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Exploiting novel genes to improve resource use efficiency in wheat

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This is the final report of a 65 month project (RD-2008-3543) which started in September 2009. This was a Sustainable Arable LINK project (LK09129) with total funding for the work of £1227,000 from Defra, BBSRC, AHDB Cereals & Oilseeds, Limagrain and BASF, which included a contract for £125,000 from AHDB Cereals & Oilseeds.

CONTENTS

1. Abstract

Resource use efficiency can be improved by either maintaining yield with lower crop inputs or increasing yield with the same, or reduced, inputs. Increasing yield is likely to be the most sustainable approach given the need to ensure global food security and the limited scope for expanding the cropped area without further degrading the environment. Achieving greater yields without increasing crop inputs will improve the use efficiency of land area, sunlight, nutrients and other crop inputs, as well as reducing greenhouse gas (GHG) emissions per unit of crop production. UK farm wheat yields increased rapidly after the 1970s, but the rate of yield improvement had slowed markedly by the late 1990s and has hovered at around 8 t/ha since the turn of the century. Making progress towards higher yield potentials will require the development of new varieties and changes to crop management to ensure that high genetic yield potential of new varieties are realised.

This project aimed to increase resource use efficiency by developing reliable genetic markers and a physiological understanding for Quantitative Trait Loci (QTL) that increase yield and lodging resistance without increasing the crop's requirement for inputs. This was achieved through the following objectives; i) Identify reliable genetic markers for genes that improve resource use efficiency, increase yield and affect height; ii) Understand the physiological mechanisms by which the genes increase resource use efficiency and yield; iii) Investigate which yield and height genes are in current varieties and the scope for combining them to increase yield without increasing lodging risk; iv) Quantify the responsiveness of the different height genes to Plant Growth Regulators (PGR) active ingredients.

Across 21 field experiments, this project has demonstrated that three QTL for yield on chromosomes 3A, 6A and 7D each increased yield by 0.25 to 0.49 t/ha, with the potential for a combined yield improvement of about 1 t/ha. Some QTL are not commonly present in elite germplasm and it is very unlikely that any elite varieties have all three yield enhancing QTL, which demonstrates there is scope to continue to increase wheat yield. Nitrogen (N) fertiliser treatments showed no evidence that the yield effects would necessitate more N fertiliser, but they would require action to reduce a greater lodging risk since some yield QTL also increased height. The yield QTL were often associated with increases in total crop biomass, more grains or both more and larger grains, with most growth improvements occurring after flowering.

One of the drawbacks of combining the QTL for greater yield using existing genetic markers is that this will increase crop height by several centimetres. To investigate whether the increase in height could be mitigated a specific breeding population was made to stack the three yield QTL within a double-dwarf background (*Rht1* and *Rht2*; NB current elite varieties possess one of these dwarfing genes, but not both). The resulting genetic lines were 60 to 80 cm tall, therefore showing that the yield QTL could be combined into one variety without causing a high lodging risk.

Near inbred lines (NILs) are pairs of lines differing only for the region of the chromosome containing the yield or height QTL of interest. This project focussed on NILs for three yield QTL

and three height QTL. The project developed new NILs for the yield QTL on chromosome 7D and further refined and multiplied up existing NILs which will provide valuable pre-breeding materials. This project has developed new and more reliable genetic markers for yield and height that are amenable for high throughput use in commercial breeding programmes. Gene candidates have been proposed for some QTLs which will help to find even more reliable genetic markers which are necessary to understand whether yield/height effects can be uncoupled.

Varieties were shown to undergo significantly different amounts of shortening (up to 2-fold) in response to PGRs. However PGR effects commonly interacted strongly with the environment which made it difficult to identify consistent varietal differences. There was no consistent evidence that varietal differences in PGR sensitivity were related to crop height, the presence of specific height genes, or differences in leaf waxiness. If a variety was sensitive to a PGR then it tended to be sensitive to a wide range of PGR active substances.

2. Introduction

Background and purpose

The problem

Resource use efficiency can be improved by either maintaining yield with lower crop inputs or increasing yield with the same, or reduced, inputs. Increasing yield is likely to be the most sustainable approach given the need to ensure global food security and the limited scope for expanding the cropped area without further degrading the environment. Achieving greater yields without increasing crop inputs will improve the use efficiency of land area, sunlight, nutrients and other crop inputs, as well as reducing greenhouse gas (GHG) emissions per unit of crop production (Berry *et al*., 2008).

UK farm wheat yields increased rapidly after the 1970s, but the rate of yield improvement had slowed markedly by the late 1990s and has hovered at around 8 t/ha since the turn of the century (Defra statistics). Physiological analyses (Sylvester-Bradley *et al.*, 2004; Berry *et al*., 2011) show that the UK environment could support a doubling of wheat yields, so there is substantial scope for improving yields. Making progress towards higher yield potentials will require the development of new varieties and changes to crop management to ensure that high genetic yield potential of new varieties are realised.

Yield is determined by a large number of genes each conferring a relatively small effect, hence genetic improvements in yield have tended to be incremental. However, there have been instances of major genes which have had a large effect on yield. These include 1) the semi-dwarfing genes (*Rht-B1b* and *Rht-D1b*) introduced during the 1970s and 80s which reduced lodging risk, allowing greater fertiliser use, as well as conferring a direct effect on sink size and yield at the same fertiliser level (Miralles and Slafer, 1995; Flintham *et al*., 1997), and 2) the 1RS.1BL wheat-rye translocation introduced during the late 1980s and early 1990s, which increased overall biomass (Shearman *et al*., 2005). The semi-dwarfing genes have been estimated to increase yield by 0.4 to 1.0 t/ha (Flintham *et al*., 1997), with about 0.6 t/ha associated with the 1RS.1BL (Carver & Rayburn, 1994).

The recent LINK Project LK0958 '*Identification of genetic markers for lodging resistance in wheat*', investigated a Rialto x Savannah doubled haploid (DH) population and identified two major QTL on chromosomes 3A and 7D that were associated with increased resource use efficiency resulting from yield increases (at a given level of inputs) of 0.43 t/ha and 0.29 t/ha, respectively, across three site-seasons. Both of these QTL were also associated with height increases of 4 cm to 7 cm, thinner stem walls, and one QTL was associated with a smaller root plate and 0.4 t/ha more straw. Two recent studies have also identified QTL for increased grain yield and height within similar

regions of the 3A and 7D chromosomes (Dilbirligi *et al*., 2006; Roder *et al*., 2008). A QTL for greater yield has also been detected within a similar region of 7D in a proprietary DH population of Brigadier x Alcedo (Brigadier is one of the parents of Savannah). Project LK0958 also identified a major QTL on chromosome 6A which increased height by 70 mm but had a less consistent effect on yield with an average increase of 0.15 t/ha over the 3 years. Spielmeyer *et al.* (2007) also detected a strong height QTL within a similar region of 6A, but did not measure yield; whereas Snape *et al*. (2007) showed this region affected thousand grain weight.

