
		Mean	Rank	Mean	Rank
Height at centre of gravity					
	2011	0.94	0.91	0.96	0.95
	2012	0.96	0.92	1.06	0.96
	2013	0.90	0.87	0.93	0.94
Ear number per plant					
	2011	0.64	0.61	0.65	0.78
	2012	0.83	0.81	0.74	0.90
	2013	0.83	0.86	0.72	0.93
Natural frequency					
	2011	0.94	0.84	1.00	0.92
	2012	0.98	0.95	0.96	0.97
	2013	0.98	0.94	0.96	0.97
Plant height					
	2011	0.95	0.92	0.96	0.96
	2012	0.98	0.94	0.99	0.97
	2013	0.98	0.96	0.93	0.98
Internode length					
	2011	0.87	0.83	0.78	0.91
	2012	0.93	0.87	0.93	0.93
	2013	0.79	0.77	0.77	0.88
Internode diameter					
	2011	0.88	0.86	0.91	0.93
	2012	0.96	0.96	0.97	0.98
	2013	0.96	0.92	0.93	0.96
Internode wall width					
	2011	0.91	0.81	0.91	0.90
	2012	0.94	0.91	0.80	0.95
	2013	0.96	0.93	0.93	0.97
Internode breaking strength					
	2011	0.87	0.87	0.86	0.93
	2012	0.92	0.85	0.9	0.92
	2013	0.91	0.86	0.85	0.93
Root plate spread					
	2011	0.64	0.53	0.69	0.73
	2012	0.93	0.91	1.03	0.96
	2013	0.89	0.83	0.87	0.91
Structural rooting depth					
	2011	0.77	0.72	0.79	0.86
	2012	0.97	0.94	1.03	0.97
	2013	0.94	0.91	0.92	0.96
Anchorage failure wind speed					
	2011	0.79	0.75	0.82	0.87
	2012	0.97	0.96	1.03	0.98
	2013	0.91	0.83	0.91	0.91
Stem failure wind speed					
	2011	0.89	0.86	0.90	0.93
	2012	0.90	0.88	0.86	0.94
	2013	0.97	0.96	0.97	0.98

5.4.2 Optimizing number of traits required to evaluate lodging

Measurements of traits related to stem strength are normally done in the first two bottom internodes (internodes 1 and 2) of the main stem of a wheat plant. Regression analyses indicated strong association between IL, ID, SWW, IBS, IDW and SS measured on internodes 1 and 2. The variation accounted for indicated by the regression had coefficients of determination in the range of 0.63 for internode length to 0.92 for internode strength (Figure 5.1). The strong correlations for traits such as stem strength, diameter and wall width indicates that measurement on one internode only will be sufficient to detect genotypic differences in stem strength and its sub-traits.

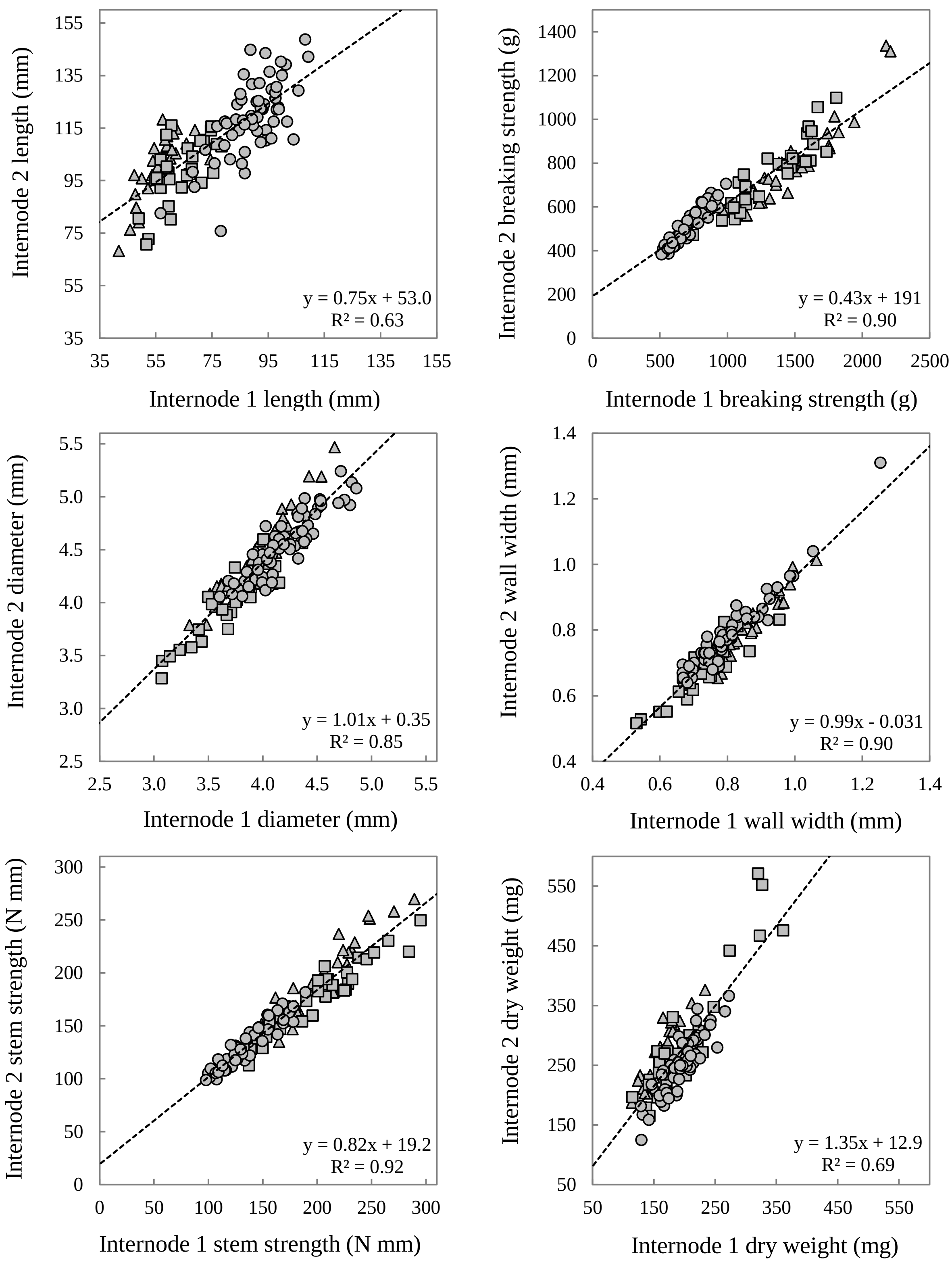

Fig. 5.1 Relationships between the length, wall width, diameter, breaking strength, stem strength and dry weight of internodes 1 and 2 for means of spring wheat cultivars in 2011 (circles, 60 cultivars), 2012 (triangles, 30 cultivars) and 2013 (squares, 30 cultivars) experiments. All regression lines had 118 d. f. and *P* < 0.001.

Reducing the number of traits required to estimate the leverage of the shoot and plant could be another option to contribute to the reduction of time to evaluate lodging resistance. Plant height is a very straight forward trait that can be measured directly in the field and has a strong impact on the shoot/plant leverage. Linear regression analyses described a linear relationship between PH and CG with the same equation for 2012 and 2013 dataset ($R^2 = 0.59$, $P < 0.001$) (Figure 2) (data from 2011 was not included in this analysis due to differences in the methods used to calculate CG in this experiment, see Chapter 3). Association between PH and NF was described with a single equation for all experiment datasets ($R^2 = 0.47$, $P < 0.001$) (Figure 5.2). Non-significant associations were found between ENPP and EA with PH, except for EA during 2011 and cross-year means gave a correlation coefficient of 0.34 ($P < 0.01$) and 0.27 ($P < 0.05$), respectively. These results indicated that it would be safe to omit CG and perhaps NF.

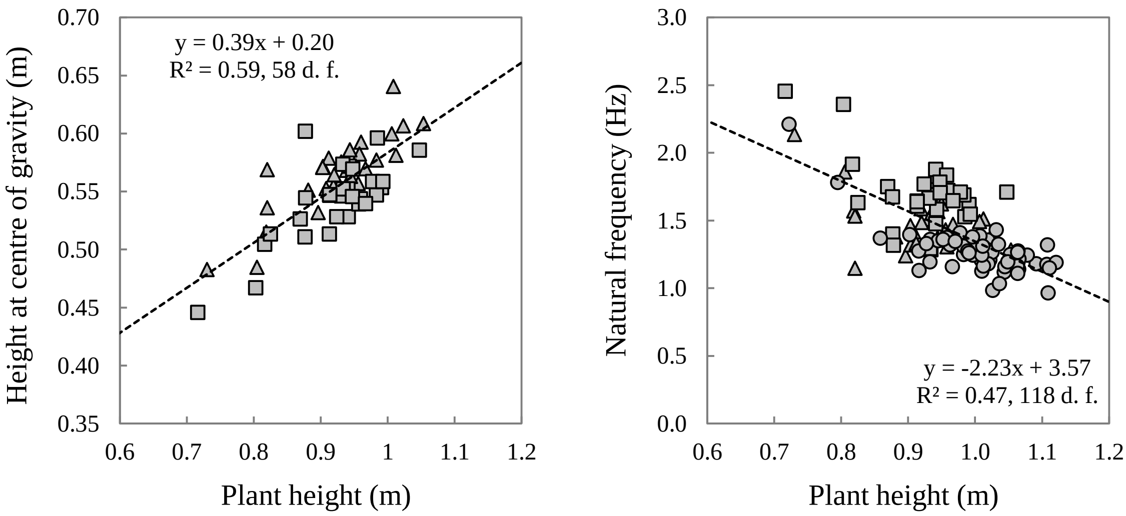

Fig. 5.2 Relationships between plant height and height at centre of gravity and natural frequency for means of spring wheat cultivars in 2011 (circles, 60 cultivars), 2012 (triangles, 30 cultivars) and 2013 (squares, 30 cultivars) experiments. All regression lines had a *P* < 0.001.

Root plate spread is the main component of the anchorage strength. Correlation coefficients between RPS and AFM of 0.52, 0.85 and 0.79 ($P < 0.001$) were estimated for experiments 2011, 2012 and 2013, respectively. RPS was also correlated to RDW with correlation coefficients of 0.40, 0.82 and 0.66 ($P < 0.01 – 0.001$) estimated for experiments 2011, 2012 and 2013, respectively. Measuring only RPS can be another approach to reduce the time-consuming nature of the lodging performance assessment.

5.4.3 Optimizing methodologies for measuring root plate spread and structural rooting depth

During the 2014 experiment the main components of the anchorage strength (RPS and SRD) were evaluated with a standard method in the laboratory and with a new method in the field (SRPS and SSRD). Then the standard laboratory measurements of RPS and SRD were compared with field measurements of SRPS and SSRD. Analysis of variance indicated significant genetic differences between cultivars only for SRPS and SSRD (Table 5.4). Despite the lack of genetic differences in RPS, ranking of the widest and narrower root plate spread were the same as with SRPS. Regression analysis showed strong correlations between SRPS and RPS ($R^2 = 0.94$, $P < 0.001$) and SSRD and SRD ($R^2 = 0.90$, $P < 0.001$) (Figure 5.3).

Table 5.4 Mean values for root plate spread, structural rooting depth, soil root plate spread and soil structural rooting depth for a subset of five cultivars (G) from CIMCOG panel grown at NWM during 2014.

Genotype	Root plate spread (mm)	Structural rooting depth (mm)	Soil root plate spread (mm)	Soil structural rooting depth (mm)
7	46.9	44.6	64.9	63.3
19	51.4	48.3	82.8	72.8
24	51.4	44.0	68.3	60.4
57	48.5	44.6	71.8	61.0
60	50.1	45.1	79.0	65.4
SED (G)	3.36	1.97	3.75	2.96
P value	ns	Ns	< 0.01	< 0.05
CV (%)	8.3	5.3	6.3	5.6

SED, standard error of differences; CV, coefficient of variation; ns, not significant

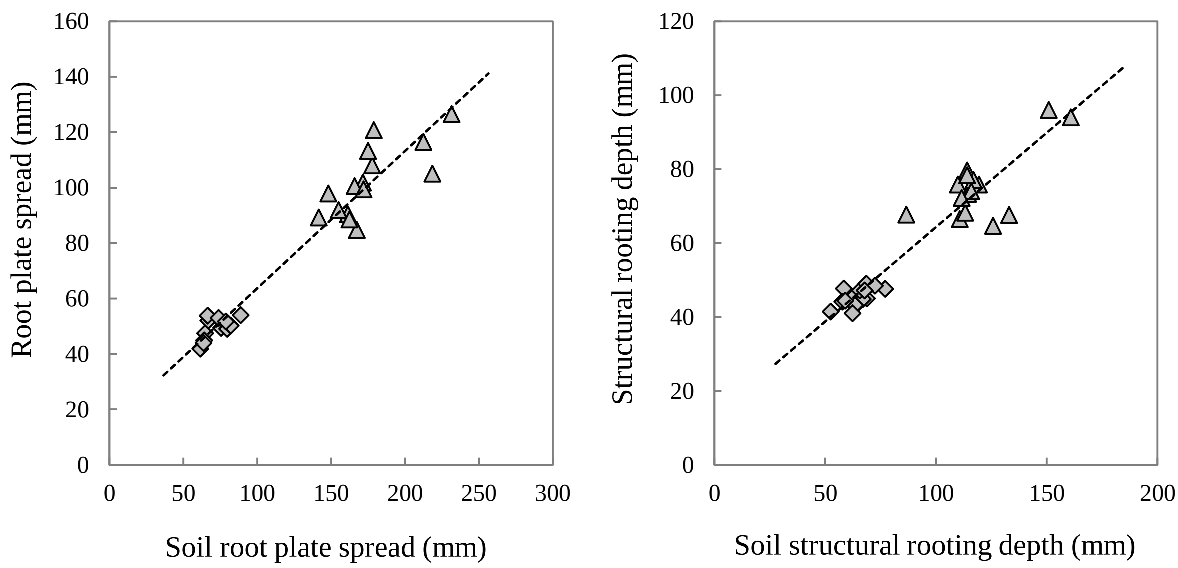

Fig. 5.3 Root plate spread plotted against soil root plate spread (y = 0.50x + 14.3; R^2 = 0.94, *P* < 0.001, 28 d. f.) and structural rooting depth plotted against soil structural rooting depth (y = 0.51x + 13.3; R^2 = 0.90, *P* < 0.001, 28 d. f.) for plot means of five cultivars growing isolated (triangles) and at 175 seed m^{-2} (squares) in 2014.

5.4.4 Association between NDVI and leverage and stem strength components

NDVI is widely used at ground level and from air or satellite level (remote sensing technology) to measure the green vegetation density or canopy photosynthetic size. This vegetation index measured during stem elongation was positively correlated with PH (r_p = 0.46 – 0.59, P < 0.05 – 0.001) and CG (r_p = 0.29, P = 0.05, to 0.60, P < 0.001) and negatively correlated with NF (r_p = -0.31 to -0.50, P < 0.05 – 0.01). Moreover, the genetic correlation coefficients were similar or higher indicating an important genetic effect shared by NDVI with both leverage characters. Phenotypic correlations between NDVI with stem strength characters (IL1, IBS1 and SS) were lower and for some cases not significant. For instance, IL1 was significant for 2011 and 2012 (r_p = 0.31 – 0.39, P < 0.05) and not significant for 2013 means (0.27, ns). Genetic effect shared by IL1 and NDVI indicated that the genetic correlation coefficient was considerably higher for 2012 (r_g = 0.59, P < 0.001). Less consistent were the correlation coefficients found between NDVI and IBS1. However, weak or strong this correlation was negative in all cases. Phenotypic correlation coefficients between NDVI and IBS1 were only significant for 2012 (r_p = -0.37, P < 0.05). The genetic correlation coefficient was higher than the phenotypic correlations in all experiments, which indicated shared genetic effects between NDVI and IBS1. Despite the associations between the NDVI and IL and IBS, the overall stem strength (SS) showed no significant correlations with NDVI (Table 5.5). Table 5.5 also showed that there was genetic effect shared by RPS and NDVI in the 2012 and 2013 experiments, although no significant phenotypic correlations were found. Covariance analysis showed that the seed rate used in each experiment did not explain any correlations between NDVI, IBS, SS or RPS, further indicating that NDVI was detecting genetic variation in these traits.

Table 5.5 Phenotypic (r_p) and genetic (r_g) correlations between NDVI at elongation and leverage (PH, CG and NF), stem strength (SIL, IBS and SS) and anchorage strength (RPS) components for spring wheat growing at NWM during 2011, 2012 and 2013.

Character	Correlation coefficients with NDVI at elongation					
	2011 (58 d. f.)		2012 (28 d. f.)		2013 (28 d. f.)	
	r_p	r_g	r_p	r_g	r_p	r_g
PH	0.46***	0.54***	0.59***	0.64***	0.46*	0.50**
CG	0.29*	0.33*	0.60***	0.63***	0.34†	0.39*
NF	-0.31*	-0.35**	-0.50**	-0.49**	-0.40*	-0.41*
SIL	0.31*	0.31*	0.39*	0.59***	0.27ns	0.33ns
IBS	-0.16ns	-0.29*	-0.37*	-0.43*	-0.32ns	-0.36*
SS	-0.16ns	-0.29*	0.00ns	0.13ns	-0.10ns	-0.10ns
RPS	-0.01ns	0.13ns	-0.28ns	-0.39*	-0.30ns	-0.45*

PH, plant height; CG, height at centre of gravity; NF, natural frequency; SIL, internode 1 length; IBS, internode 1 breaking strength; SS, internode 1 stem strength; RPS, root plate spread; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; †, P = 0.05; ns, not significant

5.5 Discussion

Optimisation of standard field and laboratory protocols for measuring lodging characters (Berry et al., 2003c, 2000) must be done in order to reduce its time consuming nature, which restricts the capacity to evaluate a large number of genotypes. One of the strategies proposed in this chapter was estimating the minimum plant sample number required to maintain sufficient precision and power to detect genetic differences among cultivars. Results indicated that measuring most of the lodging related traits can be done on fewer plants than the current plant sample numbers of 10 plants per plot (3 replicates) and 15 plants per plot (2 replicates). Reducing plot sample size to seven plants could be implemented for all traits involved in the lodging risk performance assessment. Coefficient of variation or relative residual variance and F test have indicated that this reduction will not compromise the ability of the REML to detect genetic differences while maintaining low residual variance in the spring wheat population used in this paper. Additionally, regression analysis plotting varietal ranks and absolute means calculated with seven and the largest plot sample size in each experiment has indicated R^2 above 0.9 with slopes differing by 5% or less from the 1:1-line slope for all traits, except for ENPP. This is an important finding that makes a plot sample size of seven plants reliable enough to obtain not only sensible varietal ranks but also a precise estimation of absolute varietal means. This will be important for breeders because the achievement of a lodging-resistant ideotype requires not only the best cultivars but also their precise lodging-trait dimensions.