It should be recognised that lodging caused by taller plants may reduce the size of the yield effects and therefore the effects on yield associated with each QTL may be greater in the absence of lodging. The association of greater yield and taller plants (within a semi-dwarf background) has been observed previously (Law *et al*., 1978) and may help to explain why breeders have recently found it difficult to simultaneously increase yield and reduce height/lodging risk. It is likely that the same genes affect yield and height (Law *et al*., 1978), but this has never been proven conclusively.

LK0958 found a further four major height QTL on chromosomes 1D, 2A, 2D and 4D, which were not located in regions already known to contain reduced height (*Rht*) genes. These QTL had individual effects on height of 3 cm to 6 cm and did not affect yield. It was found that the 5 largest height QTL could be combined to alter height by 26 cm. Meta studies have identified several other minor height QTL segregating within European wheat germsplasm in addition to the ones identified by LK0958 (Griffiths *et al*., 2012; Wurschum *et al*., 2015). LK0958 also showed that some height QTL were twice as responsive as others to shortening by a PGR programme, consisting predominantly of active ingredients that inhibit the synthesis of gibberellic acid. Several authors have suggested that gibberellin biosynthesis underlie some height QTL (Roder *et al*., 2008; Rademacher, 2000), which may explain why certain varieties appear to be more responsive to PGRs than others.

Knowledge of pedigrees and analysis of several DH populations indicate that either very few, or none, of the current wheat varieties have inherited the major yield/height increasing 7D allele from Brigadier/Savannah and a proportion, perhaps half, have inherited the yield/height increasing 3A and 6A alleles. It would be advantageous to develop new varieties with these major QTL on 3A, 6A and 7D as this would increase resource use efficiency by increasing yield additively by at least 0.7 t/ha. However, this is not straight forward because: 1) the genetic markers are not close enough to the genes in question to reliably identify the presence of the positive alleles in a range of genetic backgrounds and 2) the QTL for increased yield are also associated with greater height, a smaller root plate and thinner stem walls which increase lodging risk. In order to compensate for this greater lodging risk it will also be necessary to incorporate other QTL for reduced height which had no effect on yield. Again more tightly linked markers would be required to achieve this.

This project aimed to find reliable genetic markers and develop new precise genetic stocks, making it possible to clone the genes to give ‘perfect’ markers for each allele at these loci. Within the project it was important to identify whether the simultaneous increases in yield and height (and effects on other traits) are caused by the same gene (pleiotropy) or independent genes in close proximity (linkage). If the latter is true then breeders should be able to separate the effects of yield and height through recombination. Diagnostic markers for height genes will also enable breeders to design crosses to breed new varieties with optimum height, as well as for predicting varietal responsiveness to PGRs.

In order to fully exploit the genes for improved resource use efficiency it will be necessary to understand the physiological mechanisms by which they increase yield so that crop management can be optimised to allow the greater yield potential to be realised or to achieve an even greater yield with the minimum inputs. For example if the higher yielding varieties are shown to be more sink limited than source limited, then greater emphasis must be placed on maximising shoot and grain numbers. This will mean that the timing of nutritional inputs may have to be brought forward and the importance of protecting the green canopy during early crop development will increase. Understanding yield formation will also help to predict how altering inputs will affect resource use efficiency and GHG emissions.

Another important area of variety/ crop management interactions which requires improved understanding is whether the height reducing effect of a PGR is affected by the presence or absence of particular height QTL in different varieties. Understanding whether specific height QTL affect a plant’s sensitivity to different PGR active substances will enable PGRs to be targeted at specific varieties for greatest reduction in lodging risk.

3. Aim and objectives

3.1. Project Aim

Improve resource use efficiency and reduce GHG emissions by developing reliable genetic markers and physiological understanding for QTL that increase yield without increasing the crop’s requirement for inputs (e.g. N fertiliser or PGRs).

3.2. Specific Objectives

SO1 – Develop near isogenic lines for QTL that increase resource use efficiency.
SO2 – Identify more diagnostic markers for the QTL that increase resource use efficiency.
SO3 – Understand the physiological mechanisms by which these QTL act and quantify effects on resource use efficiency and GHG emissions.

SO4 – Investigate which yield and height QTL are in current varieties and the scope for combining them to increase resource use efficiency.

SO5 – Quantify the responsiveness of the different height QTL to different PGR active ingredients.

4. Identify reliable genetic markers for genes that improve resource use efficiency, increase yield and affect height

4.1. Develop near isogenic lines for QTL that increase resource use efficiency

4.1.1. Introduction

Plant height is an important trait affecting resource use and yield in wheat; tall plants may be affected by lodging, while shorter plants may have a reduced yield. Therefore, the identification of genes affecting crop height, while not affecting crop yield, is extremely important.

The Avalon x Cadenza DH population (A x C) was one of several developed to represent a broad spectrum of the variation present in the UK elite winter germplasm pool and is now the UK reference population under the UK Department of Environment, Food and Rural Affairs (DEFRA) Wheat Genetic Improvement Network (WGIN) and was developed as part of WGIN 1. The A x C population has been extensively phenotyped in field trials in Norfolk from 2005-2008. A number of QTL's affecting crop height had previously been identified in the A x C population (Griffiths *et al.,* 2012) and a subset of these are studied here including QTL on chromosomes 2D, 3A, 3B, 6A and 6B.

4.1.2. Materials and Methods

Selection of Recombinants

Individual genetic lines (streams) carrying a single QTL affecting plant height were generated from the A x C DH lines. These genetic lines were backcrossed to Cadenza to produce BC2 and self-fertilised to give BC2F2. The lines were screened with Simple Sequence Repeats (SSR) genetic markers flanking the QTL (**Table 4.1**) at BC2F2 and BC2F3 for the required recombinant genotypes. Markers were chosen from the IPK Gatersleben (gwm/gdm), Wheat Microsatellite Consortium (wmc), Beltsville Agricultural Research Station (barc) and INRA (cfd/cfa) collections (see GrainGenes website for genetic maps, http://www.wheat.pw.usda.gov/). Genotyping was carried out at John Innes Centre (JIC) or Limagrain (LG). A total of 15,603 plants were generated and analysed (**Table 4.2)** and a subset of these lines, for each QTL, selected for further analysis (**Table 4.3)**. All these NIL-derived recombinants have either Avalon or Cadenza germplasm across the height QTL region, in a background of Cadenza.

For the fine mapping of the QTL on 3A additional recombinants (around genetic marker barc19) were generated from the AC179-E27-2 stream – these were genotyped at Limagrain using their in-house markers close to barc19.

Table 4.1. Flanking SSR markers

†QTL location	SSR markers flanking QTL	
2D	cfd36	gwm261
3A	barc19	wmc264
3B	gwm285	wmc326
6A	barc171	gwm570
6B	wmc105	gwm219

† **chromosome location**

Table 4.2. Plants generated and analysed for each height QTL

†QTL location	F2 screened	F3 Het RC's identified	F3 screened	F3 un-screened	F4 Hom RC's identified	F4 phenotyped	F4 not phenotyped (sibs)
2Do	475	60	42	18	84	41	44
2Da	480	72	50	22	107	50	57
3A	454	131	84	47	211	76	135
3A	1480 (LG)	178	94	84	1104 (LG)	164	940
3A	2880	778	639	134	807	807	0
3B	500	169	109	60	120	100	20
6A	476	110	50	60	100	49	51
6B	474	95	25	70	58	56	2
TOTAL	7239	1593	1093	495	2591	1343	1249

† **chromosome location**

Table 4.3. Height QTL streams and families of selected BC2F4 recombinants

QTL location	Increasing height allele	Stream	Families	Number of selected BC2F4 Recombinants
2D (2Do)	Cadenza	AC162-E24-4	AC162-E24-4-1	9
			AC162-E24-4-9	6
			AC162-E24-4-10	7
			AC162-E24-4-15	8
(2Da)		AC113-E67-9	AC113-E67-9-7	14
		AC113-E67-17	AC113-E67-17-7	16
		AC113-E67-30	AC113-E67-30-7	20
3A	Cadenza	AC179-E27-2	AC179-E27-2-8	27
			AC179-E27-2-15	49
3B	Avalon	AC160-E28-4	AC160-E28-4-2	27
			AC160-E28-4-3	27

			AC160-E28-4-13	15
			AC160-E28-4-23	31
6A	Avalon	AC89-E5-1	AC89-E5-1-1	4
			AC89-E5-1-3	9
			AC89-E5-1-14	14
			AC89-E5-1-16	8
			AC89-E5-1-23	12
6B	Cadenza	AC75-E101-3	AC75-E101-3-9	13
			AC75-E101-3-11	9
			AC75-E101-3-19	13
			AC75-E101-3-22	20

Field trials

Field trials of the recombinant lines were conducted at Church Farm, Bawburgh, Norfolk, UK in 2013 (one monodrill trial) and 2014 (one monodrill and one/two Hege 90 trials) (**Table 4.4**, **Images 4.1** and **4.2)**. Each recombinant was grown in three replicates, in plots with a fully randomised design, and grown according to standard agronomic practice, except PGRs were not used. At least one row or plot of Avalon, Cadenza and the NIL parents for each QTL were included in each replicate. Crop height was measured in cm from ground level to the collar (2013 only) and top of the ear and the mean of the three reps used in the analysis (unless otherwise stated). For each QTL there was a clear difference between the NIL parents carrying the Avalon and Cadenza alleles.

Table 4.4. Trial names and types, and phenotypic data recorded

Trial Name (*Sown)*	Gen.	Number of Lines	Trial Type	Reps	Phenotypic data recorded	Comment
(Feb 2013)						
2DoHt2013	BC2F4	41	Field Single Row Spacing 12.5cm 1x2m plot*****	3	Height at collar and top of ear, all plants	
3AHt2013	BC2F4	76	Field Single Row Spacing 12.5cm 1x2m plot*****	3	Height at collar and top of ear, all plants	
3BHt2013	BC2F4	100	Field Single Row Spacing 12.5cm 1x2m plot*****	3	Height at collar and top of ear, all plants	
6AHt2013	BC2F4	49	Field Single Row Spacing 12.5cm 1x2m plot*****	3	Height at collar and top of ear, all plants	
6BHt2013	BC2F4	56	Field Single Row Spacing 12.5cm 1x2m plot*****	3	Height at collar and top of ear, all plants	
(Oct/Nov 2013)						
2DoHt2014	BC2F4	41	Field Single Row Spacing 12.5cm 1x2m plot*	3	x	Repeat trial. *Failed*
3AHt2014m	BC2F4	76	Field Single Row Spacing 12.5cm 1x2m plot*	3	Height at top of ear, mean of row	Repeat trial *Poor growth*
3BHt2014m	BC2F4	100	Field Single Row Spacing 12.5cm 1x2m plot*	3	Height at top of ear, mean of row	Repeat trial
6AHt2014m	BC2F4	49	Field Single Row Spacing 12.5cm 1x2m plot*	3	Height at top of ear, mean of row	Repeat trial
6BHt2014m	BC2F4	56	Field Single Row Spacing 12.5cm 1x2m plot*	3	Height at top of ear, mean of row	Repeat trial
2DoHt2014H90	BC2F5	41	Hege90 1 metre square**^**	3	Height at top of ear, mean of row	
3AHt2014H90	BC2F5	76	Hege90 1 metre square**^**	3	Height at top of ear, mean of row	
3BHt2014H90	BC2F5	100	Hege90 1 metre square**^**	3	Height at top of ear, mean of row	
6AHt2014H90	BC2F5	49	Hege90 1 metre square**^**	3	Height at top of ear, mean of row	
6BHt2014H90	BC2F5	56	Hege90 1 metre square**^**	3	Height at top of ear, mean of row	
3AHt2014F4B1	3A F4 Batch 2	400	Field Single Row Spacing 12.5cm 1x2m plot*	3	x	Repeat trial. *Failed*
(Mar 2014)						
2DaHt2014s	BC2F4	50	Haldrup30 1 metre square, 3 rows**+**	3	Height at top of ear, mean of row	
3AHt2014F4B2s	3A F4 Batch 2	418	Haldrup30 1 metre square, 3 rows**+**	3	Height at top of ear, mean of row	Incomplete vernalisation; data not shown

***** 4 rows with 'Soisson' and dwarf (*Rht-12*) or Avalon as markers

^ 6 rows with rows 1 and 6 being 'Soisson' to prevent edge effects and rows 2-5 being the BC2F5. 'Soisson' and dwarf (*Rht-12*) were used as markers

+ 6 rows with a 3 row offset. 'Soisson' was used as marker.