A second strategy was to find the minimum number of traits that enables the lodging model to estimate cultivar lodging susceptibility performances. According to the standard protocols the stem strength measurements are normally done in both of the bottom two internodes of the stem base in the laboratory. The strong association between measurements of IL, ID, IBS, SWW, IDW and SS from both internodes was demonstrated by linear regression analysis. These results can be used to infer that stem strength traits can be measured in one internode, thus reducing the time required by 50 %. Leverage characters' interrelationships also indicated that some measurements can be omitted from the standard protocol. From these characters PH is the most straightforward measurement and can be done directly in the field. PH was strongly correlated with CG and moderately correlated with NF. This analysis showed that CG could be omitted from the measurement protocol. Finally, strong positive correlation between RPS, SRD and RDW used to calculate anchorage strength indicated that protocols can focus on RPS as the root resistance selection trait.

The amount of time required to implement the standard field/laboratory lodging trait assessment on a plot basis per person was from 100 to 150 minutes when 10 to 15 plants per plot were measured. This included plant extraction from the field, washing of roots and measurements five stem strength characters (done in both internodes), three anchorage strength characters and five leverage characters done in the laboratory (except for NF which was done in the field). If we consider that the number of traits from the standard protocol can be reduced from 18 to 9 (measuring only EA, ENPP, PH, RPS and ID, IL, IBS, SWW and IDW of one internode), then we can reduce time by 50%. This reduction indicates a time requirement of 50 to 75 minutes per plot. This new time requirement can be further decreased by minimizing the number of plants per plot to seven or even below. If we consider a plot sample size of seven plants this time requirement can be reduced to 39 minutes per plot. Thus, reducing the number of traits and number of plants per plot to 8 and 7, respectively, would increase the plot measurement capacity from 1 plot to 2.6 or 3.8 plots per 100 or 150 minutes from the standard protocol. Thus, for every technician work shift of 8 h, approximately 12 plots can be assessed which is considerable higher than the 3.2 to 4.8 that can be done under the standard protocol.

Removing the soil surrounding the crown roots to assess root anchorage strength is one of the processes that requires a considerable amount of time, water and human work. Avoiding the removal of soil surrounding the crown roots can save human effort, water resources and time. A key aspect to consider is the moisture on the soil when the extraction of the plants is done. Dry or saturated soil will complicate the plant extraction, thus, a soil moisture condition between field capacity and permanent wilting point will be the ideal. This will greatly increase the likelihood that the surrounding soil sticks to the crown roots when the plant extraction is done and allowing the technician to measure the dimensions of the root/soil 'ball'. If we consider this approach we can further reduce the 39 minutes estimated to measure lodging traits per plot previously perhaps to 25 – 30 minutes per plot. This method must be tested on a range of soil types as it may not work as well on soils with a lower clay content that are less cohesive.

Large or dense wheat canopies and excessive nitrogen applications were reported to increase lodging in wheat (Berry et al., 2000). Sparkes and King (2008) indicated that stem strength was reduced when wheat plants were grown under shading due to changes in the quantity and quality of the light conditions. Considering these two aspects, focus on the canopy size and nitrogen content could be a useful indicator of some lodging traits. NDVI has been reported to correlate positively with wheat plant biomass (Reynolds et al.1999; Cabrera-Bosquet et al. 2011) and nitrogen content (Cabrera-Bosquet et al., 2011). This study has indicated correlations between NDVI at stem elongation with key leverage (PH, CG and NF) and stem strength characters (IL, IBS). Nevertheless, only correlation between NDVI and PH was strong enough and consistent. Since PH is an easy and common trait measured in most experiments the usefulness of the NDVI is limited. However, further investigations focussing on NDVI and key lodging-traits associations might be justified to fully understand if spectral reflectance indices can give better predictions.

5.6 Conclusion

Considering the results discussed in this chapter, it can be concluded that optimising standard protocols to measure lodging resistance in spring wheat can be achieved by:

- Reducing to seven the number of plants measured per plot when three replicates are used to calculate cultivar means while maintaining power and precision to detect genetic differences.
- Reducing the number of lodging characters from 18 to 8 to assess lodging based on its genetic linkages. Measurements that can be omitted would be IL, IBS, SWW, ID and IDW in one of the basal internodes, CG and NF on the main shoot and RPS and RDW from the root system.
- Implementing novel measurements such as measuring of the dimensions of the surrounding soil of the root crowns to infer in the anchorage strength dimension; however, further investigation will be necessary.

Further investigation of the associations between NDVI and lodging characters will be necessary to fully comprehend to what extent NDVI can be used to predict lodging-traits.

5.7 References

Berry, P.M., 1998. Predicting lodging in wheat. Ph. D. Thesis. The University of Nottingham, UK, 210 pp.

Berry, P.M., Berry, S.T., 2015. Understanding the genetic control of lodging-associated plant characters in winter wheat (Triticum aestivum L.). Euphytica. doi:10.1007/s10681-015-1387-2

Berry, P.M., Griffin, J.M., Sylvester-bradley, R., Scott, R.K., Spink, J.H., Baker, C.J., Clare, R.W., 2000. Controlling plant form through husbandry to minimise lodging in wheat. F. Crop. Res. 67, 59–81.

Berry, P.M., Spink, J., 2012. Predicting yield losses caused by lodging in wheat. F. Crop. Res. 137, 19–26. doi:10.1016/j.fcr.2012.07.019

Berry, P.M., Spink, J., Sterling, M., Pickett, A.A., 2003a. Methods for Rapidly Measuring the Lodging Resistance of Wheat Cultivars. J. Agron. Crop Sci. 189, 390–401. doi:10.1046/j.0931-2250.2003.00062.x

Berry, P.M., Spink, J.H., Gay, A.P., Craigon, J., 2003b. A comparison of root and stem lodging risks among winter wheat cultivars. J. Agric. Sci. 141, 191–202. doi:10.1017/S002185960300354X

Berry, P.M., Sterling, M., Baker, C.J., Spink, J., Sparkes, D.L., 2003c. A calibrated model of wheat lodging compared with field measurements. Agric. For. Meteorol. 119, 167–180. doi:10.1016/S0168-1923(03)00139-4

Berry, P.M., Sterling, M., Spink, J.H., Baker, C.J., Sylvester-Bradley, R., Mooney, S.J., Tams, A.R., Ennos, A.R., 2004. Understanding and reducing lodging in cereals. Adv. Agron. 84, 217–271. doi:10.1016/S0065-2113(04)84005-7

Berry, P.M., Sylvester-Bradley, R., Berry, S., 2007. Ideotype design for lodging-resistant wheat. Euphytica 154, 165–179. doi:10.1007/s10681-006-9284-3

Cabrera-Bosquet, L., Molero, G., Stellacci, a. M., Bort, J., Nogués, S., Araus, J.L., 2011. NDVI as a potential tool for predicting biomass, plant nitrogen content and growth in wheat genotypes subjected to different water and nitrogen conditions. Cereal Res. Commun. 39, 147–159. doi:10.1556/CRC.39.2011.1.15

Hai, L., Guo, H., Xiao, S., Jiang, G., Zhang, X., Yan, C., Xin, Z., Jia, J., 2005. Quantitative trait loci (QTL) of stem strength and related traits in a doubled-haploid population of wheat (Triticum aestivum L.). Euphytica 141, 1–9. doi:10.1007/s10681-005-4713-2

Kashiwagi, T., Ishimaru, K., 2004. Identification and functional analysis of a locus for improvement of lodging resistance in rice. Plant Physiol. 134, 676–683. doi:10.1104/pp.103.029355.inherited

Keller, M., Karutz, C., Schmid, J.E., Stamp, P., Winzeler, M., Keller, B., Messmer, M.M., 1999. Quantitative trait loci for lodging resistance in a segregating wheat x spelt population. TAG 98, 1171–1182.

Niu, L., Feng, S., Ru, Z., Li, G., Zhang, Z., Wang, Z., 2012. Rapid determination of single-stalk and population lodging resistance strengths and an assessment of the stem lodging wind speeds for winter wheat. F. Crop. Res. 139, 1–8. doi:10.1016/j.fcr.2012.10.014

Pask, A., Pietragalla, J., Mullan, D., Reynolds, M.P., 2012. Physiological breeding II: a field guide to wheat phenotyping. CIMMYT, Mexico, D. F.

Sparkes, D.L., King, M., 2008. Disentangling the effects of PAR and R:FR on lodging-associated characters of wheat (Triticum aestivum). Ann. Appl. Biol. 152, 1–9. doi:10.1111/j.1744-7348.2007.00184.x

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Reserach 14, 415–421.

5.8 Supplementary material

Table S5.1 Standard error differences (SED) and coefficient of variation percentages (CV) of lodging-related characters for cultivar means estimated with different plot sample size for spring wheat growing at NWM during 2011, 2012 and 2013 experiments

Exp.	Plant no.	CG		ENPP		NF		PH		IL		ID		SWW		IBS		RPS		SRD	
		SED	CV	SED	CV	SED	CV	SED	CV	SED	CV	SED	CV	SED	CV	SED	CV	SED	CV	SED	CV
2011	1	3.09**	8.06	0.89ns	33.6	0.18***	14.1	6.54***	7.47	13.0**	14.6	0.42ns	9.95	0.10***	13.0	219*	30.0	5.38ns	16.7	6.13ns	16.5
	2	2.17***	5.64	0.72ns	26.8	0.19**	14.7	4.42***	5.02	13.0*	14.2	0.31***	7.30	0.10***	12.8	178*	24.8	4.30ns	13.0	2.48**	6.73
	3	1.74***	4.51	0.51*	18.8	0.15***	11.7	3.99***	4.53	9.10***	10.3	0.28***	6.66	0.10***	11.9	147*	21.1	3.40ns	10.3	3.33ns	9.05
	4	1.40***	3.64	0.52ns	18.9	0.13***	9.79	3.20***	3.65	8.67***	9.64	0.20***	4.65	0.08***	9.82	126**	17.8	2.92ns	8.90	2.72**	7.43
	5	1.33***	3.48	0.55ns	19.6	0.14***	11.1	2.98***	3.41	8.18***	9.04	0.23***	5.45	0.07***	8.68	124**	17.5	3.05ns	9.14	2.74**	7.39
	6	1.38***	3.61	0.47ns	17.3	0.15***	11.3	3.11***	3.55	6.78***	7.54	0.17***	4.04	0.06***	7.16	130*	18.7	2.78ns	8.40	2.49**	6.72
	7	1.41***	3.69	0.46ns	16.8	0.13***	10.2	2.78***	3.17	6.47***	7.24	0.20***	4.81	0.08***	9.93	120**	17.0	2.87ns	8.68	2.75*	7.45
	8	1.42***	3.72	0.41*	14.9	0.13***	9.57	2.76***	3.15	7.01***	7.83	0.21***	4.90	0.06***	7.94	121*	17.0	2.68ns	8.15	2.48**	6.75
	9	1.18***	3.07	0.42ns	15.3	0.13***	9.71	2.69***	3.06	7.12***	7.90	0.18***	4.27	0.06***	7.96	116**	16.5	2.55ns	7.75	2.13**	5.79
	10	1.27***	3.32	0.38*	13.8	0.13***	10.1	2.56***	2.93	6.41***	7.14	0.17***	3.95	0.06***	7.84	112**	18.9	2.70ns	8.16	2.48**	6.73
	11	1.24***	3.23	0.39*	14.1	0.12***	9.34	2.65***	3.02	6.63***	7.41	0.17***	4.00	0.06***	7.41	110**	18.7	2.65ns	8.01	2.32***	6.27
	12	1.22***	3.18	0.39*	14.1	0.13***	9.76	2.56***	2.92	6.38***	7.13	0.17***	4.11	0.06***	7.47	118*	16.9	2.61ns	7.93	2.29**	6.20
	13	1.19***	3.11	0.36*	13.2	0.12***	9.49	2.49***	2.84	5.99***	6.68	0.16***	3.90	0.06***	7.85	108**	15.4	2.49ns	7.55	2.36**	6.43
	14	1.15***	2.97	0.36*	13.3	0.12***	9.26	2.64***	3.02	5.64***	6.25	0.16***	3.78	0.06***	7.32	105***	14.9	2.49ns	7.55	2.25***	6.12
	15	1.14***	2.97	0.36*	13.2	0.12***	9.22	2.41***	2.75	5.64***	6.29	0.16***	3.73	0.06***	7.43	106**	15.1	2.51ns	7.62	2.21***	6.01
2012	1	3.47***	6.16	0.72ns	27.3	0.19***	12.1	4.36***	4.71	13.5ns	23.6	0.31***	7.80	0.13ns	15.6	558ns	36.8	5.25*	14.3	4.63**	12.0
	2	1.85***	3.27	0.49*	17.8	0.11***	7.00	2.54***	2.74	8.60**	15.2	0.21***	5.32	0.08***	9.64	344**	22.8	4.79**	12.8	3.77**	9.91
	3	2.14***	3.79	0.39ns	14.7	0.12***	7.75	3.59***	3.90	6.56***	11.4	0.23***	5.72	0.06***	6.92	250***	16.9	4.49**	12.3	3.13***	8.15
	4	1.72***	3.05	0.41*	14.8	0.11***	7.42	2.77***	3.00	6.21***	11.0	0.20***	5.13	0.07***	7.99	240**	15.9	3.91**	10.5	3.03***	7.81

	5	1.38***	2.45	0.30***	11.2	0.11***	7.36	2.75***	2.97	5.64***	10.1	0.18***	4.45	0.06***	7.09	226***	14.7	3.86**	10.5	2.74***	7.11
	6	1.34***	2.37	0.33**	12.3	0.11***	7.06	2.21***	2.40	5.29***	9.17	0.18***	4.58	0.06***	6.90	236***	15.8	3.96**	10.7	2.68***	6.94
	7	1.27***	2.24	0.32*	11.9	0.11***	7.04	2.10***	2.28	5.29***	9.33	0.18***	4.46	0.06***	6.88	231***	15.3	3.80***	10.3	2.56***	6.65
	8	1.36***	2.40	0.31**	11.5	0.09***	6.22	2.43***	2.63	4.63***	8.12	0.17***	4.28	0.05***	6.33	199***	13.2	3.98**	10.8	2.80***	7.22
	9	1.22***	2.15	0.29**	10.8	0.10***	6.32	2.20***	2.38	4.60***	8.12	0.17***	4.33	0.05***	6.10	203***	13.4	3.76**	10.2	2.56***	6.63
	10	1.22***	2.17	0.29**	10.8	0.10***	6.37	2.15***	2.33	4.63***	8.15	0.17***	4.18	0.05***	5.97	192***	12.7	3.77**	10.2	2.55***	6.62
2013	1	2.88***	5.34	0.67ns	26.2	0.18***	10.5	3.85***	4.19	14.7ns	24.3	0.36**	9.55	0.11ns	15.4	456*	31.4	5.12ns	11.5	3.72*	9.48
	2	3.31**	6.11	0.44ns	16.4	0.17***	9.84	4.05***	4.41	11.5ns	19.3	0.25***	6.65	0.07**	10.2	264*	18.9	3.55**	8.02	2.80**	7.23
	3	1.96***	3.63	0.35*	13.3	0.15***	8.74	2.41***	2.62	8.11***	13.2	0.19***	5.09	0.08***	10.7	209***	15.1	3.43**	7.77	2.73**	7.04
	4	2.57***	4.78	0.30ns	11.5	0.13***	7.46	2.24***	2.42	7.74***	12.2	0.19***	4.95	0.07***	9.54	180***	13.4	3.58*	8.09	2.68*	6.93
	5	1.99***	3.66	0.31**	11.6	0.14***	8.03	2.45***	2.66	7.73***	12.3	0.20***	5.25	0.07***	9.30	200***	14.6	3.21*	7.24	2.32***	5.98
	6	2.28***	4.23	0.30*	11.5	0.13***	7.71	1.95***	2.11	7.05***	11.1	0.18***	4.92	0.06***	8.84	174***	12.8	3.01*	6.79	2.03***	5.22
	7	1.84***	3.40	0.29*	11.1	0.13***	7.89	2.30***	2.50	6.26***	9.96	0.17***	4.48	0.07***	9.54	170***	12.7	2.63***	5.93	2.05***	5.28
	8	1.73***	3.19	0.27*	9.68	0.13***	7.68	1.92***	2.08	6.40***	10.0	0.16***	4.26	0.07***	9.38	180***	13.6	2.79**	6.27	2.02***	5.20
	9	1.73***	3.20	0.25*	9.54	0.13***	7.47	1.99***	2.16	5.58***	8.78	0.14***	3.81	0.07***	9.40	170***	12.7	2.58***	5.82	1.95***	5.01
	10	1.65***	3.05	0.25**	9.31	0.13***	7.56	1.95***	2.12	5.50***	8.68	0.15***	4.03	0.07***	9.00	163***	12.2	2.69**	6.03	1.97***	5.07

CG, height at centre of gravity; ENPP, ear number per plant; NF, natural frequency; PH, plant height; IL, internode length; ID, internode diameter; SWW, internode wall width; IBS, internode breaking strength; RPS, root plate spread; SRD, structural rooting depth: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant

Table S5.2 Standard error differences (SED) and coefficient of variation percentages (CV) of anchorage failure wind speed and stem failure wind speed for spring wheat growing at NWM during 2011, 2012 and 2013 experiments

Experiment	Plant no.	Anchorage failure wind speed		Stem failure wind speed	
		SED	CV	SED	CV
2011	1	3.61ns	35.6	2.03ns	17.4
	2	2.97ns	29.6	1.62*	13.7
	3	2.16ns	21.6	1.50*	13.0
	4	2.15ns	21.7	1.24**	10.5
	5	1.91*	18.7	1.19***	10.1
	6	1.78**	17.4	1.18***	10.0
	7	1.90*	18.8	1.20**	10.2
	8	1.80**	18.1	1.23**	10.4
	9	1.66*	16.7	1.12***	9.42
	10	1.87*	18.5	1.13***	9.54
	11	1.89*	18.7	1.11***	9.40
	12	1.70**	16.9	1.19**	10.1
	13	1.74*	17.4	1.07***	9.07
	14	1.71**	17.0	1.06***	9.00
	15	1.70**	17.0	1.05***	8.94
2012	1	2.90***	26.6	1.40**	11.7
	2	2.57***	24.0	1.03**	8.45
	3	2.28***	21.6	0.92*	7.48
	4	2.09***	19.5	0.71***	5.74
	5	2.12***	19.9	0.78***	6.33
	6	2.18***	20.4	0.70***	5.67
	7	2.05***	19.3	0.71***	5.68
	8	2.06***	19.1	0.72***	5.76
	9	1.98***	18.6	0.66***	5.31
	10	1.95***	18.2	0.63***	5.06
2013	1	3.60*	21.6	1.47**	10.9
	2	2.16***	13.8	1.07***	7.86
	3	2.12***	13.6	0.91***	6.59
	4	2.07***	13.2	0.77***	5.57
	5	1.98***	12.6	0.83***	5.93
	6	1.64***	10.4	0.74***	5.29
	7	1.61***	10.2	0.70***	5.00
	8	1.62***	10.2	0.66***	4.73
	9	1.57***	9.90	0.68***	4.84
	10	1.50***	9.53	0.65***	4.67

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant

Table S5.3 Regression coefficient and slope for the relationship between cultivar means and ranks estimated with 10 or 15 plants per plot and with lower different plant number per plot for leverage traits at 2011, 2012 and 2013.