Image 4.1. Trial overview of recombinant lines

Image 4.2. Near Inbred Lines with and without the height QTL on chromosome 2D

(Images courtesy Oscar Gonzalez).

Both Avalon and Cadenza carry the recessive photoperiod sensitive alleles of *Ppd-D1* and *Ppd-B1*. Avalon is a winter type; Avalon carries recessive alleles for all *Vrn-1* homoeoalleles of *vrn-A1*, *vrn-B1*, and *vrn-D1* (alleles affecting vernalisation requirement) while Cadenza carries the dominant spring allele *Vrn-A1a* together with *vrn-B1* and *vrn-D1*. The *Vrn-1A* gene was observed to be segregating in some families of some of the selected lines (3A, 6A and 6B) and was considered unlikely to affect height if the crop was fully vernalised. However some of the results obtained suggest that incomplete vernalisation, due to the spring sowing in 2013, has had an influence on height in one of these trials.

Weather data for the duration of the 2013 and 2014 field trials is shown in **Figure 4.1**. This data is courtesy of the Buxton Weather Station (http://www.buxton-weather.co.uk/weather.htm), approximately 15 km from Bawburgh.

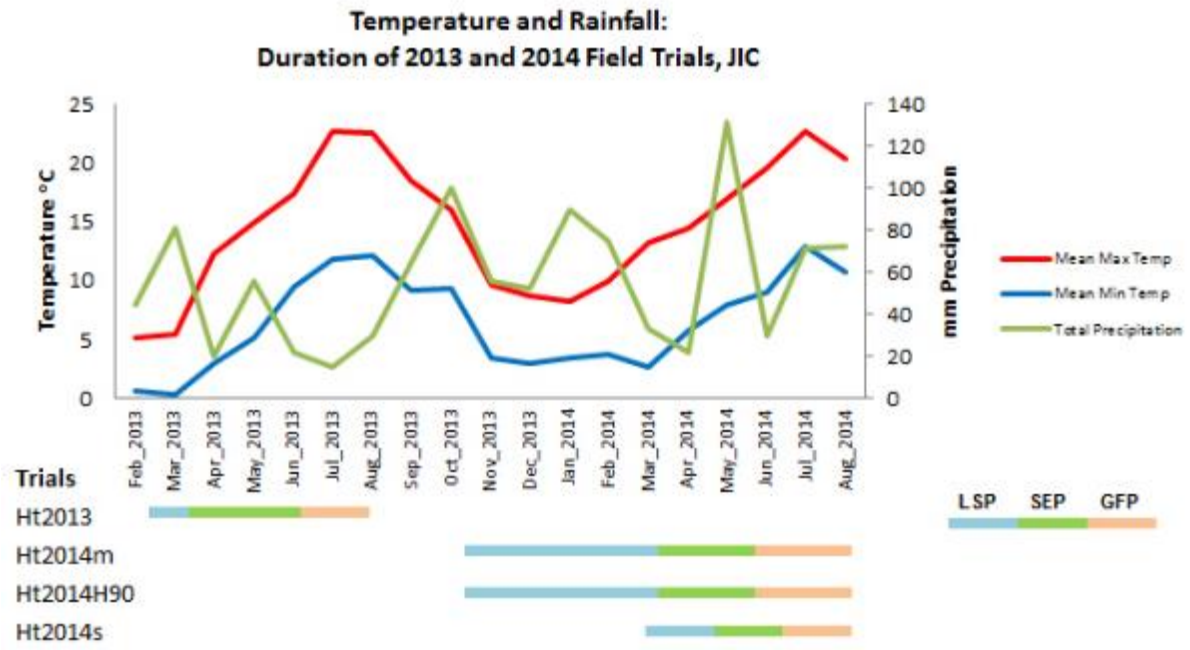


Figure 4.1. Summary of temperature and rainfall during the 2013 and 2014 experiments
LSP = period from seedling emergence to first node detectable; SEP = from first node detectable to anthesis; GFP = the period from anthesis to physiological maturity

The temperature data for the **Ht2013** BC2F4 trials suggest that there may not have been a sufficient period of vernalisation for those lines with the *vrn-A1*. The **Ht2014m** and **Ht2014H90** trials had sufficient time for the vernalisation requirement to be met. The **Ht2014s** trials received very little vernalisation and those plants in the **3AHt2014F4B2s** trial carrying the *vrn-A1* never reached maturity so data from this trial is not shown.

Due to insufficient BC2F4 seed, the additional 3A recombinants (around barc19) could not be included in the 2013 and 2014 field trials. However, this germplasm of recombinants very close to

the height QTL on chromosome 3A will provide a useful future resource for fine-mapping this region.

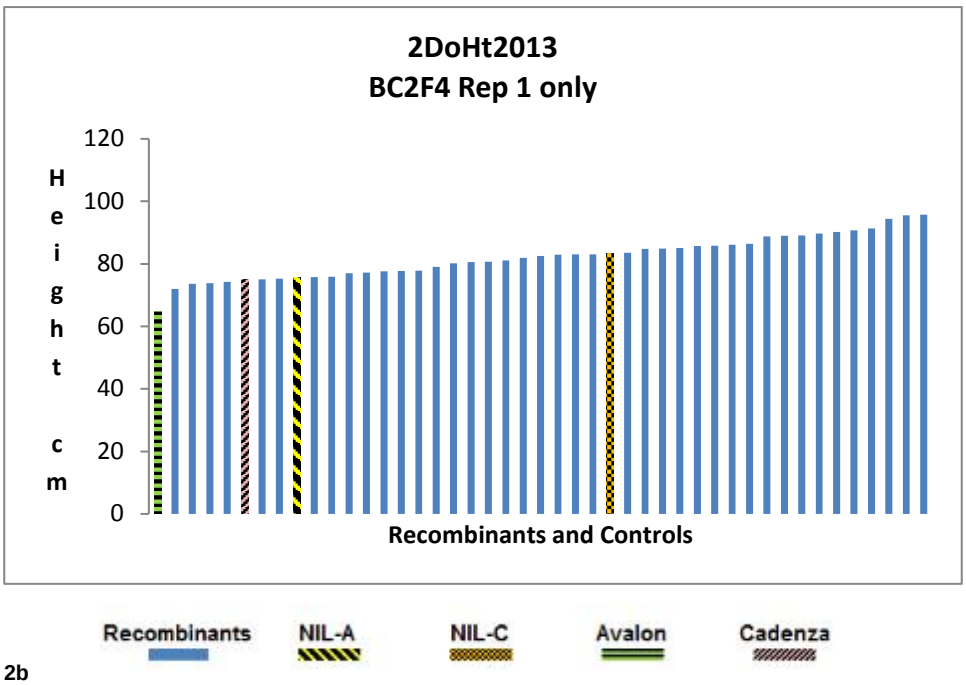
The spring 2013 trials (**Ht2013**) were drilled with a new Wintersteiger Monoseed B drill but insufficient optimisation of the equipment and poor weather resulted in some mixing of seed between adjacent plots or poor drilling. This led to the exclusion of some data in the analyses below. Seed was harvested from the field-grown BC2F4 plants, where we believed least mixing had occurred, and this BC2F5 seed was used in the **Ht2014H90** trials. Given the 2013 drilling problems there was also concern about the provenance of some BC2F5 seed; leaf material was harvested from the BC2F5 field plants and genotyped, any lines which did not give the expected genotype (from the BC2F4 genotyping) were also excluded.

As some of the BC2F4 seed stocks used for the **Ht2014m** trials were low the addition of seed from the phenotypically distinct ‘Soisson’ were used to raise the plant numbers in each row, so as to prevent ‘edge effects’ in a sparse row, these were not included in the phenotyping. There was also a problem with the top third of these trials (especially **3AHt2014m**) in which the soil composition changed to a more sandy texture and this caused a general reduction in plant growth.

4.1.3. Results

Height data (top of ear) from the field trials was analysed using Microsoft Excel to produce histograms (see **Figures 4.2–4.16**). In cases where single gene segregation is occurring, all other genes controlling height are fixed, and environmental variance is low, we expect to see a bimodal segregation for crop height. In these perfect cases we are able to move from quantitative to qualitative genetics, effectively scoring lines as ‘tall’ or ‘short’. In less clear cut cases a bimodal segregation might not be achieved and techniques of quantitative genetics are required to assess correlation of phenotype with genotype. Height data to the collar (2013 trial) produced results so similar to full crop height that it was considered duplication and therefore discontinued. Data from all trials, for each QTL, is presented. Data from all streams for each QTL have been combined.

2a



2b

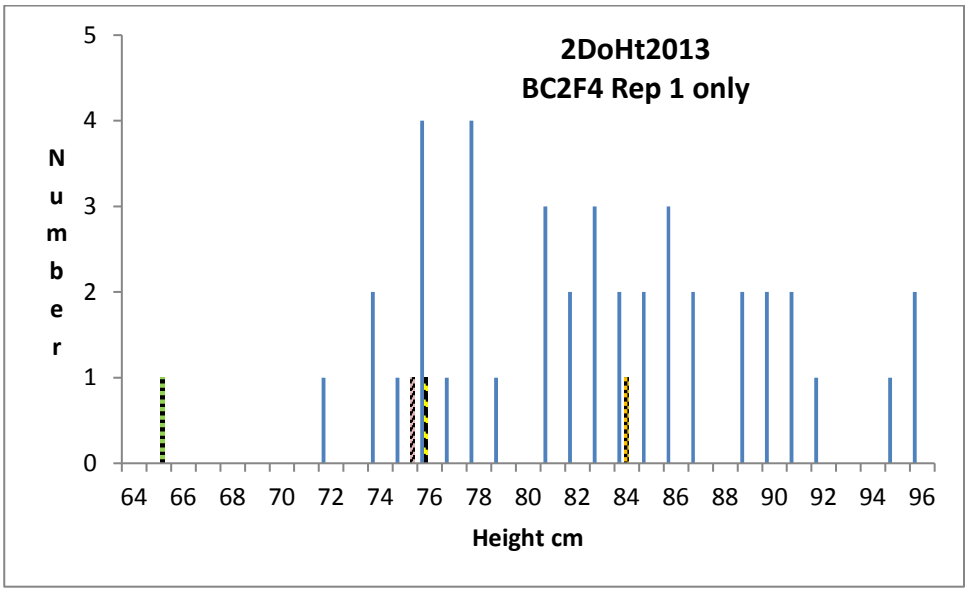
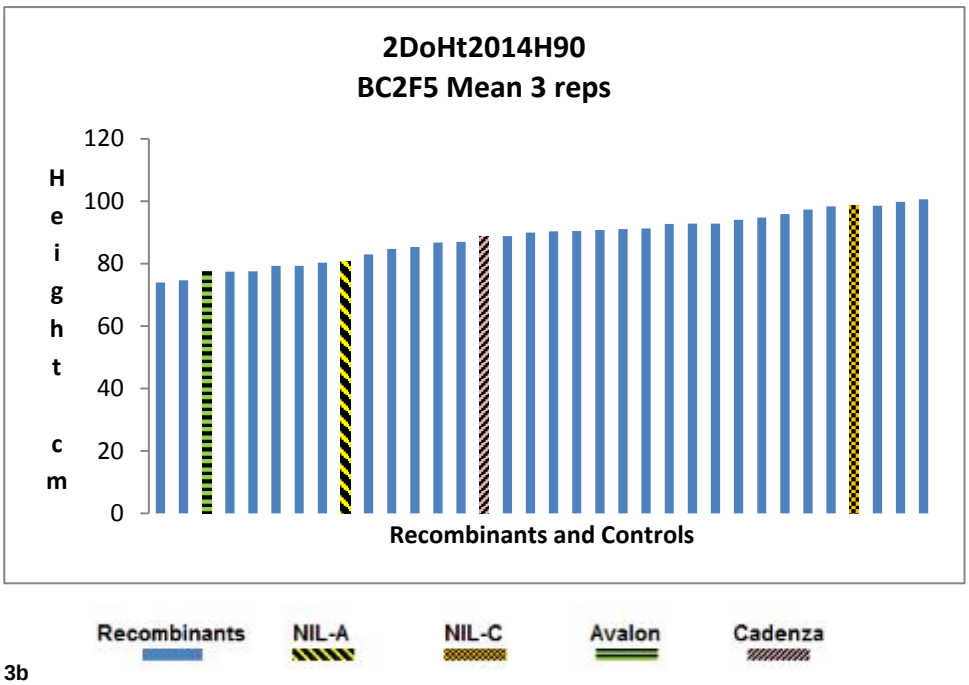


Figure 4.2. 2DoHt2013 trial for recombinant lines around height QTL 2D

Only data from **2DoHt2013** Rep1 was used in the analysis due to the problem affecting the drilling of Reps 2 and 3 in this trial. **Figure 4.2a** shows a clear distinction in the height of the 2D NIL-A and NIL-C parents, with NIL-C carrying the increasing allele. A spread of height is observed in the BC2F4 recombinants, from 72.0 to 95.8 cm, and considerable transgression is observed; particularly there are many recombinants taller than NIL-C. **Figure 4.2b** shows a poor bimodal distribution of height but the parental NILs are located in different peaks. The Avalon control is short as expected but the Cadenza control is unexpectedly quite short, probably due to its position in a poorly drilled area of the field. (The Avalon and Cadenza controls are the same for *all* the **Ht2013** trials).