Exp.	Plant no.	Height at centre of gravity				Ear number per plant				Natural frequency				Plant height			
		R^2		Slope		R^2		Slope		R^2		Slope		R^2		Slope	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
2011	1	0.63	0.58	0.63	0.77	0.16	0.12	0.19	0.39	0.72	0.32	0.67	0.56	0.75	0.68	0.67	0.83
	2	0.78	0.61	0.86	0.78	0.40	0.35	0.38	0.60	0.81	0.51	0.80	0.71	0.83	0.65	0.87	0.80
	3	0.89	0.81	0.94	0.90	0.55	0.52	0.52	0.72	0.88	0.75	0.93	0.87	0.91	0.85	0.93	0.92
	4	0.91	0.89	0.91	0.94	0.65	0.58	0.59	0.76	0.92	0.82	0.87	0.91	0.95	0.93	0.93	0.97
	5	0.94	0.91	0.96	0.95	0.64	0.61	0.65	0.78	0.94	0.84	1.00	0.92	0.95	0.92	0.96	0.96
	6	0.93	0.90	0.97	0.95	0.98	0.59	0.74	0.77	0.96	0.87	0.94	0.94	0.92	0.88	0.94	0.94
	7	0.95	0.91	0.95	0.96	0.85	0.82	0.76	0.90	0.97	0.95	1.00	0.97	0.97	0.93	0.96	0.97
	8	0.97	0.95	0.97	0.97	0.84	0.83	0.82	0.92	0.96	0.92	1.01	0.96	0.98	0.96	0.94	0.98
	9	0.98	0.96	1.00	0.98	0.85	0.82	0.95	0.90	0.97	0.91	0.95	0.95	0.98	0.96	0.98	0.98
	10	0.98	0.96	0.98	0.98	0.91	0.89	0.89	0.93	0.97	0.92	0.97	0.96	0.99	0.97	0.99	0.98
	11	0.99	0.97	0.98	0.99	0.94	0.94	0.89	0.97	0.99	0.96	0.96	0.98	0.99	0.97	1.00	0.98
	12	0.99	0.98	1.01	0.99	0.94	0.93	0.94	0.97	0.99	0.96	1.03	0.98	0.99	0.98	1.01	0.99
	13	1.00	0.99	0.98	0.99	0.96	0.95	0.96	0.98	0.99	0.99	1.01	0.99	1.00	0.98	0.98	0.99
	14	1.00	0.99	1.00	1.00	0.98	0.97	0.97	0.98	1.00	0.99	1.01	1.00	0.99	0.99	1.00	0.99
	15	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2012	1	0.70	0.59	0.62	0.77	0.39	0.35	0.29	0.59	0.85	0.74	0.82	0.86	0.84	0.75	0.79	0.86
	2	0.88	0.80	0.86	0.90	0.42	0.42	0.41	0.65	0.93	0.86	0.85	0.93	0.94	0.90	0.89	0.95
	3	0.92	0.86	0.86	0.93	0.56	0.61	0.74	0.78	0.93	0.85	1.00	0.92	0.93	0.91	0.97	0.95
	4	0.92	0.75	0.88	0.86	0.76	0.75	0.66	0.86	0.97	0.94	0.83	0.97	0.94	0.87	0.90	0.93
	5	0.96	0.92	1.06	0.96	0.83	0.81	0.74	0.90	0.98	0.95	0.96	0.97	0.98	0.94	0.99	0.97
	6	0.98	0.94	0.96	0.97	0.86	0.81	0.79	0.90	0.98	0.97	0.93	0.98	0.98	0.95	0.98	0.97
	7	0.98	0.97	0.99	0.98	0.91	0.89	0.95	0.94	0.99	0.96	0.93	0.98	0.99	0.98	0.98	0.99

	8	0.99	0.98	0.99	0.99	0.93	0.88	0.96	0.94	0.99	0.98	0.97	0.99	0.99	0.98	0.99	0.99
	9	1.00	0.99	0.99	1.00	0.98	0.95	0.94	0.98	1.00	0.99	0.99	1.00	1.00	0.99	0.99	1.00
	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2013	1	0.85	0.75	0.80	0.86	0.28	0.24	0.29	0.51	0.87	0.65	0.76	0.81	0.80	0.73	0.84	0.85
	2	0.69	0.66	0.68	0.81	0.30	0.34	0.50	0.59	0.92	0.83	0.93	0.91	0.90	0.84	0.81	0.92
	3	0.94	0.90	0.94	0.95	0.71	0.68	0.66	0.82	0.95	0.85	0.98	0.92	0.97	0.92	0.94	0.96
	4	0.85	0.79	0.81	0.89	0.76	0.68	0.83	0.83	0.98	0.87	1.03	0.93	0.97	0.92	0.98	0.96
	5	0.90	0.87	0.93	0.94	0.83	0.86	0.72	0.93	0.98	0.94	0.96	0.97	0.98	0.96	0.93	0.98
	6	0.91	0.75	0.95	0.87	0.94	0.92	0.82	0.95	0.99	0.96	1.04	0.98	0.99	0.98	1.00	0.99
	7	0.99	0.91	0.98	0.96	0.92	0.91	0.86	0.94	0.99	0.98	0.98	0.99	0.99	0.99	1.00	0.99
	8	0.97	0.88	0.99	0.94	0.92	0.87	0.95	0.94	1.00	0.99	0.98	0.99	0.99	0.97	1.01	0.99
	9	0.98	0.93	0.99	0.96	0.97	0.94	0.98	0.97	1.00	0.99	1.01	1.00	1.00	0.99	1.00	1.00
	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table S5.4 Regression coefficient and slope for the relationship between cultivar means and ranks estimated with 10 or 15 plants per plot and with lower different plant number per plot for stem and anchorage stem strength traits at 2011, 2012 and 2013.

Exp.	Plant no.	Internode length				Internode diameter				Internode wall width				Internode breaking strength				Root plate spread				Structural rooting depth			
		R^2		Slope		R^2		Slope		R^2		Slope		R^2		Slope		R^2		Slope		R^2		Slope	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
2011	1	0.44	0.38	0.48	0.62	0.47	0.40	0.54	0.63	0.75	0.54	0.59	0.74	0.56	0.59	0.44	0.77	0.17	0.17	0.21	0.42	0.41	0.36	0.39	0.60
	2	0.74	0.63	0.66	0.79	0.75	0.75	0.67	0.86	0.79	0.70	0.77	0.84	0.63	0.58	0.55	0.76	0.46	0.41	0.43	0.64	0.63	0.51	0.60	0.72
	3	0.64	0.52	0.74	0.72	0.77	0.81	0.74	0.90	0.88	0.78	0.79	0.89	0.75	0.77	0.69	0.88	0.40	0.42	0.49	0.65	0.48	0.43	0.62	0.66
	4	0.83	0.79	0.84	0.89	0.86	0.81	0.84	0.90	0.89	0.80	1.01	0.89	0.76	0.79	0.81	0.89	0.66	0.54	0.69	0.74	0.83	0.77	0.82	0.88
	5	0.87	0.83	0.78	0.91	0.88	0.86	0.91	0.93	0.91	0.81	0.91	0.90	0.87	0.87	0.86	0.93	0.64	0.53	0.69	0.73	0.77	0.72	0.79	0.86
	6	0.89	0.82	0.92	0.91	0.90	0.91	0.88	0.95	0.92	0.87	0.89	0.93	0.85	0.85	0.86	0.92	0.74	0.66	0.76	0.81	0.83	0.74	0.79	0.86
	7	0.93	0.91	0.94	0.96	0.91	0.89	0.96	0.94	0.95	0.89	0.98	0.95	0.90	0.87	0.91	0.93	0.85	0.79	0.93	0.89	0.93	0.90	0.93	0.95
	8	0.95	0.95	0.94	0.98	0.95	0.95	0.97	0.97	0.98	0.94	0.99	0.97	0.93	0.91	0.94	0.95	0.83	0.75	0.84	0.87	0.89	0.88	0.86	0.94
	9	0.96	0.96	0.96	0.98	0.96	0.97	0.96	0.98	0.98	0.94	0.94	0.97	0.95	0.94	0.90	0.97	0.87	0.80	0.95	0.89	0.90	0.89	1.01	0.95
	10	0.97	0.97	0.97	0.98	0.97	0.97	0.92	0.98	0.98	0.94	0.97	0.97	0.96	0.94	0.98	0.97	0.91	0.86	0.90	0.93	0.96	0.94	0.91	0.97
	11	0.97	0.96	0.99	0.98	0.98	0.98	0.99	0.99	0.99	0.95	0.98	0.98	0.97	0.97	1.03	0.98	0.92	0.89	0.90	0.94	0.96	0.95	0.91	0.98
	12	0.98	0.96	0.99	0.98	0.98	0.98	0.98	0.99	0.99	0.96	1.00	0.98	0.98	0.97	0.98	0.98	0.95	0.92	1.02	0.96	0.96	0.95	0.98	0.98
	13	0.99	0.98	0.98	0.99	0.99	0.97	1.01	0.99	0.99	0.97	0.99	0.99	0.98	0.98	0.99	0.99	0.95	0.92	1.02	0.96	0.98	0.97	0.99	0.99
	14	0.99	0.99	1.00	0.99	0.99	0.99	1.00	0.99	1.00	0.98	0.99	0.99	0.99	0.99	0.98	1.00	0.99	0.97	0.98	0.99	0.99	0.99	0.98	1.00
	15	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2012	1	0.56	0.48	0.60	0.69	0.70	0.62	0.64	0.79	0.62	0.53	0.55	0.73	0.25	0.28	0.36	0.53	0.74	0.78	0.70	0.88	0.71	0.61	0.64	0.80
	2	0.66	0.70	0.67	0.84	0.88	0.89	0.76	0.94	0.77	0.72	0.60	0.85	0.77	0.79	0.67	0.89	0.81	0.80	0.75	0.89	0.84	0.61	0.84	0.78
	3	0.75	0.72	0.72	0.85	0.88	0.82	0.89	0.90	0.81	0.82	0.71	0.90	0.86	0.84	0.70	0.92	0.92	0.94	0.83	0.97	0.84	0.74	0.73	0.86
	4	0.80	0.76	0.85	0.87	0.93	0.91	0.91	0.95	0.88	0.83	0.92	0.91	0.82	0.76	0.97	0.88	0.91	0.88	0.93	0.94	0.88	0.86	0.97	0.92
	5	0.93	0.87	0.93	0.93	0.96	0.96	0.97	0.98	0.94	0.91	0.80	0.95	0.92	0.85	0.90	0.92	0.93	0.91	1.03	0.96	0.97	0.94	1.03	0.97

	6	0.93	0.93	0.90	0.96	0.98	0.97	0.96	0.99	0.94	0.97	0.80	0.98	0.90	0.90	0.86	0.95	0.98	0.95	0.91	0.98	0.96	0.93	0.89	0.97
	7	0.96	0.94	0.98	0.97	0.99	0.98	0.98	0.99	0.93	0.94	0.93	0.97	0.95	0.93	0.96	0.96	0.98	0.97	0.96	0.98	0.98	0.93	0.94	0.96
	8	0.97	0.93	0.94	0.96	0.99	0.99	1.01	1.00	0.98	0.96	0.91	0.98	0.99	0.97	0.93	0.99	0.99	0.97	0.97	0.98	0.99	0.98	0.95	0.99
	9	0.98	0.96	1.00	0.98	0.99	0.98	1.01	0.99	0.99	0.95	0.98	0.98	0.99	0.98	0.99	0.99	0.99	0.98	0.98	0.99	0.99	0.99	1.00	0.99
	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2013	1	0.20	0.12	0.32	0.35	0.64	0.56	0.69	0.75	0.50	0.51	0.61	0.71	0.42	0.48	0.40	0.69	0.66	0.56	0.56	0.75	0.60	0.36	0.60	0.60
	2	0.71	0.67	0.71	0.82	0.92	0.89	0.94	0.94	0.76	0.72	1.00	0.85	0.70	0.70	0.82	0.84	0.72	0.63	0.61	0.80	0.81	0.73	0.71	0.87
	3	0.75	0.72	0.64	0.85	0.89	0.86	0.99	0.93	0.87	0.85	0.83	0.92	0.87	0.83	0.71	0.91	0.80	0.76	0.75	0.87	0.84	0.82	0.86	0.92
	4	0.78	0.71	0.76	0.84	0.93	0.96	0.92	0.98	0.94	0.86	0.88	0.92	0.88	0.86	0.84	0.93	0.84	0.80	0.83	0.90	0.89	0.81	0.92	0.90
	5	0.79	0.77	0.77	0.88	0.96	0.92	0.93	0.96	0.96	0.93	0.93	0.97	0.91	0.86	0.85	0.93	0.89	0.83	0.87	0.91	0.94	0.91	0.92	0.96
	6	0.93	0.94	0.92	0.97	0.99	0.98	0.96	0.99	0.96	0.94	0.90	0.97	0.95	0.94	0.89	0.97	0.93	0.90	0.98	0.95	0.94	0.89	0.94	0.95
	7	0.94	0.90	1.03	0.95	0.99	0.99	0.97	0.99	0.97	0.97	1.02	0.99	0.97	0.97	0.96	0.98	0.96	0.94	0.94	0.97	0.98	0.95	0.99	0.99
	8	0.94	0.90	0.94	0.95	0.99	0.98	1.01	0.99	0.99	0.98	0.99	0.99	0.99	0.97	0.99	0.99	0.98	0.97	0.99	0.99	0.98	0.96	1.01	0.99
	9	0.98	0.98	0.99	0.99	1.00	0.99	0.99	1.00	0.99	0.99	0.98	1.00	0.99	0.99	0.96	0.99	0.99	0.98	0.99	0.99	0.99	0.98	1.01	1.00
	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table S5.5 Regression coefficient and slope for the relationship between cultivar means and ranks estimated with 10 and 15 plants per plot and with lower different plant number per plot for stem and anchorage failure wind speed at 2011, 2012 and 2013.

Exp.	Plant no.	Anchorage failure wind speed				Stem failure wind speed			
		R^2		Slope		R^2		Slope	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
2011	1	0.42	0.39	0.43	0.62	0.52	0.42	0.53	0.65
	2	0.58	0.47	0.60	0.69	0.62	0.62	0.69	0.79
	3	0.57	0.47	0.70	0.68	0.85	0.83	0.81	0.91
	4	0.73	0.69	0.82	0.83	0.85	0.84	0.86	0.92
	5	0.79	0.75	0.82	0.87	0.89	0.86	0.90	0.93
	6	0.87	0.81	0.82	0.90	0.90	0.86	0.88	0.93
	7	0.93	0.92	0.90	0.96	0.91	0.90	0.97	0.95
	8	0.89	0.89	0.86	0.94	0.95	0.93	0.99	0.96
	9	0.93	0.91	1.03	0.96	0.95	0.94	0.90	0.97
	10	0.95	0.92	0.92	0.96	0.97	0.95	0.98	0.97
	11	0.97	0.95	0.94	0.97	0.97	0.97	1.01	0.98
	12	0.97	0.95	0.99	0.97	0.98	0.95	0.99	0.98
	13	0.98	0.97	1.01	0.98	0.99	0.98	1.00	0.99
	14	0.99	0.99	1.01	0.99	0.99	0.99	0.98	0.99
	15	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2012	1	0.83	0.82	0.74	0.91	0.51	0.44	0.41	0.66
	2	0.87	0.88	0.84	0.94	0.77	0.69	0.72	0.83
	3	0.90	0.88	0.84	0.94	0.69	0.57	0.83	0.76
	4	0.92	0.91	0.99	0.95	0.82	0.80	0.75	0.90
	5	0.97	0.96	1.03	0.98	0.90	0.88	0.86	0.94
	6	0.96	0.95	0.93	0.97	0.92	0.87	0.94	0.93
	7	0.98	0.99	0.97	0.99	0.93	0.92	0.94	0.96
	8	0.99	0.99	0.97	0.99	0.97	0.93	0.96	0.97
	9	1.00	0.98	0.99	0.99	0.98	0.95	0.94	0.97
	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2013	1	0.63	0.36	0.57	0.60	0.74	0.65	0.73	0.81
	2	0.80	0.68	0.68	0.82	0.85	0.75	0.95	0.87
	3	0.84	0.71	0.80	0.84	0.91	0.92	0.93	0.96
	4	0.90	0.80	0.91	0.89	0.90	0.93	0.94	0.97
	5	0.91	0.83	0.91	0.91	0.97	0.96	0.97	0.98
	6	0.92	0.90	0.93	0.95	0.97	0.96	0.99	0.98
	7	0.98	0.93	0.95	0.96	0.98	0.98	0.95	0.99
	8	0.97	0.95	1.00	0.98	0.99	0.98	1.00	0.99
	9	0.99	0.98	0.96	0.99	0.99	0.99	1.01	0.99
	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Chapter 6 Quantitative trait loci analysis for lodging-associated traits in wheat

6.1 Abstract

The implementation of rapid methodologies to enable plant breeders to select for greater lodging resistance in wheat will be a major breakthrough that will help guarantee future grain yield increases. Lodging is a persistent issue affecting grain quality and grain yield. Standard methods for measuring the biophysical properties of the stem base and anchorage system related to lodging resistance (stem and anchorage strength) are too laborious to be incorporated in breeding programs especially at early stages of selection. Thus, developing molecular markers to rapidly select for these properties should be considered. The aim of this chapter was the identification of QTLs associated with stem and anchorage strength characters in the UK reference wheat population Avalon x Cadenza. Our results indicated a wide genetic variation and significant G×E interactions ($P < 0.05$) for the lodging-related characters. However, stem strength traits were highly heritable while root or anchorage strength traits were less heritable. The implementation of two QTL for stem strength identified on chromosome 3B (explaining 44 % of the phenotypic variation) and chromosome 1D (explaining 6.4 % of the phenotypic variation) can counteract this shortcoming of labour intensive phenotyping. A QTL for root plate spread on chromosome 5B (explaining 33 % of the phenotypic variation) will be helpful to achieve an increased root lodging resistant ideotype. Further validation and/or fine mapping of QTLs on chromosomes 3B and 5B will be necessary to develop molecular markers to enable marker-

assisted selection (MAS) to be used for stem and root lodging resistance, respectively.

6.2 Introduction

Reducing lodging in wheat remains an important strategy to maintain high yields and grain quality. Leverage exerted on the stem base and anchorage system (shoot and plant leverage) diminished as a consequence of semi-dwarf wheats produced after the Green revolution in the 1960s and 70s. Since then continued grain yield increases, together with either no further change in plant height or increasing of plant height in semi-dwarf cultivars in key breeding programs worldwide, have contributed to increasing shoot and plant leverage. For instance, in the UK there was not a significant trend towards the reduction of plant height of winter wheat after 1990's (Berry et al. 2015) and it has been found that plant height of spring semi-dwarf wheat cultivars developed by CIMMYT breeding programs has increased in the last decades (Aisawi et al., 2015). Thus, novel ways to counteract this increased stem and plant leverage will be needed. The most recent detailed lodging literature review by Berry et al. (2004) has indicated that to counteract these leverage forces it is necessary to strengthen the stem base and anchorage system. This can be done by exploiting the biophysical properties of these components of the plant (lodging-related traits) which include root plate spread, structural rooting depth and anchorage strength of the root system and diameter, wall width, overall strength and material strength of the stem base. Berry et al. (2007) reduced the number of traits to a set of key traits which included the root plate spread (anchorage strength), diameter, material strength, wall width and stem strength of the stem base (stem strength) and plant height (leverage). Genetic variation of lodging-related traits was found for winter wheat crops grown in the UK (Berry and Berry, 2015; Berry et al., 2007, 2003b) as well as for spring wheat grown in NW Mexico (See Chapter 3). Additionally, several other studies have indicated genetic variation for some of the traits in winter and/or spring wheat (Hai et al., 2005; A. J. Kelbert et al., 2004; Keller et al., 1999; Tripathi et al., 2003; Verma et al., 2004; Wiersma et al., 2011). Berry et al. (2007) and this present investigation (See Chapter 2), respectively, estimated the dimensions of a lodging-proof crop for the UK (winter wheat) and NW Mexico (spring wheat) growing conditions. Achieving this lodging-proof crop will depend on the inter-relationships of lodging-related traits and the combination of key trait targets in a single variety. In this regard, it has been found that genetic linkages between lodging-related traits will not represent a great barrier to achieve this lodging resistant crop in both winter (Berry and Berry 2015) and spring wheat (See Chapter 3). However, strong genetic linkage between stem strength and stem wall width will increase the biomass cost for stronger stems in spring wheat (See Chapter 3). This will represent a trade-off between stem strength and yield and yield formation processes. Another key limitation will be that current genetic ranges for root plate spread are not sufficient to achieve the ideotype target (See Chapter 3) and may compromise breeding for root lodging resistance. Thus, it will be required to find material with this dimension in more diverse germplasm.

The amount of time required to asses lodging traits will constrain the capacity of plant breeders to select for lodging resistance in wheat. Optimizing number of traits and plant sample size (See Chapter 5) together with automation of lodging devices for direct measurements in the field (Berry et al., 2003a; Niu et al., 2012b) can increase this selection capacity. Despite the reduction of time for lodging assessment conferred by these approaches, the use of molecular markers perhaps represents the most efficient long-term strategy to advance lodging resistance together with increases in yield.

Molecular markers in wheat are currently being implemented by modern breeding programmes where the use of genetic markers is replacing phenotypic selection to accelerate development and delivery of improved cultivars (Ribaut et al., 2010). However, to develop these genetic markers it is necessary to carry out phenotypic screening and genetic mapping of wheat genotypes segregating for the target trait in order to identify quantitative trait loci (QTLs) that can be further developed into reliable genetic markers. The genetic control of lodging-related traits, particularly, those associated with stem and anchorage strength has not been fully understood, in fact, investigations were mostly done on a limited range of germplasm and seasons and some key traits such as root plate spread were not measured in field-grown crops (Berry and Berry, 2015). These authors also identified some associations of lodging traits with grain yield that will need further investigation (e.g. with root plate spread and structural rooting depth). Identifying QTLs related to lodging-resistance traits represents a key target to accelerate selection of lodging resistance in wheat. Recently, QTLs linked to stem diameter (Hai et al 2005; Berry and Berry 2015), stem wall width (Berry and Berry, 2015; Hai et al., 2005), stem strength, stem material strength, root plate spread and root plate depth (Berry and Berry, 2015) have been identified. The gene TaCM, involved in the biosynthesis of lignin, was also associated with the stem strength and lodging index (Ma, 2009). The trade-off between stem strength and grain yield indicated above can be minimized if QTL linked to both yield and straw biomass (Berry et al., 2008; Li et al., 2014) can be exploited.

The aim of this chapter was to understand the genetic basis of the key lodging characters by identifying quantitative trait loci (QTLs) related to grain yield, plant height and dimensions of the stem and anchorage strength. This will help in the development of genetic markers that will enable breeders to select rapidly for these traits, for example, in marker-assisted selection (MAS) or whole genome selection, fine mapping and cloning, and eventually to explore genetic resources for new sources of lodging resistance.

6.3 Materials and Methods

6.3.1 Field experiments and plant material

Plant material included in this study consisted of 84 lines from the doubled-haploid (DH) mapping population derived from an F1 progeny of a cross Avalon x Cadenza. It was developed by Clare Ellerbrook, Liz Sayers and the late Tony Worland (John Innes Centre), as part of a Department of Environment, Food and Rural Affairs (DEFRA) funded project led by ADAS. The parents were originally chosen (to contrast for canopy architecture traits) by Steve Parker (CSL), Tony Worland and Darren Lovell (Rothamsted Research). Avalon is UK semi-dwarf winter wheat released in 1979 (National Association of British and Irish Millers (NABIM) group 1, bread making), and Cadenza a UK tall spring wheat released in 1991 (NABIM group 2, bread making potential but not suited to all grists). Avalon x Cadenza DH mapping population has been considered as the UK reference population under the UK DEFRA Wheat Genetic Improvement Network (WGIN). Field experiments were conducted during 2012-13 near ADAS High Mowthorpe, North Yorkshire (54.1°N, 0.5°W) during 2013-2014 at Sutton Bonington, University of Nottingham farm (52.8°N, 1.24°W) (henceforth referred as High Mowthorpe and Sutton Bonington, respectively). The soil types were both sandy loam. The DH lines were arranged in a resolvable incomplete block design (Alpha Design). The High Mowthorpe experiment consisted of each DH line replicated three times in plots measuring 6 x 2 m, each replicate contained 12 blocks and each block contained seven DH lines. At Sutton Bonington, each treatment was replicated three times in plots measuring 6 x 1.65 m, each replicate contained 8 blocks and each block contained 11 DH lines. The experiments were sown on 21 October in 2012 at High Mowthorpe and 8 October in 2013 at Sutton Bonington. The seed rate was standardized according to thousand grain weight and was 350 seeds m^{-2} for both sites. Nitrogen fertilizer was provided in both experiments in amounts of 190 kg ha^{-1} for High Mowthorpe and 180 kg ha^{-1} for Sutton Bonington as ammonium nitrate prill. Preventive control of disease, weed and pest was done when necessary to assure optimum conditions for crop growth and development. Herbicidies, fungicides and insecticides were applied as required to minimize effects of weeds and pathogens and pests.

6.3.2 Weather conditions

Long-term mean (LTM) and seasonal means (2012-13, High Mowthorpe; 2013-14, Sutton Bonington) for average temperature and rainfall were obtained from weather stations at or near to the experiment sites (historic data obtained from http://www.metoffice.gov.uk). Weather station used for High Mowthorpe experiment was located at ADAS High Mowthorpe (within 10 km from the field trial), this weather station spanned a 30-year period from 1962 to 2014; weather station located at Sutton Bonington (within 1 km from the field trial) spanned a 56-year period from 1959 to 2014. LTM and seasonal means were compared within each site and between sites. On average, the Sutton Bonington experiment occurred under warmer conditions with more precipitation, whereas the High Mowthorpe experiment occurred in cooler and drier conditions. The experiment at High Mowthorpe had colder conditions between autumn and spring (September – May) than average (LTM), specifically at the end of winter and beginning of spring, whereas summer was warmer. On the other hand, for the experiment at Sutton Bonington had warmer conditions than average, except for November in autumn and August in summer. Seasonal rainfall pattern was the weather parameter least consistent with the LTM in both sites. For High Mowthorpe seasonal rainfall was far below the average with a very dry autumn-winter period (November-February) and also with a dryer spring (March-April), except for May at the end of this period. For Sutton Bonington seasonal rainfall was slightly above the LTM. More rainfall occurred for January and February during winter and May at the end of spring than the LTM. However, there were dryer months such as November during autumn, December during winter, March and April during spring and June and July during summer (Fig. 6.1).

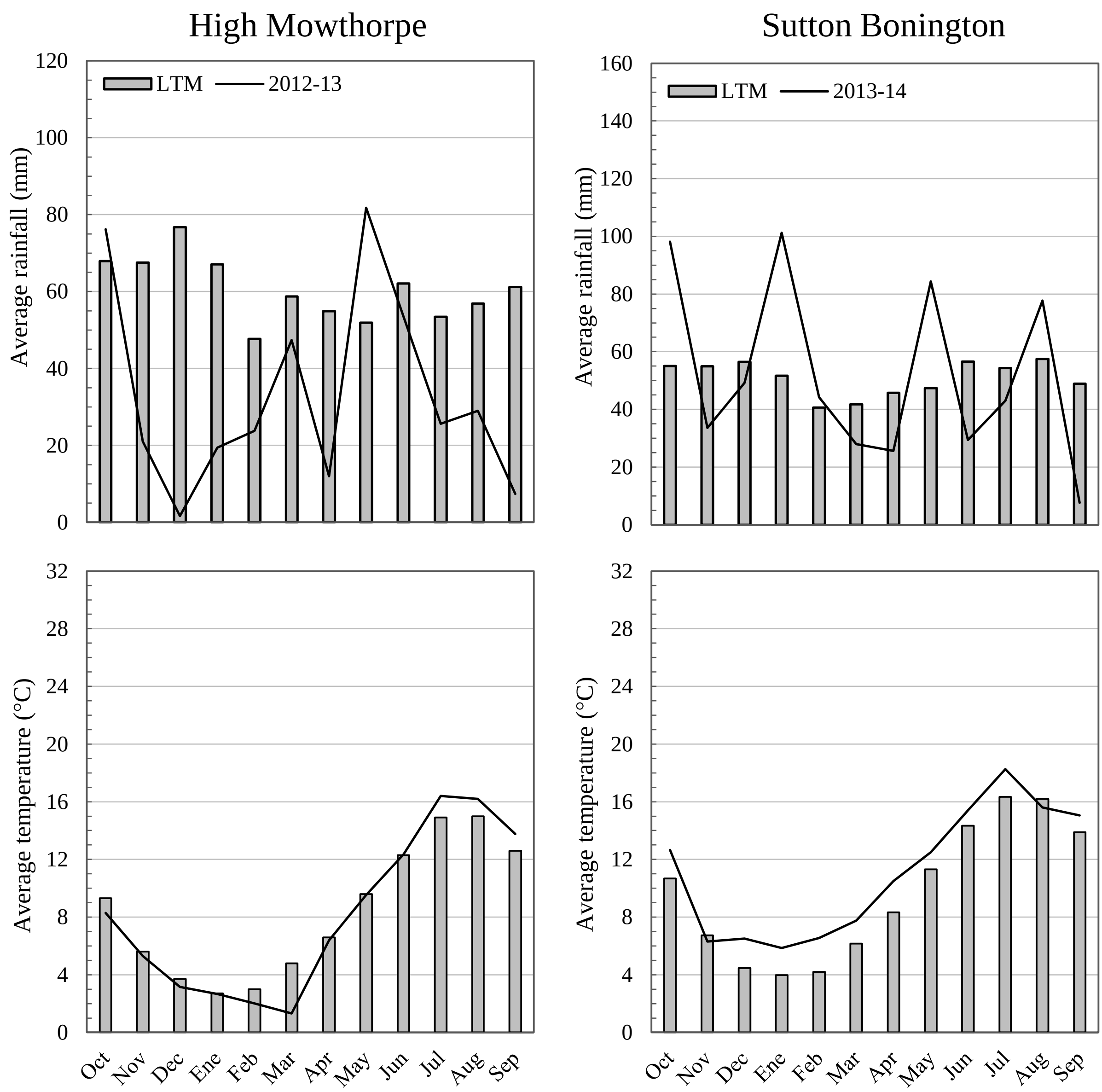

Fig. 6.1 Rainfall and average temperature for High Mowthorpe (2012-13) and Sutton Bonington (2013-14). Long term mean (LTM) for High Mowthorpe from 1971 – 200 and for Sutton Bonington from 1962 – 2014.

6.3.3 Measurements and Calculations

Measurements of the plant characters associated with lodging were made after flowering and during early grain filling (GS65 – GS79) (Zadoks et al., 1974). Seven plants were sampled randomly, avoiding any border effect of the plot, and excavated with a hand fork to recover roots to a depth of 100 mm (crown roots). Measurements associated with lodging resistance were done on each plant included the root plate spread (mm) (RPS) and structural rooting depth (mm) (SRD) of the plant root system. Fertile ear number per plant (ENPP) and height to ear tip (mm) (PH) from the main stem were also measured. Internode 1 in the main stem was identified, defined as the first internode of more than 10 mm, originating at or just below the ground surface and without crown roots emerging from its upper node. Subsequent internodes ascending the stem were numbered two, three, four etc., with the uppermost internode referred to as the peduncle. After identifying the bottom internode, the diameter (mm) (SD), length (mm) (SIL), wall width (mm) (SWW) and breaking strength (Newtons) (SIBS) of internode 2 were measured. The detailed methods for these measurements were described in Chapter 3, although, to reduce time, an optimization of these methods was implemented in Chapter 5.

Calculations included the stem failure moment (B_s) which is the force required to bend a stem (N mm) (referred in the rest of the thesis after its initial explanation in Chapter 3 as stem strength, SS), stem material strength (σ) (MPa) (SMS). The equations for these parameters were described in Chapter 2.

All plots were harvested with a combine harvester to measure grain yield (t ha^{-1}) (GY) in both High Mowthorpe and Sutton Bonington experiments and moisture content recorded on a grain subsample and yield adjusted to 100% DM.

6.3.4 Statistical analyses

Restricted maximum likelihood (REML) of a resolvable incomplete block design (Alpha Design) was performed to test for differences between the treatments for each parameter for both individual and combined experiment data, considering genotype as fixed effect while replicates, sub-blocks, environment and genotype x environment interaction (G×E) were the random effects. Adjusted means were calculated for each trait by experiment and by combining data from both experiments. Average standard error of difference (SED) between cultivar means was calculated for individual experiments. Broad sense heritability (H^2) was calculated from variance components (Eq. 6.1) from a mixed model performed for combined data from both experiments where genotype, replicates, sub-blocks, environment and genotype x environment interaction (G×E) were considered as random effects.

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{ge}^2/nlocs + \sigma_e^2/(nlocs\ x\ nreps)} \qquad (6.1)$$

Equation 6.1 components σ_g^2, σ_{ge}^2 and σ_e^2 were the genotype, genotype by interaction and error variance components, respectively, and nreps and nlocs number of replications and number of locations or environments, respectively. Phenotypic correlations (r_p) between traits were simple Pearson correlations. All the analyses mentioned were performed using SAS software version 9.