3a



3b

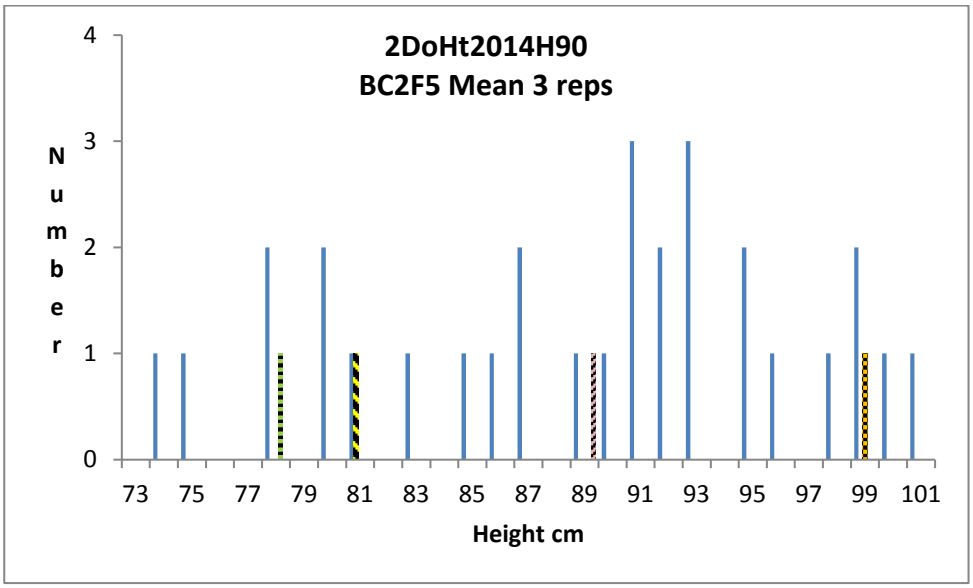
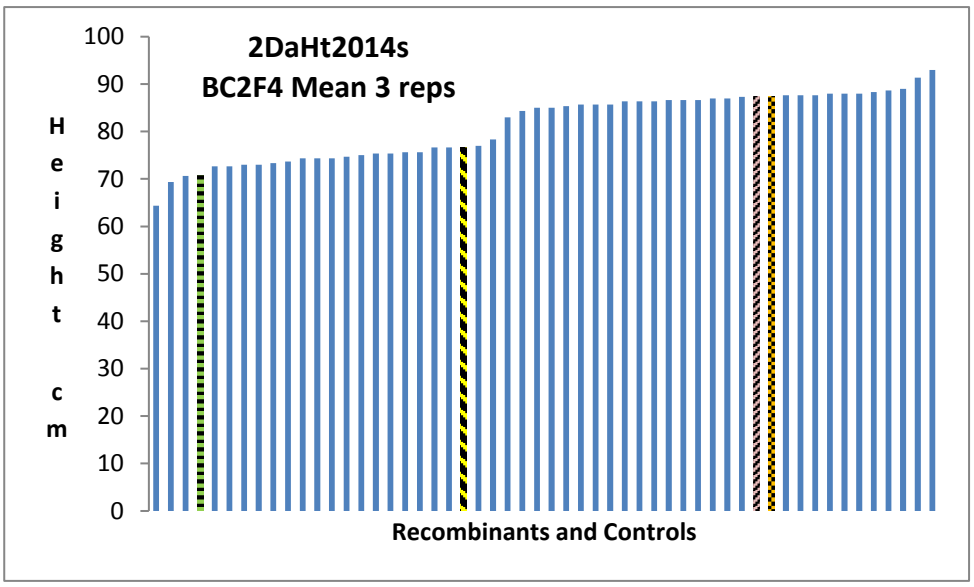


Figure 4.3. 2DoHt2014H90 trial for recombinant lines around height QTL 2D

Data from all three reps of the **2DoHt2014H90** trial were used in this analysis however as the seed used in this trial was harvested from the **2DoHt2013** field trial there was concern about the data from approximately 25% of the lines and these were excluded from the analysis. **Figure 4.3a** shows a clear distinction in the height of the 2D NIL-A and NIL-C parents, with NIL-C carrying the increasing allele. A spread of height was observed in the BC2F5 recombinants, from 74.0 to 100.6 cm, and some transgression is observed.

Figure 4.3b shows a weak bimodal distribution of height but with the NIL parents in separate peaks, as are the Avalon and Cadenza controls.