6.3.5 Linkage map and QTL analysis

The genetic linkage map of the full Avalon x Cadenza DH mapping population described before by Ma et al. (2015) and available at http:www.cerealsdb.uk.net consisted of 4,021 markers already grouped in 26 linkage groups. Each linkage group was further mapped separately in Joinmap v4.1 (Van Ooijen, 2011) eliminating any redundant markers or markers closer than 0.5 cM. A genetic linkage map with a total of 1,484 loci, consisting of 1,190 SNPs, 148 SSRs, 81 DArTs and 65 perfect markers, was obtained. This map covered a total of 4,686 cM with an average marker interval of 3.21 cM. The genetic linkage map together with phenotypic scores (adjusted means across experiments) for each trait were subsequently used for QTL identification by composite interval mapping (Zeng, 1994) function of windows QTL cartographer version 2.5 using default settings (S. Wang et al., 2012). Threshold value to declare a significant (P < 0.05) QTL was determined by 1000 permutations test (Churchill and Doerge, 1994). The LOD values obtained were in the range 3.3 – 3.5. The confidence interval for QTL position was projected in the respective genetic map segment were a LOD drop-off of one unit occurred (Lander and Botstein, 1989). Denomination of QTL found was composed by "q" (abbreviation of QTL), trait abbreviation and linkage group (multiple QTLs for the same trait in the same linkage group were differentiated by adding a number in ascendant order) (Yang et al., 2010). For Supplementary Table S5.1 experiment abbreviation (HM or SB) was added after QTL abbreviation to identify whether the QTL was identified for one or the other experiment.

6.4 Results

6.4.1 Genetic variation

Descriptive statistics for the traits for each experiment and variance components and broad sense heritability for cross-experiment analysis are given in Table 6.1. The Avalon x Cadenza DH population showed large and statistically significant genetic differences in all characters in each experiment. Genetic variance for cross-experiment analysis showed large statistically significant differences ($P < 0.001$). G×E interaction variance was also found for all traits across experiments ($P < 0.05 – 0.001$). Variance components (genetic, G×E and residual variances) across experiments were used to calculate broad sense heritability values which ranged from 0.64 to 0.95 for traits measured on the stem internode and related to stem strength (SS, SMS, SIL, SD and SWW). Heritability for RPS and SRD (anchorage strength related characters) was considerably lower with values of 0.15 and 0.21, respectively. ENPP (leverage characters) indicated a heritability of 0.30. These results indicate that the phenotypic variation found for RPS, SRD and ENPP was to a great extent due to the environment, but environmental effects were less important for the rest of the target traits. Heritability for GY was 0.46. In summary, for all lodging target traits, apart from root traits, a significant percentage of phenotypic variance was accounted for by genetic effects.

Table 6.1. Descriptive statistics for lodging traits (SS, stem strength; SMS, stem material strength; SIL, stem internode length; SD, stem internode diameter; SWW, stem internode wall width; RPS, root plate spread; SRD, structural rooting depth; PH, plant height; ENPP, ear number per plant) and grain yield (GY) for the Avalon x Cadenza DH population (84 lines) for High Mowthorpe and Sutton Bonington and variance components for combined analysis across experiments.

Character	High Mowthorpe			Sutton Bonington			Variance components			H^2
	Mean	Range	SED	Mean	Range	SED	σ_g^2	σ_{ge}^2	σ_e^2	
SS (N mm)	254	165 – 354	21.4	173	131 – 248	22.1	535	392	661	0.64
SMS (MPa)	45.8	22.5 – 66.8	4.12	33.8	18.9 – 54.8	4.64	61.6	15.4	27.2	0.83
SIL (mm)	84.9	65.7 – 109	5.39	66.3	49.1 – 88.7	4.07	43.4	12.6	31.8	0.79
SD (mm)	4.02	3.51 – 5.07	0.13	3.94	3.34 – 4.72	0.12	0.096	0.0029	0.022	0.95
SWW (mm)	0.94	0.66 – 1.30	0.068	0.86	0.60 – 1.26	0.060	0.031	0.0012	0.0059	0.90
RPS (mm)	44.5	38.7 – 50.3	2.66	43.3	35.4 – 52.9	2.66	0.57	3.62	9.26	0.15
SRD (mm)	36.8	32.9 – 41.4	1.95	42.3	38.7 – 47.1	1.98	0.40	1.30	5.17	0.21
PH (cm)	85.0	64.6 – 111	2.74	76.3	57.9 – 104	2.01	127	12.2	7.73	0.95
ENPP	2.62	2.08 – 3.89	0.27	2.87	1.98 – 3.58	0.39	0.019	0.039	0.16	0.30
GY (t ha^{-1})	6.61	5.40 – 7.82	0.30	8.16	6.60 – 9.89	0.71	0.11	0.12	0.43	0.46

SED, standard error of differences (P < 0.001, except for SRD at Sutton Bonington, P < 0.01); σ_g^2, genetic variance (P < 0.001); σ_{ge}^2, genetic x environment variance (P < 0.01 – 0.001, except for SD, P < 0.05); σ_e^2, residual variance; H^2, broad sense heritability

6.4.2 Association among traits

Pearson correlation coefficients (r) given in Table 6.2 were used to describe association among traits for cross-experiment means. SS was positively correlated with SWW (r = 0.78, P < 0.001) and SMS (r = 0.65, P < 0.001) and negatively correlated with SD (r = -0.32, P < 0.01). Associations between stem strength traits (SMS, SIL, SD and SWW) were also found. For instance, SMS and SWW were positively associated (r = 0.67, P < 0.001) while negative correlations were identified between SD and SWW (r = -0.53, P < 0.001) and SD and SMS (r = -0.90, P < 0.001). Correlation between RPS and SRD (anchorage strength traits) indicated a strong positive association (r = 0.67, P < 0.001). A trend between RPS and SRD with ENPP was also found (r = 0.20, P = 0.07). PH was highly correlated positively with SIL (r = 0.72, P < 0.001) while a negative association was found when compared against SWW (r = -0.31, P < 0.01). There were also positive correlations between GY and SIL (r = 0.45, P < 0.001) and PH (r = 0.61, P < 0.001) and a negative correlation between GY and SWW (r = -0.24, P < 0.05). Correlation analysis was also done using cultivar means by experiment and showed consistent correlations with the analysis above (Table S6.1).

Table 6.2 Phenotypic correlation coefficients between cross-experiment means of lodging traits (SS, stem strength; SMS, stem material strength; SIL, stem internode length; SD, stem internode diameter; SWW, stem internode wall width; RPS, root plate spread; SRD, structural rooting depth; PH, plant height; ENPP, ear number per plant) and grain yield of the Avalon x Cadenza DH population (84 lines) grown at High Mowthorpe and Sutton Bonington.

Character	SS	SMS	SIL	SD	SWW	RPS	SRD	PH	ENPP	GY
SS	1.00									
SMS	0.65***	1.00								
SIL	-0.01	0.01	1.00							
SD	-0.32**	-0.90***	0.00	1.00						
SWW	0.78***	0.67***	-0.21	-0.53***	1.00					
RPS	-0.02	-0.14	-0.05	0.11	-0.02	1.00				
SRD	-0.02	-0.21	0.01	0.25*	-0.18	0.67***	1.00			
PH	-0.06	0.18	0.72***	-0.17	-0.31**	0.03	-0.04	1.00		
ENPP	-0.18	0.01	0.06	-0.13	0.03	0.20	0.20	-0.05	1.00	
GY	-0.18	0.03	0.45***	-0.12	-0.24*	0.07	0.03	0.61***	0.04	1.00

*, P < 0.05; **, P < 0.01; ***, P < 0.001

6.4.3 QTL analysis

A total of 25 significant QTLs were identified for the Avalon x Cadenza DH population for the lodging-related traits and grain yield across the two years of experiments. These QTLs were detected by composite interval mapping using adjusted cultivar means across the two experiments (HM-SB). A summary of the location, significance and effects for these QTLs is given in Table 6.3. For stem strength characters (SS, SMS, SIL, SD and SWW) 15 QTLs were identified and were clustered on chromosomes 1A, 1D, 2D, 3A, 3B, 4B and 4D. Only two QTLs for anchorage strength traits (RPS and SRD) were found on chromosomes 5B and 6B. For leverage characters (PH and ENPP) seven QTLs were identified on chromosomes 1A, 2D, 3A, 3B, 4A and 4D. Only one QTL was detected for GY in linkage group 2D. All significant QTLs detected by composite interval mapping using adjusted means of each individual experiment (HM and SB) are presented in Table 6.4. The High Mowthorpe experiment showed 30 QTLs distributed between chromosomes 1A, 2B, 2D, 3A, 3B, 4A, 4B, 4D, 5B, 5D, 6A, 7B and 7D and Sutton Bonington experiment showed 22 QTLs distributed between chromosomes 2D, 3A, 3B, 4D, 5B, 6B and 7A. Major findings will be described in the following text and these included mainly the major QTLs identified for key lodging traits with consistency across experiments.

Proximal QTLs identified on chromosome 3B for combined means across experiments were found for SS (PVE = 42 %), SD (PVE = 30 %), SWW (PVE = 82 %) and SMS (PVE = 49 %). Consistency across experiments was indicated by the QTLs located on the same region of chromosome 3B for SS (HM, PVE = 53 %; SB, PVE = 12 %), SD (HM, PVE = 33 %; SB, PVE = 27 %), SWW (HM, PVE = 83 %; SB, PVE = 68 %) and SMS (HM, PVE = 54 %;

SB, PVE = 29 %) on each experiment. Except for QTLs for SD, all the positive additive effects came from the Cadenza allele. A QTL with minor effects on PH (PVE = 5.9 %) co-located with all the QTLs mentioned above (Figure 6.2) (cross-experiment mean). This QTL was only identified for SB experiment (PVE = 6.8 %) and not for HM experiment perhaps due to G×E interaction. Increasing alleles for these QTLs came from the parent Avalon.

Three co-located QTLs were identified on chromosome 2D for SD (PVE = 24 %), SMS (PVE = 13 %) and GY (PVE = 28 %). Proximal to these QTLs a QTL with effects on PH (PVE = 22 %) was also found. Consistency across experiments was found only for QTLs affecting SD (HM, PVE = 24 %; SB, PVE = 22 %) and PH (HM, PVE = 22 %; SB, PVE = 18 %). The QTLs for SMS and GY were not identified for SB and HM experiments, respectively. However, QTLs on SMS for HM (PVE = 9.5 %) and on GY for SB (PVE = 13 %) were consistent with QTLs found for combined means. In all cases, except for SD, the increasing alleles came from the parent Cadenza.

Effects on PH and SIL were found in one and two proximal QTLs, respectively, located in the region delimited between 134 and 166 cM of chromosomes 3A. Two of these QTLs co-located for PH (*qPH3A*) and SIL (*qSIL3A-1*) explaining about 16 and 45 % of the phenotypic variation. These QTLs on PH (HM, PVE = 11 %; SB, PVE = 24 %) and SIL (HM, PVE = 33 %; SB, PVE = 43 %) were consistently detected for individual analysis of each experiment. In all cases the increasing alleles came from the parent Cadenza.

Chromosome 4D showed two significant QTLs for SWW and one for PH. Figure 1 showed co-located loci *qSWW4D-1* (PVE = 3.3 %) and *qPH4D* (PVE = 23 %) for SWW and PH. QTLs for PH (HM, PVE = 30 %; SB, PVE = 13 %)

were also identified at the same position on chromosome 4D for individual analysis by experiment. QTLs for SWW were inconsistent and were only identified for SB (PVE = 4.6 %). Increasing effects came from the Cadenza allele for PH and from Avalon allele for SWW.

Major QTLs for RPS were detected on chromosome 5B (PVE = 33 %). A QTL with lower effect was found for SRD on chromosome 6B (PVE = 12 %). QTLs for RPS were consistent across experiments for this region of the 5B chromosome (HM, PVE = 25 %; SB, PVE = 28 %). The QTL for SRD was not consistent and was only found for SB experiment (PVE = 15 %). Increasing alleles for these QTLs were conferred by the parent Cadenza.

Regions on chromosomes 3B and 5B have indicated the presence of major QTLs affecting key stem strength and anchorage traits. Despite the G×E interaction identified for these traits, these QTLs were not affected and were identified whether the QTL analysis was done by using combined means or individual experiment means. These findings are promising and further validation/fine mapping will be required.

Table 6.3. Summary of the QTL detected for cross-experiment means

Trait	Chr	QTL	Position (cM)	CI (cM)	LOD	PVE (%)	Additive effect[a]	Peak marker
SS	1D	*qSS1D*	37.1	33.8 – 38.5	3.33	6.39	-7.80	*cfd19*
	3B	*qSS3B*	284	282 – 285	15.6	42.3	-19.5	*wPt-4412*
SMS	2D	*qSMS2D*	55.2	48.7 – 56.0	7.44	12.8	-3.25	*BS00022730*
	3B	*qSMS3B*	282	282 – 284	19.2	48.7	-6.05	*BS00003884*
SIL	1A	*qSIL1A*	139	137 – 146	4.43	9.93	-2.40	*BS00023118*
	3A	*qSIL3A-1*	152	148 – 152	14.3	45.4	-5.15	*BS00022691*
	3A	*qSIL3A-2*	162	161 – 166	14.5	43.5	-5.05	*bArc19*
SD	2D	*qSD2D*	55.2	49.3 – 56.7	11.7	23.8	0.16	*BS00022730*
	3B	*qSD3B*	285	283 – 287	13.6	30.4	0.18	*cos4Gb*
	4B	*qSD4B-1*	51.6	50.0 – 52.9	4.02	6.88	0.086	*BS000022972*
	4B	*qSD4B-2*	57.9	54.3 – 60.3	4.56	7.45	0.090	*BS00009272*
SWW	3B	*qSWW3B*	279	277 – 280	34.5	81.7	-0.17	*BS00022401*
	4B	*qSWW4B*	156	147 – 161	4.38	3.82	0.032	*BS00022847*
	4D	*qSWW4D-1*	72.2	63.2 – 76.8	3.81	3.26	0.033	*BS00021924*
	4D	*qSWW4D-2*	83.4	72.4 – 103	3.76	3.22	0.032	*BS00064001*
RPS	5B	*qRPS5B*	180	180 – 186	10.1	32.6	-1.20	*BS00023078*
SRD	6B	*qSRD6B*	153	151 – 162	3.52	12.0	-0.50	*wPt-4858*
PH	2D	*qPH2D*	35.4	28.1 – 46.9	10.2	21.9	-5.55	*cos2Q*
	3A	*qPH3A*	145	143 – 153	8.38	16.1	-4.70	*wPt-9215*
	3B	*qPH3B*	282	271 – 285	3.57	5.89	2.87	*BS00088726*
	4D	*qPH4D*	63.3	59.3 – 67.3	10.6	23.3	-5.65	*RhtMrkD1*
ENPP	1A	*qENPP1A*	11.2	108 – 121	5.50	17.5	0.11	*BS00012226*
	4A	*qENPP4A*	48.1	45.3 – 53.0	6.93	23.1	-0.13	*BS00010339*
	4D	*qENPP4D*	30	18.9 – 47.6	3.74	9.93	0.1	*BS00054978B*
GY	2D	*qGY2D*	55.2	47.8 – 74.1	8.41	27.6	-0.27	*BS00022730*

Chr, chromosome or linkage group; CI, confidence interval (LOD ±1); LOD, logarithm of odds; PVE, phenotypic variation explained; [a], additive effect with negative sign indicates that the positive alleles come from parent Cadenza, while a positive sign indicates that positive alleles come from Avalon; SS, stem strength; SMS, stem material strength; SIL, stem internode length; SD, stem internode diameter; SWW, stem internode wall width; RPS, root plate spread; SRD, structural rooting depth; PH, plant height; ENPP, ear number per plant; GY, grain yield

Table 6.4. List of all QTL identified by experiment

Trait	Chr	Exp.	QTL	Position (cM)	CI (cM)	LOD	PVE (%)	Additive effect	Peak marker
SS	3B	HM	*qHMSS3B*	284	282 – 285	20.0	53.4	-30.2	*wPt-4412*
	3B	SB	*qSBSS3B*	273	272 – 277	4.28	12.4	-10.0	*BS000062859*
	4D	HM	*qHMSS4D*	23.0	16.4 – 40.8	6.15	12.1	-14.9	*BS00022283*
	4D	SB	*qSBSS4D*	127	120 – 135	4.08	13.1	10.7	*BS00023934*
	6A	HM	*qHMSS6A*	156	155 – 162	5.15	9.02	12.4	*BS00021999*
SMS	2D	HM	*qHMSMS2D*	55.2	49.4 – 56.7	7.10	9.54	-3.52	*BS00022730*
	3B	HM	*qHMSMS3B*	284	282 – 285	23.9	53.8	-7.97	*wPt-4412*
	3B	SB	*qSBSMS3B*	282	279 – 285	9.42	28.9	-4.14	*BS00073411*
	7A	SB	*qSBSMS7A*	227	216 – 237	3.90	16.1	3.08	*bArc49A*
SIL	1A	HM	*qHMSIL1A*	141	137 – 148	4.08	10.1	-2.99	*Tagluten*
	3A	HM	*qHMSIL3A*	173	169 – 176	10.5	32.5	-5.29	*BS00022148*
	3A	SB	*qSBSIL3A*	162	162 – 164	15.2	42.7	-4.93	*bArc19*
	4D	HM	*qHMSIL4D*	60.3	58.9 – 68.2	3.75	9.68	-2.86	*BS99999977*
	5D	HM	*qHMSIL5D*	35.4	30.8 – 38.9	4.25	10.5	2.98	*qwm159*
SD	2D	HM	*qHMID2D*	55.2	49.9 – 56.9	11.0	23.8	0.17	*BS00022730*
	2D	SB	*qSBSD2D*	55.2	49.6 – 114	10.2	21.7	0.15	*BS00022730*
	3B	HM	*qHMSD3B*	282	282 – 285	13.7	32.9	0.20	*BS00003884*
	3B	SB	*qSBSD3B*	282	279 – 285	11.9	27.3	0.17	*BS00003884*
	4B	HM	*qHMSD4B-1*	56.9	56.5 – 58.9	3.51	6.10	0.087	*BS00004407*
	4B	HM	*qHMSD4B-2*	63.2	61.0 – 67.4	3.49	6.25	0.088	*BS000049964*
	4B	SB	*qSBSD4B*	64.9	62.4 – 70.3	3.45	5.93	0.079	*BS000087301*
SWW	3B	HM	*qHMSWW3B*	279	277 – 281	34.1	82.8	-0.18	*BS00022401*
	3B	SB	*qSBSWW3B*	284	282 – 285	26.0	68.3	-0.16	*wPt-4412*
	4D	SB	*qSBSWW4D*	72.2	63.6 – 76.8	3.96	4.64	0.040	*BS00021924*
RPS	2D	SB	*qSBRPS2D*	144	143 – 145	3.35	9.24	-0.94	*BS00014647*
	4A	HM	*qHMRPS4A*	80.2	75.1 – 89.6	5.15	17.5	1.07	*wmc258*
	5B	HM	*qHMRPS5B-1*	171	170 – 175	3.61	11.4	-0.86	*BS00014954*
	5B	HM	*qHMRPS5B-2*	184	179 – 187	4.45	13.7	-0.94	*BS00022763*
	5B	SB	*qSBRPS5B-1*	197	195 – 200	5.76	17.8	-5.86	*Ex42-7B*
	5B	SB	*qSBRPS5B-2*	208	205 – 210	3.63	10.1	4.30	*BS00031991*
SRD	2D	SB	*qSBSRD2D*	143	142 – 144	4.84	14.2	-0.73	*BS000162725*
	3A	HM	*qHMSRD3A*	36.9	32.3 – 38.5	3.70	13.4	0.71	*BS00030652*
	4D	SB	*qSBSRD4D*	26.0	14.7 – 39.8	4.19	13.4	0.70	*BS00054978B*
	6B	SB	*qSBSRD6B*	177	172 – 186	4.67	14.8	-0.73	*wPt-4930*
PH	2D	HM	*qHMPH2D*	51.4	47.8 – 53.4	10.7	25.8	-6.39	*gwm132*
	2D	SB	*qSBPH2D*	37.2	27.8 – 46.9	9.58	17.5	-4.88	*gwm261*
	3A	HM	*qHMPH3A*	148	145 – 153	5.90	11.4	-4.23	*BS00022691*
	3A	SB	*qSBPH3A*	145	143 – 156	12.0	23.6	-5.58	*wPt-9215*
	3B	SB	*qSBPH3B*	284	273 – 285	4.34	6.81	3.02	*wPt-4412*
	4A	HM	*qHMPH4A*	94.9	91.8 – 97.0	3.77	5.57	3.00	*BS00088726*
	4D	HM	*qHMPH4D*	63.3	59.6 – 67.2	12.2	29.8	-6.89	*RhtMrkD1*
	4D	SB	*qSBPH4D*	63.3	57.6 – 68.9	7.45	13.3	-4.19	*RhtMrkD1*
	7B	HM	*qHMPH7B*	98.0	91.6 – 104	3.46	6.16	3.17	*BS00003649*
ENPP	5B	HM	*qHMENPP5B*	73.6	71.8 – 76.7	5.52	20.5	-0.24	*BS00003592*
	7D	HM	*qHMENPP7D*	134	119 – 138	3.60	12.1	-0.11	*wPt-4555*
GY	1A	HM	*qHMGY1A-1*	23.9	22.8 – 26.4	4.76	13.0	-0.19	*BS00070580*
	1A	HM	*qHMGY1A-2*	33.6	30.7 – 35.2	3.49	10.2	-0.17	*BS00021758*
	2B	HM	*qHMGY2B-1*	155	153 – 158	4.04	10.9	-0.17	*wPt-3132*
	2B	HM	*qHMGY2B-2*	166	160 – 167	3.36	9.22	-0.16	*BS00074390*
	2D	SB	*qSBGY2D*	55.2	44.5 – 56.9	4.36	13.3	-0.26	*BS00022730*
	4A	HM	*qHMGY4A*	96.6	94.3 – 103	5.94	16.8	0.21	*BS00022837*
	4D	SB	*qSBGY4D*	54.3	43.1 – 60.1	5.07	17.5	-0.31	*BS99999977*

Chr, chromosome or linkage group; CI, confidence interval (LOD ±1); LOD, logarithm of odds; PVE, phenotypic variation explained; [a], additive effect with negative sign indicates that the positive alleles come from parent Cadenza, while a positive sign indicates that positive alleles come from Avalon; SS, stem strength; SMS, stem material strength; SIL, stem internode length; SD, stem internode diameter; SWW, stem internode wall width; RPS, root plate spread; SRD, structural rooting depth; PH, plant height; ENPP, ear number per plant; GY, grain yield

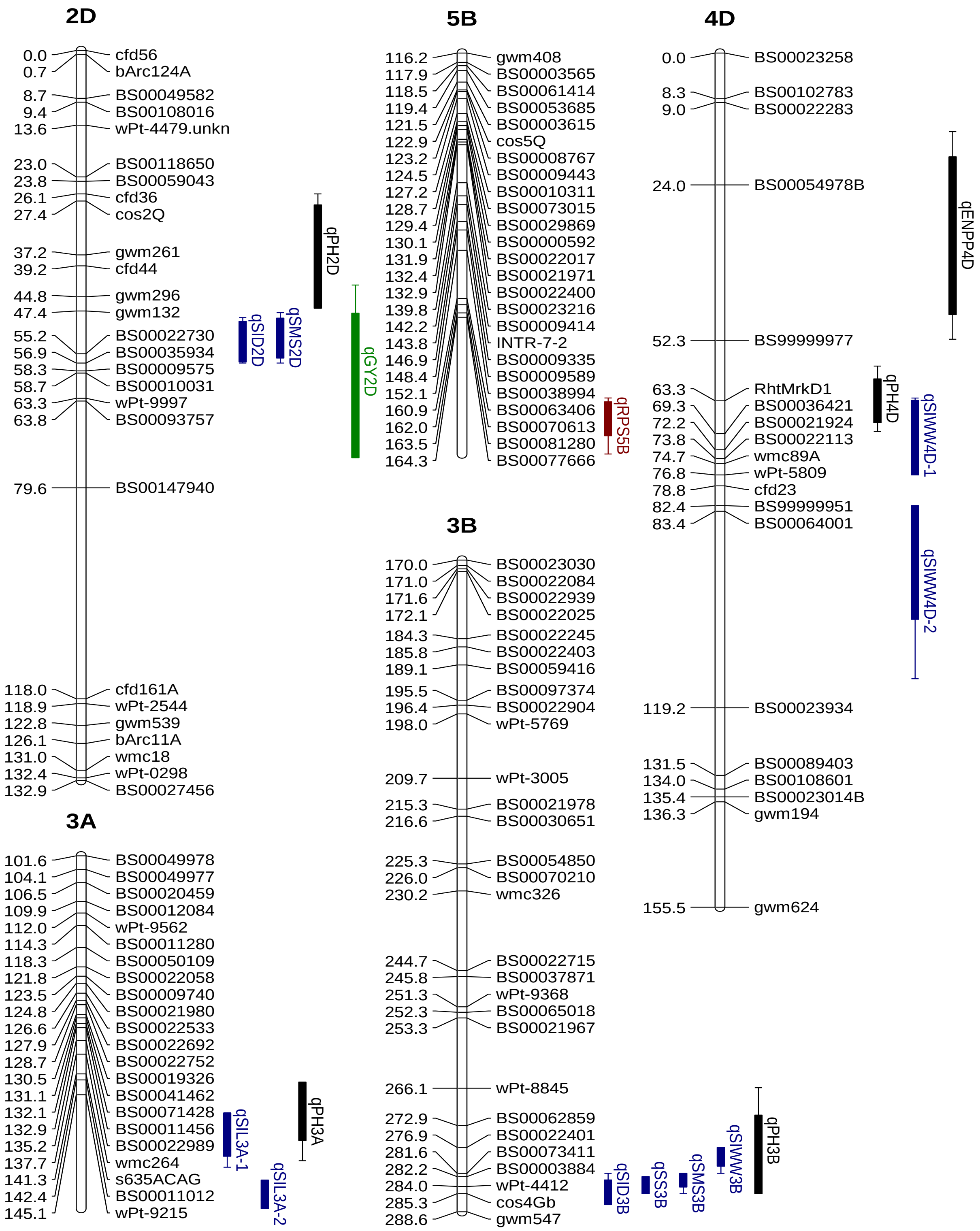

Fig. 6.2 Relevant QTLs identified for stem strength characters (blue), root plate spread (carmine), leverage characters (black) and grain yield (green). Data are adjusted cross-experiment means for 84 lines of the Avalon x Cadenza doubled haploid population growing at High Mowthorpe 2013 and Sutton Bonington 2014.

6.5 Discussion

Standard current methods for assessing lodging in wheat described in the literature (Berry et al., 2003c, 2000) require a great amount of time because of the necessity of sampling and measuring stems and roots (Berry et al., 2003a). These absence of rapid screening methodologies to asses lodging resistance demonstrates the necessity to explore and develop more reliable methods that can confer enough selection capacity to breeding programs to improve lodging resistance in wheat. This study attempts to better understand the genetic control of key lodging traits in order to assess the possibility of developing reliable genetic markers for these traits. In order to achieve this goal, key lodging traits have been targeted to develop a phenotypic database for the Avalon x Cadenza doubled-haploid population on a two-year experiment in the UK. Together with this database, genetic information of public domain (http:www.cerealsdb.uk.net) have been used to implement QTL mapping tools that enabled the identification of a set of QTLs with important genetic effects on stem strength characters (SS, SIL, SD and SWW), anchorage strength characters (RPS and SRD), leverage characters (PH and ENPP) and grain yield (GY).

Large genetic differences were detected for all the lodging traits and GY in this study (Table 5.1). This wide genetic range has been described before for winter (Berry and Berry, 2015; Berry et al., 2007, 2003b) and spring wheat (See Chapter 3) for the lodging traits used in this chapter. Significant G×E interactions have been described by Berry and Berry (2015) and in this study in Chapter 3 which can reduce heritability across environments. Heritability values for lodging traits in the range of 0.73 – 0.98 have been calculated for winter wheat (Berry et al., 2007) and in the range of 0.11 –

0.96 for spring wheat (See Chapter 3). Lower values have been also found for winter wheat ranging 0.17 – 0.90 (Berry and Berry, 2015). Heritability values found in this study for the Avalon x Cadenza population ranged 0.15 – 0.95. Anchorage strength characters showed lowest heritability values in most of these studies. Interestingly, averaging the heritability values across all the aforementioned studies gives mean heritability values of above 0.60 for stem strength and 0.30 for root plate spread. These two traits have the greatest effects on strengthening the stem base and anchorage system and it's clear that an important percentage of the phenotypic variation is due to genetic effects. Reduced plant height has been described as having great effect on decreasing the plant leverage (decreased lodging risk) (Berry et al., 2004) and as a highly heritable trait (in these studies values above 0.90 were always described). Thus, focusing on SS, RPS and PH can be an important strategy to improve lodging resistance. However, PH has to be optimised carefully because high yields might not be compatible with reduction of plant height further than 0.7 m (Allan, 1986; Balyan and Singh, 1994; Berry et al., 2014; Flintham et al., 1997; Kertesz et al., 1991; D J Miralles and Slafer, 1995; Richards, 1992). Additionally, Flintham et al. (1997) indicated that extreme dwarfism can reduce overall biomass which counteracts improvements in grain yield thorough increased biomass (Reynolds et al., 2011).

Inter-relationships among lodging traits have been described to not represent a significant barrier to combine lodging proof dimensions in a single wheat genotype (Berry et al. 2003b; Berry et al. 2007; Berry and Berry 2015; See Chapter 6 too). Nonetheless, for the Avalon x Cadenza population negative correlations between SD and each of SMS and SWW were identified. A previous study has stated that the ideal strategy to increase stem strength in winter wheat would be to increase SD and SMS (maintaining a minimum SWW) in order to improve overall SS while investing the minimum amount of biomass (Berry et al., 2007). However, for spring wheat a very strong linkage between SS and SWW has been found that will be difficult to break, which may infer that more than one option to combine trait dimensions has to be employed to strengthen the stem base (see Chapter 3). This strong linkage between SS and SWW has also been found for the Avalon x Cadenza doubled-haploid population.

Coincidence of QTLs affecting SS, SMS, SD and SWW distributed in the interval 272 – 285 cM of chromosome 3B (Table 6.3 and 6.4) may indicate this is a region with pleiotropic effects on the overall SS and its components or a region with genes tightly linked that individually are controlling each trait. Also it should be highlighted an important phenotypic variation explained by this QTL region for stem strength traits (e.g. overall stem strength) indicates that solely this area of chromosome 3B can substantially increase lodging resistance. This pleiotropic effect or tight genetic linkage identified for this 3B region might also include effects on PH (*qPH3B*). Interestingly, the positive alleles came from parent Cadenza for SS, SMS and SWW and from parent Avalon for SD and PH. These results are consistent with the negative correlations identified between SD with SMS and SWW and between PH and SWW. An example of how this genomic region can increase the overall stem strength is using the loci *qSS3B* which has a mean QTL effect of 48 N mm (size of the mean QTL effect equivalent to twice the additive effect). Designed ideotypes for winter wheat (grain yield of 8 t ha^{-1} under 18 m s^{-1} wind gust) (Berry et al., 2007) and spring wheat (6 t ha^{-1} under 22 m s^{-1} wind gust) (See Chapter 2) with a height of 0.7 m require 250 and 268 N mm of stem strength. If grain yield is increased to 10 t ha^{-1} in winter wheat and 8 t ha^{-1} in spring wheat the stem strength requirement will be 302 and 298 for spring wheat. This will be close to shortening the plant to 0.5 m in both winter (190 N mm SS requirement) and spring wheat (190 N mm SS requirement). This analysis puts the large benefit of a QTL that increases stem strength by 48 N mm into context.

It has been discussed that increasing stem strength with the minimum investment in biomass should be done by increasing SMS or SD rather than SWW. Thus, implications to find the optimum strategy to increase the overall SS by targeting chromosome 3B will be complicated with the allelic opposite direction conferred for SS, SMS, SWW (Cadenza allele) and SD (Avalon allele). However, accepting additional investment of biomass to improve SS and possible trade-offs with GY and yield formation processes may be a more sensible breeding target. Nonetheless, selecting the Cadenza allele at 3B (*qSS3B, qSMS3B and qSWW3B*) and Avalon allele at 2D (*qSD2D*) could be a criterion for designing a target genotype with stronger stems that can counteract these trade-offs. The region on chromosome 3B offers also the advantage of a decreasing effect on PH which in turn can reduce lodging risk (Berry et al. 2015) and stem biomass (Chapter 2; Berry et al. 2007). Polygenic control of PH identified for the Avalon x Cadenza population is in agreement with previous studies (Griffiths et al., 2012; Ma et al., 2015). This can help to optimise PH independently of target stem characters. A major finding of this study has been also the identification of QTLs affecting RPS in the linkage group 5B. RPS has been described as the target anchorage strength trait to increase root lodging resistance (Berry et al. 2007).

In the last decades, QTLs with effects on SS, SD, SWW, SMS, RPS and SRD inferred that increased lodging resistance in wheat can be accelerated by the implementation of molecular markers. In fact, QTLs affecting straw strength, SWW and SD can be found on chromosomes 6A and 3B (Al-Qaudhy et al., 1988) and higher expression of gene *TaCM* has already been linked to a lodging resistant wheat variety (Ma, 2009). A summary of the most recent studies and linkage groups or chromosomes where QTL regions affecting lodging characters is presented in Table 5.4. Coincidences between chromosomes with QTLs affecting the lodging-related traits assessed in the present study and the studies below can be identified. For instance, QTLs with effects on SS and SD were found on the chromosome 3B for Avalon x Cadenza in the UK and can be comparable with the QTLs affecting SS and SD identified for winter wheat CA9613 x H1488 DH population located on the same chromosome under Northeast China growing conditions (Hai, 2006; Hai et al., 2005). Microsatellite marker *gwm547* of chromosome 3BL has been related to stem solidness (SWW) (Cook et al., 2004). This was similar to the QTL located in chromosome 3B of Avalon x Cadenza which was very close to marker *gwm547*.

QTLs affecting RPS were identified on chromosome 5B for Avalon x Cadenza and Solstice x Xi19 DH populations in the UK (Berry and Berry, 2015; Berry et al., 2008). All chromosomes with QTLs affecting PH identified for Avalon x Cadenza in this study can be matched with the chromosomes for PH meta-QTLs from four doubled-haploid populations reported by Griffiths et al. (2012). It is also relevant that for QTLs found in the QTL analysis for the HM experiment means (Table S6.2), a QTL on chromosome 6A affecting SS reported by Berry and Berry (2015) (see also Berry et al. 2008) for Savannah x Rialto and Solstice x Xi19 DH populations is comparable with a QTL affecting SS for the Avalon x Cadenza DH population. Additionally, a single QTL analysis on SB experiment means detected a QTL affecting SRD on chromosome 2D. Verma et al. (2004) also reported a comparable QTL affecting SRD located on the same chromosome.

In conclusion, this chapter has described the genetic variation and associations within the lodging-related characters targeted for an improved lodging resistance in wheat. Broad genetic ranges were identified for the Avalon x Cadenza doubled-haploid population, although G×E interactions were also found. Stem strength traits were highly heritable while root or anchorage strength traits had low heritability. Inter-relationships between traits have implications for achieving a lodging-resistant ideotype with the minimum investment of biomass. This was inferred from the negative correlation between SD with SS, SMS and SWW and the strong positive correlation between SS and SWW. However, possible strategies have emerged from the QTL mapping of this doubled-haploid population to counteract these implications. In general, QTLs for SS on chromosome 3B and SD on chromosome 2D could help to find a stem lodging-resistant ideotype with an optimum investment of biomass and QTLs for RPS on chromosome 5B similarly could help to find an increased root lodging resistant ideotype. More importantly, these QTLs on chromosomes 3B and 5B have been shown to be stable across environments which potentially make them more reliable for breeding purposes.