4a



4b

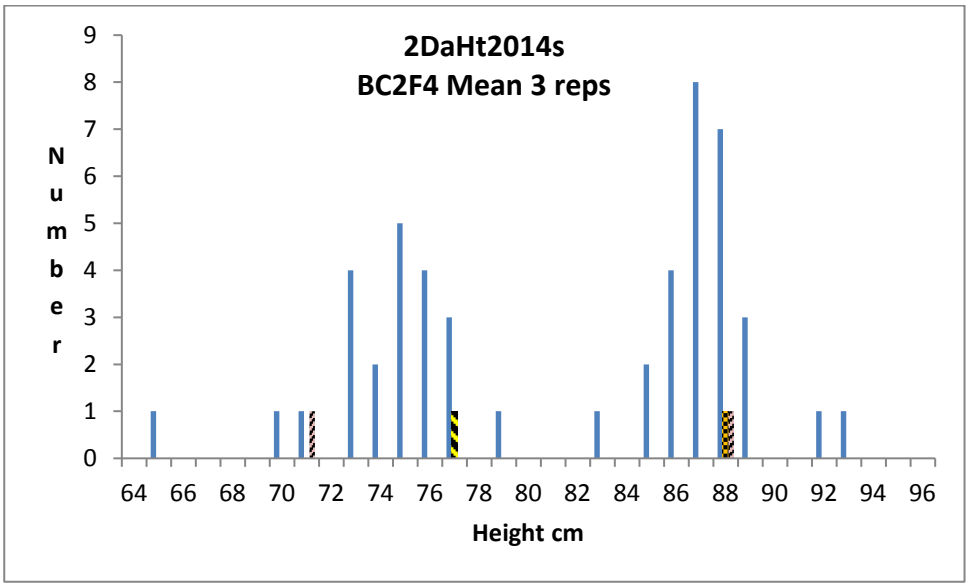
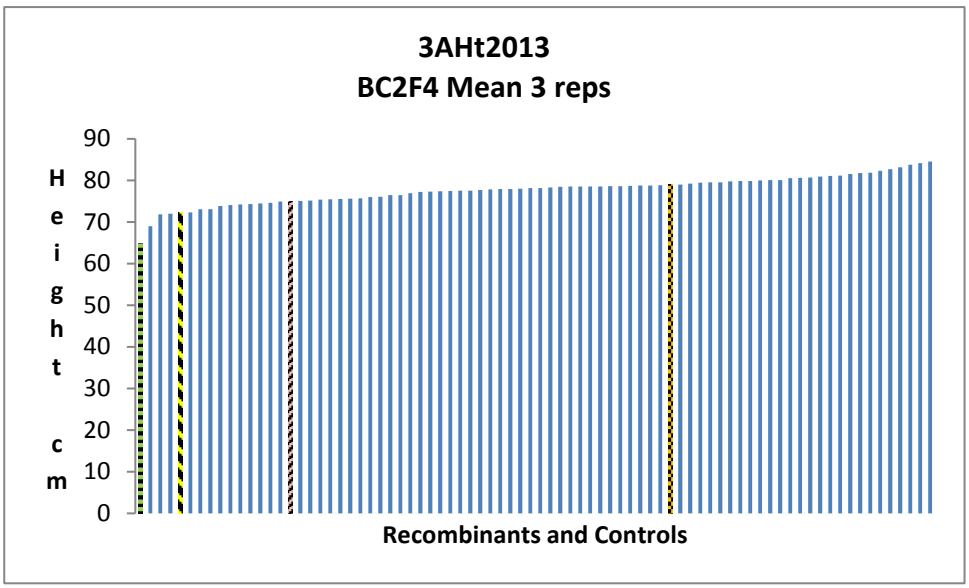


Figure 4.4. 2DaHt2014 trial for recombinant lines around height QTL 2D

The *2Da* BC2F4 recombinants are additional 2D lines which were not included in the original field trials of 2013 and originate from different streams. **Figure 4.4a** shows a clear distinction in the height of the 2D NIL-A and NIL-C parents, with NIL-C carrying the increasing allele. A spread of height is seen in the BC2F4 recombinants, from 64.3 to 93.0 cm, and considerable transgression was observed with many recombinants either shorter than NIL-A or taller than NIL-C. **Figure 4.4b** shows a clear bimodal distribution with the NIL parents in separate peaks, as are the Avalon and Cadenza controls.

5a



5b

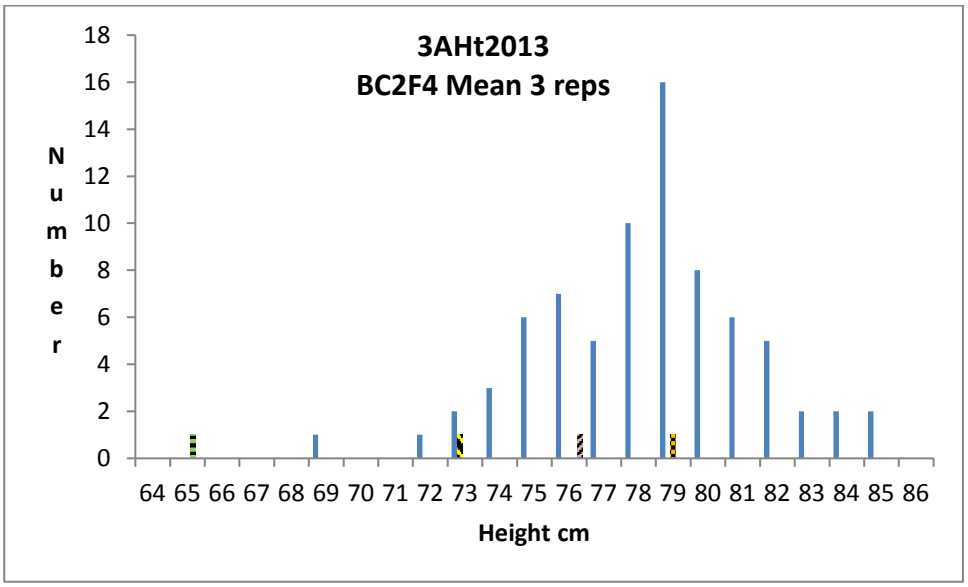
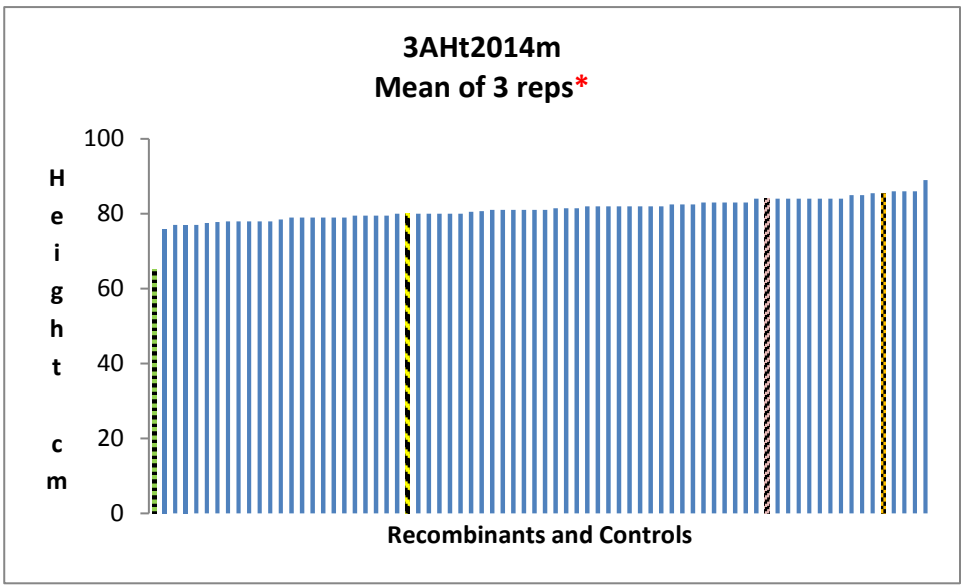


Figure 4.5. 3AHt2013 trial for recombinant lines around height QTL 3A

Figure 4.5a shows a clear distinction in the height of the 3A NIL-A and NIL-C parents, with NIL-C carrying the increasing allele. A spread of height was observed in the BC2F4 recombinants, from 69.0 to 84.6 cm, and some transgression was observed with many plants taller than NIL-C; 20% of these taller plants carry the *vrn-A1* and it is possible that insufficient vernalisation meant these plants grew taller before flowering. **Figure 4.5b** shows a weak bimodal distribution but the NIL parents are in separate peaks.