Table 6.5 Review of recent studies with identified loci regions affecting lodging-related traits in wheat. Chromosome or linkage group where the QTL is located is given.

Source	Env.	Map type	Map length	Doubled haploid	SS	SMS	SIL	SD	SWW	RPS	SRD	PH
Present study	UK	SNP, SSR, DArT	4,686	Avalon x Cadenza	1D, 3B	2D, 3B	1A, 3A	2D, 3B, 4B	3B, 4B, 4D	5B	6B	2D, 3A, 3B,4D
Berry and Berry (2015)	UK	SSR, DArT	2,466	Savannah x Rialto	6A	3A	–	3A, 4D	3A, 7D	7D	7A, 7D	1D, 2A, 3A, 6A, 7D
Berry and Berry (2015)	UK	SSR, DArT	2,503	Solstice x Xi19	1A, 6A	3A	–	4D	3A, 6A, 6B	5B	–	3A, 4A, 6A
Verma et al. (2004)	UK	SSR	2,959	Milan x Catbird	2D*	–	4B	–	1B	–	2D	4B, 4D
Hai et al. (2005)	China	SSR	2,308	CA9613 x H1488	3A, 3B	–	–	3B	2D	–	–	–
Atkinson et al. (2015)	Controlled (phytotron)	SNP	–	Savannah x Rialto	–	–	–	–	–	6D	6D, 7D	–
Griffiths et al. (2012)†	UK	SSR, DArT	–	Charger x Bagder, Spark x Rialto, Savannah x Rialto, Avalon x Cadenza	–	–	–	–	–	–	–	1A, 1B, 1D, 2A, 2B, 2D. 3A, 3B, 4B, 4D, 5A, 5B, 6A, 6B, 6D

AvCa, Avalon x Cadenza; RiSa, Savannah x Rialto; SoXi, Solstice x Xi19; CAH1, CA9613 x H1488; ChBa, charger x Badger; SpRi, Spark x Rialto; *Breaking strength; †indicates meta-qtls from four populations

Based on this study and previous investigations with plant materials with different genetic backgrounds it appears that regions of chromosomes 3B and 6A have major effects on stem strength (Al-Qaudhy et al., 1988; Berry and Berry, 2015; Hai et al., 2005). Future prospects will include the validation and/or fine mapping of QTLs (Collard and Mackill, 2008) on chromosomes 3B (SS, SMS and SWW), 2D (SD) and 5B (RPS) affecting key lodging resistant traits of the Avalon x Cadenza population in order to find reliable validated markers that could be used in MAS for root and stem lodging resistance.

6.6 References

Aisawi, K.A.B., Reynolds, M.P., Singh, R.P., Foulkes, M.J., 2015. The physiological basis of the genetic progress in yield potential of CIMMYT spring wheat cultivars from 1966 to 2009. Crop Sci. doi:10.2135/cropsci2014.09.0601

Allan, R.E., 1986. Agronomic comparisons among wheat lines nearly isogenic for three reduced-height genes. Crop Sci. 26, 707–710.

Al-Qaudhy, W., Morris, R., Mumm, R.F., Hanna, M.A., 1988. Chromosomal locations of genes for traits with lodging in winter wheat. Crop Sci. 28, 631–635.

Atkinson, J. a., Wingen, L.U., Griffiths, M., Pound, M.P., Gaju, O., Foulkes, M.J., Le Gouis, J., Griffiths, S., Bennett, M.J., King, J., Wells, D.M., 2015. Phenotyping pipeline reveals major seedling root growth QTL in hexaploid wheat. J. Exp. Bot. doi:10.1093/jxb/erv006

Balyan, H.S., Singh, O., 1994. Pleiotropic effects of GA-insensitive Rht genes on grain yield and its component characters in wheat. Cereal Res. Commun. 22, 195–200.

Berry, P.M., Berry, S.T., 2015. Understanding the genetic control of lodging-associated plant characters in winter wheat (Triticum aestivum L.). Euphytica. doi:10.1007/s10681-015-1387-2

Berry, P.M., Berry, S.T., Spink, J.H., 2008. Identification of genetic markers for lodging resistance in wheat. Home-Grown Cereal. Auth. Res. Proj. No. 441 HGCA 14 pp.

Berry, P.M., Griffin, J.M., Sylvester-bradley, R., Scott, R.K., Spink, J.H., Baker, C.J., Clare, R.W., 2000. Controlling plant form through husbandry to minimise lodging in wheat. F. Crop. Res. 67, 59–81.

Berry, P.M., Kendall, S., Rutterford, Z., Orford, S., Griffiths, S., 2015. Historical analysis of the effects of breeding on the height of winter wheat (Triticum aestivum) and consequences for lodging. Euphytica 203, 375–383. doi:10.1007/s10681-014-1286-y

Berry, P.M., Kendall, S., Rutterford, Z., Orford, S., Griffiths, S., 2014. Historical analysis of the effects of breeding on the height of winter wheat (Triticum aestivum) and consequences for lodging. Euphytica. doi:10.1007/s10681-014-1286-y

Berry, P.M., Spink, J., Sterling, M., Pickett, A.A., 2003a. Methods for Rapidly Measuring the Lodging Resistance of Wheat Cultivars. J. Agron. Crop Sci. 189, 390–401. doi:10.1046/j.0931-2250.2003.00062.x

Berry, P.M., Spink, J.H., Gay, A.P., Craigon, J., 2003b. A comparison of root and stem lodging risks among winter wheat cultivars. J. Agric. Sci. 141, 191–202. doi:10.1017/S002185960300354X

Berry, P.M., Sterling, M., Baker, C.J., Spink, J., Sparkes, D.L., 2003c. A calibrated model of wheat lodging compared with field measurements. Agric. For. Meteorol. 119, 167–180. doi:10.1016/S0168-1923(03)00139-4

Berry, P.M., Sterling, M., Spink, J.H., Baker, C.J., Sylvester-Bradley, R., Mooney, S.J., Tams, A.R., Ennos, A.R., 2004. Understanding and reducing lodging in cereals. Adv. Agron. 84, 217–271. doi:10.1016/S0065-2113(04)84005-7

Berry, P.M., Sylvester-Bradley, R., Berry, S., 2007. Ideotype design for lodging-resistant wheat. Euphytica 154, 165–179.

doi:10.1007/s10681-006-9284-3

Churchill, G.A., Doerge, R.W., 1994. Empirical threshold values for quantitative trait. Genetics 138, 963–971.

Collard, B.C., Mackill, D.J., 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philos. Trans. R. Soc. B Biol. Sci. 363, 557–572. doi:10.1098/rstb.2007.2170

Cook, J.P., Wichman, D.M., Martin, J.M., Bruckner, P.L., Talbert, L.E., 2004. Identification of microsatellite markers associated with a stem solidness locus in wheat. Crop Sci. 44, 1397–1402. doi:10.2135/cropsci2004.1397

Flintham, J.E., Borner, A., Worland, A.J., Gale, M.D., 1997. Optimizing wheat grain yield: effects of Rht (gibberellin-insensitive) dwarfing genes. J. Agric. Sci. 128, 11–25.

Griffiths, S., Simmonds, J., Leverington, M., Wang, Y., Fish, L., Sayers, L., Alibert, L., Orford, S., Wingen, L., Snape, J., 2012. Meta-QTL analysis of the genetic control of crop height in elite European winter wheat germplasm. Theor. Appl. Genet. 29, 159–171. doi:10.1007/s11032-010-6534-x

Hai, L., 2006. Analysis of genetic diversity among current spring wheat varieties and breeding for improved yield stability of wheat (Triticum aestivum L.). Ph. D. Thesis. Justus-Liebig-University Giessen, Giessen, 100 pp.

Hai, L., Guo, H., Xiao, S., Jiang, G., Zhang, X., Yan, C., Xin, Z., Jia, J., 2005. Quantitative trait loci (QTL) of stem strength and related traits in a doubled-haploid population of wheat (Triticum aestivum L.). Euphytica

141, 1–9. doi:10.1007/s10681-005-4713-2

Kelbert, A.J., Spaner, D., Briggs, K.G., King, J.R., 2004. The association of culm anatomy with lodging susceptibility in modern spring wheat genotypes. Euphytica 136, 211–221. doi:10.1023/B:EUPH.0000030668.62653.0d

Keller, M., Karutz, C., Schmid, J.E., Stamp, P., Winzeler, M., Keller, B., Messmer, M.M., 1999. Quantitative trait loci for lodging resistance in a segregating wheat x spelt population. TAG 98, 1171–1182.

Kertesz, Z., Flintham, J.E., Gale, M.D., 1991. Effects of Rht dwarfing genes on wheat grain yield and its components under eastern european conditions. Cereal Res. Commun. 19, 297–304.

Lander, E.S., Botstein, S., 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121, 185.

Li, Z.K., Jiang, X.L., Peng, T., Shi, C.L., Han, S.X., Tian, B., Zhu, Z.L., 2014. Mapping quantitative trait loci with additive effects and additive x additive epistatic interactions for biomass yield, grain yield, and straw yield using a doubled haploid population of wheat (Triticum aestivum L.). Genet. Mol. Res. 13, 1412–1424.

Ma, J., Wingen, L.U., Orford, S., Fenwick, P., Wang, J., Griffiths, S., 2015. Using the UK reference population Avalon × Cadenza as a platform to compare breeding strategies in elite Western European bread wheat. Mol. Breed. 35, 70. doi:10.1007/s11032-015-0268-7

Ma, Q.-H., 2009. The expression of caffeic acid 3-O-methyltransferase in two wheat genotypes differing in lodging resistance. J. Exp. Bot. 60, 2763–71. doi:10.1093/jxb/erp132

Miralles, D.J., Slafer, G.A., 1995. Yield, biomass and yield components in dwarf, semi-dwarf and tall isogenic lines of spring wheat under recommended and late sowing dates. Plant Breed. 114, 392–396.

Niu, L., Feng, S., Ru, Z., Li, G., Zhang, Z., Wang, Z., 2012. Rapid determination of single-stalk and population lodging resistance strengths and an assessment of the stem lodging wind speeds for winter wheat. F. Crop. Res. 139, 1–8. doi:10.1016/j.fcr.2012.10.014

Reynolds, M., Bonnett, D., Chapman, S.C., Furbank, R.T., Manès, Y., Mather, D.E., Parry, M. a J., 2011. Raising yield potential of wheat. I. Overview of a consortium approach and breeding strategies. J. Exp. Bot. 62, 439–52. doi:10.1093/jxb/erq311

Ribaut, J.-M., de Vicente, M.C., Delannay, X., 2010. Molecular breeding in developing countries: challenges and perspectives. Curr. Opin. Plant Biol. 13, 213–218. doi:10.1016/j.pbi.2009.12.011

Richards, R.A., 1992. The effect of dwarfing genes in spring wheat in dry environments. I. Agronomic characteristics. Aust J. Agric Res Res 43, 517–527.

Tripathi, S.C., Sayre, K.D., Kaul, J.N., Narang, R.S., 2003. Growth and morphology of spring wheat (Triticum aestivum L.) culms and their association with lodging: effects of genotypes, N levels and ethephon. F. Crop. Res. 84, 271–290. doi:10.1016/S0378-4290(03)00095-9

Van Ooijen, J.W., 2011. JoinMap ® 4.1, Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen, Netherlands.

Verma, V., Foulkes, M.J., Worland, A.J., Sylvester-Bradley, R., Caligari, P.D.S., Snape, J.W., 2004. Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica 135, 255–263. doi:10.1023/B:EUPH.0000013255.31618.14

Wang, S., Basten, C.J., Zeng, Z.B., 2012. Windows QTL Cartographer 2.5. Department of Statistics, North Caroline State University, Raleigh, NC. doi:10.1002/tcr.201090006

Wiersma, J.J., Dai, J., Durgan, B.R., 2011. Optimum timing and rate of trinexapac-ethyl to reduce lodging in spring wheat. Agron. J. 103, 864–870. doi:10.2134/agronj2010.0398

Yang, M., Ding, G., Shi, L., Feng, J., Xu, F., Meng, J., 2010. Quantitative trait loci for root morphology in response to low phosphorus stress in Brassica napus. Theor. Appl. Genet. 121, 181–193. doi:10.1007/s00122-010-1301-1

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Reserach 14, 415–421.

Zeng, Z.B., 1994. Precision mapping of quantitative trait loci. Genetics 136, 1457–68. doi:10.1093/aob/mcr323

6.7 Supplementary material

Table S6.1 Phenotypic correlations coefficients between experimental means of lodging traits (SS, stem strength; SMS, stem material strength; SIL, stem internode length; SD, stem internode diameter; SWW, stem internode wall width; RPS, root plate spread; SRD, structural rooting depth; PH, plant height; ENPP, ear number per plant) and grain yield of the Avalon x Cadenza DH population (84 lines) growing at High Mowthorpe 2013 (upper diagonal) and Sutton Bonington 2014 (lower diagonal).

Character	SS	SMS	SIL	SD	SWW	RPS	SRD	PH	ENPP	GY
SS		0.65***	0.13	0.13	0.79***	0.02	0.09	0.08	-0.09	-0.25*
SMS	0.66***		0.08	-0.88***	0.69***	-0.26*	-0.22*	0.23*	-0.03	0.03
SIL	-0.02	-0.05		-0.03	-0.10	0.02	-0.02	0.63***	0.00	0.34**
SD	-0.16	-0.78***	0.10		-0.50***	0.31**	0.34**	-0.18	-0.01	-0.20
SWW	0.64***	0.56***	-0.26*	-0.47***		-0.05	-0.07	-0.24*	-0.01	-0.15
RPS	0.24*	0.06	0.05	0.04	0.10		0.63***	0.06	0.29**	0.00
SRD	0.16	-0.03	0.13	0.16	-0.12	0.76***		0.00	0.30**	-0.12
PH	-0.13	0.06	0.72***	-0.11	-0.36***	0.06	0.14		-0.11	0.39***
ENPP	0.05	0.04	0.17	-0.09	0.10	0.39***	0.26*	0.08		-0.14
GY	-0.10	0.04	0.31**	0.05	-0.25*	0.10	0.07	0.57***	0.05	

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Chapter 7 General discussion

7.1 Modelling a lodging-proof plant

It has been argued in this thesis that there may now be limited potential to increase lodging resistance through further decreasing plant height since the minimum height that is compatible with high yield has been reached in many environments (0.7 to 1.0 m (Allan 1986; Kertesz et al. 1991; Richards 1992; Balyan and Singh 1994; Miralles and Slafer 1995a; Flintham et al. 1997; Berry et al. 2014)). A novel strategy to increase lodging resistance has also been proposed. This involves the focus on strengthening the support plant structures, particularly the stem base and anchorage system (crown roots). A validated model that incorporates these support structures with significant accuracy for predicting timing and incidence of lodging on winter wheat (Berry et al., 2003c) was tested on spring wheat data. Interestingly, reasonable cultivar ranking prediction was found when compared with observed ranks. This model was further developed by using spring wheat phenotypic data (awned wheat) to estimate the key plant characteristics that determine base bending moment (shoot height at centre of gravity, shoot natural frequency and ear area) from plant characteristics that are more commonly measured by crop physiologists (grain yield, grain harvest index, plant height and shoots m^{-2}). This process was done before by Berry et al. (2004) using winter wheat data (awnless wheat). Despite the morphologic differences of winter and spring wheat ears in both cases it was demonstrated that lodging model inputs can be calculated from agronomic traits.

One of the main elements of the model is the effect of wind on the whole plant and individual shoots. Thus, the model is required to be tested under wind conditions of the relevant spring wheat growing areas which in our case was Ciudad Obregon in the NW Mexico region (location of wheat CIMMYT breeding program). It was found that spring wheat grown in this particular area would need to resist wind gust of 22 m s^{-1} at least once in 25 years which is superior to the 18 m s^{-1} that winter wheat has to resist in the same period of years for winter wheat growing areas such as the UK environment. However, another aspect to consider was water supply source; while the UK is a rainfed environment the NW Mexico environment requires an irrigation scheme. This irrigation input for spring wheat in NW Mexico required a model adjustment in order to calculate probabilities of root lodging risk which only occurs when the upper 10 cm of soil are wet whether there is a rainfed or irrigated environment (Baker et al., 1998; Berry et al., 2003b; Crook and Ennos, 1994; Easson et al., 1995; Fischer and Stapper, 1987; Sterling et al., 2003). This adjustment led to the estimate that spring wheat has to resist 18 m s^{-1} at least once in 25 years when the soil is moist.

Our results indicated that the lodging-resistant ideotype crop estimated for spring wheat growing in the NW Mexico environment (0.7 m tall, grain yield of 6 t ha^{-1} with 200 plant m^{-2} and 500 shoots m^{-2}) will need a stem strength equivalent to the 268 N mm of shoot leverage and anchorage strength equivalent to 448 N mm of plant leverage.

7.2 Genetic resources for improving lodging resistance

This study has identified very diverse variation in spring wheat for the majority of the traits involved in lodging resistance. The specific key traits (stem diameter, stem strength, stem wall width, stem material strength, root plate spread and plant height) to achieve a lodging resistant crop ideotype have been almost fully achieved. In the case of the stem strength the best value observed was 6 % below the target for the lodging-proof ideotype. For root plate spread, significant genetic ranges were also found, although the best value observed was 17 % below the ideotype dimension. These results imply the need to find wider root plates and stronger stems in more diverse germplasm.

In general, cultivar mean rank for lodging-traits change across environments. This is mainly due to G×E interactions observed in both winter and spring wheat (Berry et al. 2003a; Berry and Berry 2015, Chapter 3 and 6). Nevertheless, all the ideotype key traits have heritability values equal or above 0.70, except for root plate spread. This makes most key lodging traits useful for breeding selection.

Genetic correlations among lodging-related traits and grain yield have indicated that improving lodging resistance will not compromise the achievement of a lodging-resistant ideotype crop. However, strong linkage between stem wall width and stem strength may indicate that minimising investment of biomass to strengthen the stem base would not be the optimum scenario.

7.3 Lodging standard methods and QTL as novel tool to accelerate selection

Standard current methods for assessing lodging in wheat (Berry et al., 2003c, 2000) are very time-consuming. This indicates that further development of new methodologies or the optimization of current protocols will be necessary if lodging resistance selection capacity has to be improved. Chapter 5 attempted to simplify and optimize these current lodging assessment protocols. It can be argued that the findings in this chapter will not be enough if massive breeding programs are considered. However, increasing the number of plots assessed by every technician work shift of 8 h from three (standard methods) to 12 represents a great plot improvement (Chapter 5).

The implementation of genetic markers with increased lodging resistance purposes is another strategy that might accelerate the avoidance of lodging in wheat in the future. The main results obtained in the two-year investigation with the wheat Avalon x Cadenza DH population have indicated great potential for the development of these markers. These results included a genomic region in Chromosome 3B that is correlated with all the stem strength components (stem strength, diameter, wall width and material strength) and explains an important amount of the phenotypic variation. More importantly, this region has proved to be stable across environments. A major finding has been also the identification of QTLs affecting RPS in chromosome 5B which also was stable across environments. These finding resulted more relevant because QTLs for stem strength (Hai et al., 2005) and root plate spread (Berry and Berry, 2015) have been reported for the respective chromosomes.

7.4 Implications of an increased lodging resistant crop

Achieving an ideotypic lodging resistant crop might compromise increases of grain yield. This is mainly due to the strong dependence of stem and anchorage strength on stem and root biomass (Chapter 2 – 3). In both winter and spring wheat it has been found that extra biomass will be needed in order to improve lodging resistance (Berry et al. 2007: Chapter 2). Careful optimization to find the minimum investment of this biomass on lodging dimensions must be taken by breeding for wide thin walled stems (Berry et al., 2007). However, the strong linkage between stem wall width and stem strength can compromise this.

The hypothetical harvest index was estimated by Austin (1980) at 0.62. Nevertheless, this study did not account for biomass investment on plant support structures. Our estimations for a lodging proof plant during 25 years were 0.46 for spring wheat and 0.49 for winter wheat (Chapter 2) which are far below the potential harvest index. These relatively high levels of non-grain biomass and low harvest indices suggest that the high investment in this biomass may compete with yield formation and limit the rate of breeding improvement in grain yield. Another aspect that this ideotype requires is an optimum crop height of 0.7 m. If we look at the current breeding programs (e.g. CIMMYT, UK breeders) plant height is above this dimension and no tendency to further decrease it has been seen in the last two decades (Aisawi et al., 2015; Berry et al., 2014). This indicates that achieving the ideal plant height for lodging resistance can be done. However, this also have certain implications such as reduced overall stem biomass and stem storage capacity. This can also compromise grain yield and grain yield formation processes. To counteract this other crop traits must be implemented (e.g. fruiting efficiency, (Slafer et al., 2015)).

The lack of wide enough root plate spread genetic ranges found in the populations evaluated in this study together with most winter wheat investigations will complicate finding the ideotype target dimension for this trait. Thus the searching for a more diverse germplasm should be considered for further investigations. Another aspect with potential on improving anchorage strength by increasing root plate spread is establishing lower plant populations (Berry et al. 2000; Chapter 3). However, this needs to be implemented carefully to avoid poor plant establishment that can increase weed growth.

7.5 Potential for further improvements

Further investigations should focus on the optimization of standard methods to asses lodging resistance traits in order to increase selection capacity of plant breeders. This can be implemented using the modification of the standard protocols done in Chapter 5. Indirect methods to infer on lodging traits can also be further investigated (e.g. NDVI or other spectral indices).

Screening of new germplasm in order to find a wider and deeper root plate and stronger stems will also be fundamental to achieve the lodging resistance requirements.

Fine mapping and validation of genomic regions found in this study for lodging key traits will pay great dividends to accelerate an increased lodging resistance. Moreover, this can be a strategy that can reduce trade-offs between improvements on lodging resistance and grain yield.

A direct tool for producers will be the development of a set of crop husbandry practices that can help to reduce lodging risk. This has been done for UK producers but areas such as the Yaqui Valley in the NW Mexico environment have not yet optimised these.

7.6 References

Aisawi, K.A.B., Reynolds, M.P., Singh, R.P., Foulkes, M.J., 2015. The physiological basis of the genetic progress in yield potential of CIMMYT spring wheat cultivars from 1966 to 2009. Crop Sci. doi:10.2135/cropsci2014.09.0601

Allan, R.E., 1986. Agronomic comparisons among wheat lines nearly isogenic for three reduced-height genes. Crop Sci. 26, 707–710.

Austin, R.B., 1980. Physiological limitations to cereals yield and ways of reducing them by breeding, in: Hurd, R.G., Biscoe, P. V, Dennis, C. (Eds.), Opportunities for Increasing Crop Yield. Association of Applied Biology. Pitman, Boston, pp. 3–19.

Baker, C.J., Berry, P.M., Spink, J.H., Sylvester-Bradley, R., Griffin, J.M., Scott, R.K., Clare, R.W., 1998. A method for the assessment of the risk of wheat lodging. J. Theor. Biol. 194, 587–603. doi:10.1006/jtbi.1998.0778

Balyan, H.S., Singh, O., 1994. Pleiotropic effects of GA-insensitive Rht genes on grain yield and its component characters in wheat. Cereal Res. Commun. 22, 195–200.

Berry, P.M., Berry, S.T., 2015. Understanding the genetic control of lodging-associated plant characters in winter wheat (Triticum aestivum L.). Euphytica. doi:10.1007/s10681-015-1387-2

Berry, P.M., Griffin, J.M., Sylvester-bradley, R., Scott, R.K., Spink, J.H., Baker, C.J., Clare, R.W., 2000. Controlling plant form through husbandry to minimise lodging in wheat. F. Crop. Res. 67, 59–81.

Berry, P.M., Kendall, S., Rutterford, Z., Orford, S., Griffiths, S., 2014. Historical analysis of the effects of breeding on the height of winter wheat (Triticum aestivum) and consequences for lodging. Euphytica. doi:10.1007/s10681-014-1286-y

Berry, P.M., Spink, J.H., Gay, A.P., Craigon, J., 2003a. A comparison of root and stem lodging risks among winter wheat cultivars. J. Agric. Sci. 141, 191–202. doi:10.1017/S002185960300354X

Berry, P.M., Sterling, M., Baker, C.J., Spink, J., Sparkes, D.L., 2003b. A calibrated model of wheat lodging compared with field measurements. Agric. For. Meteorol. 119, 167–180. doi:10.1016/S0168-1923(03)00139-4

Berry, P.M., Sterling, M., Spink, J.H., Baker, C.J., Sylvester-Bradley, R., Mooney, S.J., Tams, A.R., Ennos, A.R., 2004. Understanding and reducing lodging in cereals. Adv. Agron. 84, 217–271. doi:10.1016/S0065-2113(04)84005-7

Berry, P.M., Sylvester-Bradley, R., Berry, S., 2007. Ideotype design for lodging-resistant wheat. Euphytica 154, 165–179. doi:10.1007/s10681-006-9284-3

Crook, M.J., Ennos, A.R., 1994. Stem and root characteristics associated with lodging resistance in four winter wheat cultivars. J. Agric. Sci. Agric. Sci. 123, 167–174. doi:10.1017/S0021859600068428

Easson, D.L., Pickles, S.J., White, E.M., 1995. A study of the tensile force required to pull wheat roots from soil. Ann. Appl. Biol. 127, 363–373.

Fischer, R.A., Stapper, M., 1987. Lodging effects on high-yielding crops of irrigated semidwarf wheat. F. Crop. Res. 17, 245–258.

Flintham, J.E., Borner, A., Worland, A.J., Gale, M.D., 1997. Optimizing wheat grain yield: effects of Rht (gibberellin-insensitive) dwarfing genes. J. Agric. Sci. 128, 11–25.

Hai, L., Guo, H., Xiao, S., Jiang, G., Zhang, X., Yan, C., Xin, Z., Jia, J., 2005. Quantitative trait loci (QTL) of stem strength and related traits in a doubled-haploid population of wheat (Triticum aestivum L.). Euphytica 141, 1–9. doi:10.1007/s10681-005-4713-2

Kertesz, Z., Flintham, J.E., Gale, M.D., 1991. Effects of Rht dwarfing genes on wheat grain yield and its components under eastern european conditions. Cereal Res. Commun. 19, 297–304.

Miralles, D.J., Slafer, G.A., 1995. Yield, biomass and yield components in dwarf, semi-dwarf and tall isogenic lines of spring wheat under recommended and late sowing dates. Plant Breed. 114, 392–396.

Richards, R.A., 1992. The effect of dwarfing genes in spring wheat in dry environments. I. Agronomic characteristics. Aust J. Agric Res Res 43, 517–527.

Slafer, G.A., Elia, M., Savin, R., García, G.A., Terrile, I.I., Ferrante, A., Miralles, D.J., González, F.G., 2015. Fruiting efficiency: an alternative trait to further rise wheat yield. Food Energy Secur. 4, 92–109. doi:10.1002/fes3.59

Sterling, M., Baker, C.J., Berry, P.M., Wade, A., 2003. An experimental investigation of the lodging of wheat. Agric. For. Meteorol. 119, 149–165. doi:10.1016/S0168-1923(03)00140-0

Chapter 8 Conclusions

The research discussed through this thesis has focussed on the potential for further improvements of lodging resistance as well as to understand its genetic control in wheat. Special attention has been given to the possible trade-offs with yield and yield formation processes that an increased lodging resistance can generate. The following major conclusions can be extracted from this research.

1. The ideotype has been estimated for a spring wheat crop able to resist lodging for 25 years (grain yield of 6 t ha^{-1}, plant height of 0.7 m, 200 plant m^{-2}, 500 shoots m^{-2}, stem strength of 268 N mm and root plate spread of 51.1 mm). This ideotype estimation resulted from the adaptation of a lodging model developed for winter wheat to spring wheat under NW Mexico growing conditions.
2. The cost of improving lodging resistance in terms of stem structural biomass indicated possible trade-offs with yield. Therefore, plant breeders must improve the overall above-ground plant biomass in order to improve both grain yield and lodging resistance.
3. Fractionation of the stem structural biomass on its main components (lignin, cellulose, hemicellulose and water soluble carbohydrates) indicated that there is not an individual positive effect on stem strength by a single component.
4. The elite spring wheat assessed in this research has indicated broad genetic ranges for most of the key lodging resistance traits. However, not all the ideotypic dimensions have been achieved in the germplasm phenotyped in this study (e.g. stem strength, root plate spread). Stem key lodging traits (stem strength, diameter, wall width

and material strength) were high heritable even though they were affected by G×E interactions. These interactions also affected root plate spread (root key lodging trait) but in this case low heritability was found.

5. Correlations among key lodging traits indicate it is feasible to combine all the ideotypic dimensions in a single variety. Nevertheless, optimization of structural biomass in order to diminish trade-offs with yield can be difficult (e.g. strong positive correlation between stem strength and wall width).
6. Rapid methods to assess lodging can be developed in order to accelerate breeding for lodging resistance. This study has shown that optimization of current standard protocols can increase selection capacity. However, the development of lodging resistance genetic markers seems to be the most sensible strategy. Major QTLs on chromosomes 3B (*qSS3B*) and 5B (*qRPS5B*) can be used to develop these markers.

Appendices

Appendix 1. Lodging trait measurement methods*

Type of character	Description
Root characters	Crown roots were identified by their inherent rigidity and the tendency for soil particles to adhere to their dense covering of root hairs, or rhizosheath. This distinguishes them from seminal roots, which number six or less, emerge directly from the seed with less rigidity, and usually have no adhering soil. The rhizosheath section of a crown root is termed the 'rigid root length', and there is usually little variation between the roots of individual plants. However, some plants had very variable rigid root lengths, so that determination of the spread of the root plate and its depth was more subjective. The point at which the majority of rigid root portions terminated was estimated visually to define 'root plate spread'. Both the maximum root plate spread and the root plate spread at 90° to the maximum (usually the smallest spread) were measured. Structural rooting depth was measured as the distance from base of the root plate to the soil surface, identified as the point where stem colour changes from white to green. Root plate spread was determined from an average of the maximum and minimum root plate spread.
Leverage characters (main shoot)	Natural frequency was measured in the field on a still day. After selection of plants, the main shoot was identified (the tallest with the largest ear) and isolated from any neighbouring shoots. The shoot was pulled back (at the collar of the ear) 5-10 cm from the vertical and released. The time required for three complete oscillations in the line of displacement was registered and the number of oscillations recorded. Natural frequency was then calculated by dividing the number of oscillations during the timed period, by the length of the time period (s). Other measurements

	included the number of fertile shoots per plant (shoots with ears), plant height to the ear tip, and area of each main shoot ear assessed with a LI-3100C area meter (LI-COR®) during 2011-12 and 2012-13. Height at the centre of gravity of the main shoot was determined by balancing the main shoot without roots on ruler with leaves and ear still attached and recording the distance from the point of balance to the base of the stem.
Stem characters	Stem measurements were only done on the main shoot. Firstly, internode one was identified, defined as the first internode of more than 10 mm, originating at or just below the ground surface and without crown roots emerging from its upper node. Subsequent internodes ascending the stem were numbered two, three, four etc., with the uppermost internode referred to as the peduncle. Measurements of the stem base were carried out on internodes one and two of each main shoot, and the lengths of these internodes were measured from the mid-point of their adjacent nodes. Stem diameter was measured at the middle of each internode using digital callipers. The breaking strength of internodes one and two was also determined using a three-point bending test (Easson et al., 1992; Graham, 1983)**; nodes adjacent to the internode were supported on the 'Y' frame, which was clamped to the bench before the hook of a Mecmessin® basic force gauge (200 N x 0.05 N) was placed around the mid-point of the internode and a pulling pressure applied at an even rate. The force just before the internode bending was considered its breaking strength. Finally, the stem wall width was measured by cutting internodes one and two at their mid-point. Once those internodes were cut, two measurements at right angles to each other were recorded and averaged.

***Berry, P.M., Griffin, J.M., Sylvester-Bradley, R., Scott, R.K., Spink, J.H., Baker, C.J., Clare, R.W., 2000. Controlling plant form through husbandry to minimise lodging in wheat. F. Crop. Res. 67, 59–81.**
****Easson, D.L., White, E.M., Pickles, S.J., 1992. A Study of Lodging in Cereals. Home-Grown Cereals Authority Research Project No. 52. HGCA, London, 73 pp.**
****Graham, J., 1983. Crop lodging in British wheats and barleys. Ph.D. Thesis. The University of Reading, Reading, Berkshire, UK, 205 pp.**

Appendix 2. Sowing, phenology and crop management

Exp*	Crop Cycle	Sowing date	Emergence date (50%)	Anthesis date	Maturity date	Herbicide		Fungicide		Insecticide		Irrigation (at FC)	Fertiliser (N-P-K)**
						Dose	**Date**	**Dose**	**Date**	**Dose**	**Date**		
2011	2010-2011	06-Dec-10	15-Dec-10	14-Mar-11	19-Apr-11	1300 ml/ha (Buctril)	04-Jan-11	500 ml/ha (Folicur)	25-Jan-11	300 ml/ha (Muralla)	01-Apr-11	At sowing	200-50-00
						750 ml/ha (Estarane)	04-Jan-11	500 ml/ha (Folicur)	21-Feb-11			08-Jan-11	
								500 ml/ha (Folicur)	25-Mar-11			09-Feb-11	
												06-Mar-11	
												08-Apr-11	
2012	2011-2012	09-Dec-11	16-Dec-11	12-Mar-12	24-Apr-12	1000 ml/ha (Buctril)	04-Jan-12	500 ml/ha (Folicur)	12-Mar-12	175 ml/ha (Muralla)	09-Feb-12	At sowing	200-50-00
						300 ml/ha (Estarane)	04-Jan-12			175 ml/ha (Muralla)	02-Mar-12	14-Jan-12	
												09-Feb-12	
												02-Mar-12	
												16-Mar-12	
												31-Mar-12	
2013	2012-2013	23-Nov-12	02-Dec-12	27-Feb-13	10-Apr-13	500 ml/ha (Axial)	13-Dec-12	500 ml/ha (Folicur)	25-Jan-13	250 ml/ha (Muralla Max)	22-Jan-13	At sowing	200-50-00
						1000 ml/ha (Adigor)	13-Dec-12	500 ml/ha (Folicur)	15-Feb-13			08-Jan-13	
						1000 ml/ha (Buctril)	20-Dec-12	500 ml/ha (Folicur)	08-Mar-13			01-Feb-13	
						350 ml/ha (Estarane)	20-Dec-12					22-Feb-13	
												15-Mar-13	
												04-Apr-13	
2014	2013-2014	22-Nov-13	01-Dec-13	18-Feb-14	27-Mar-14	1000 ml/ha (Buctril)	16-Dec-13	500 ml/ha (Folicur)	27-Jan-14	250 ml/ha (Muralla Max)	24-Jan-14	At sowing	200-50-00
						350 ml/ha (Estarane)	16-Dec-13	500 ml/ha (Folicur)	19-Feb-14	175 ml/ha (Alectus)	21-Feb-14	09-Jan-14	
								500 ml/ha (Folicur)	06-Mar-14	175 ml/ha (Muralla Max)	07-Mar-14	31-Jan-14	
												21-Feb-14	
												14-Mar-14	

*** Summer crop (*Sesbania sesban* L. Merr.) was established from late June to late August with the purpose of incorporate organic matter to the soil and to allow the germination of seeds from previous experiment**
****Urea as source of *N* applied 25% before sowing and 75% just before the second irrigation; Triple Super Phosphate as source of *P* applied before sowing**