6a



6b

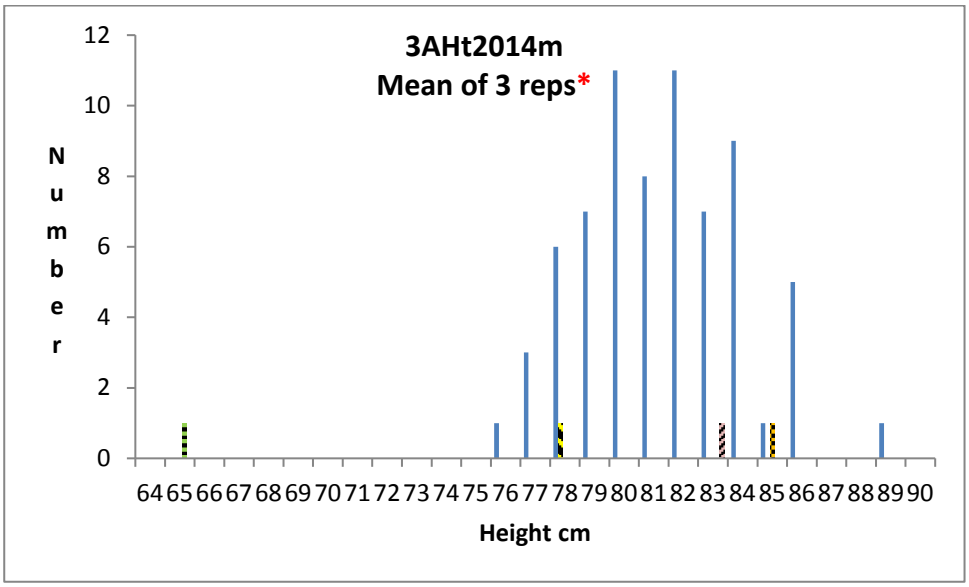
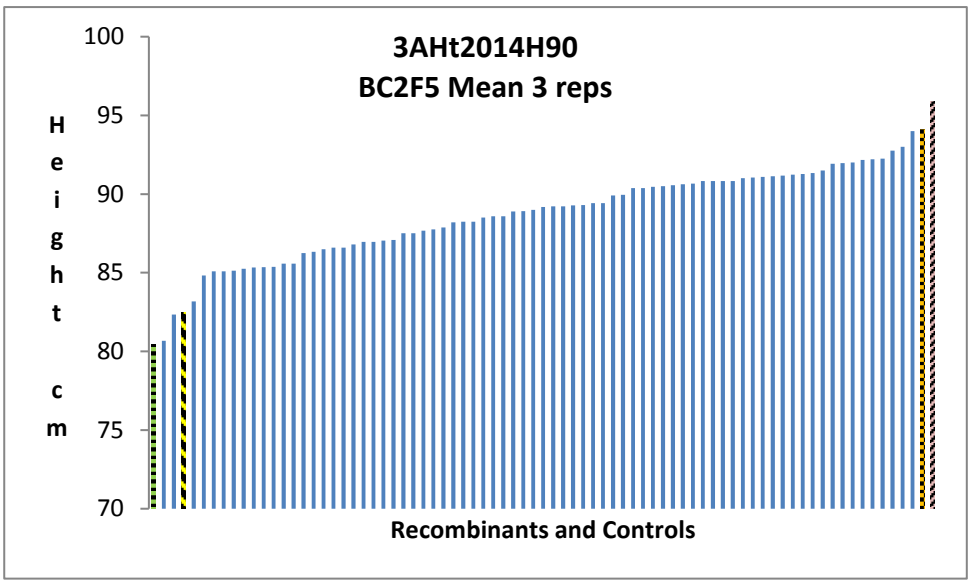


Figure 4.6. 3AHt2014m trial for recombinant lines around height QTL 3A

The data from the 3AHt2014m trial is poor due to drilling problems and variable soil composition. **Figure 4.6a** shows a clear distinction in the height of the 3A NIL-A and NIL-C parents, with NIL-C carrying the increasing allele. A spread of height is observed in the BC2F4 recombinants, from 76.0 to 89.0 cm, and some transgression is observed with many plants shorter than NIL-A. All those lines carrying the *vrn-A1* were shorter than NIL-C (compared to **3AHt2013**). **Figure 4.6b** shows a poor bimodal distribution.

7a



7b

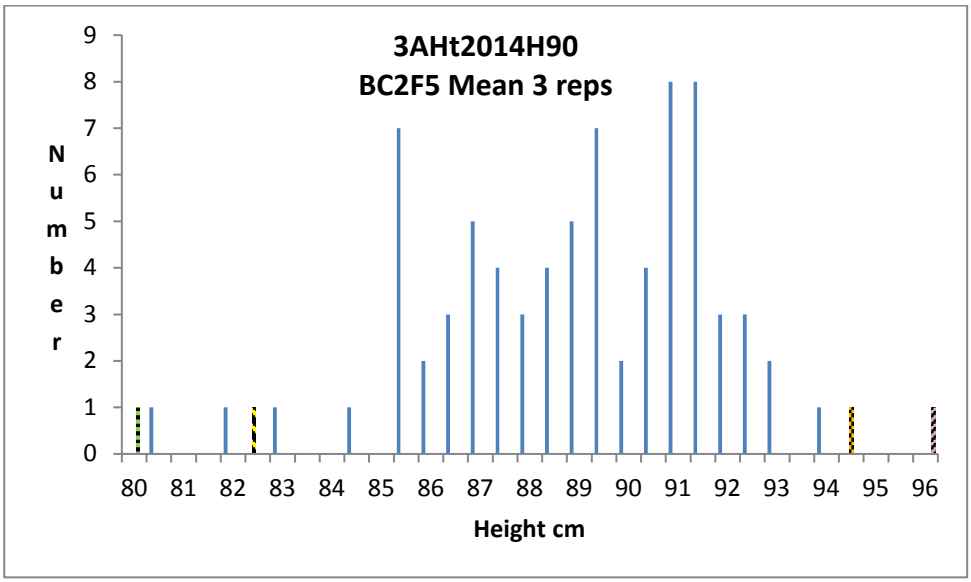
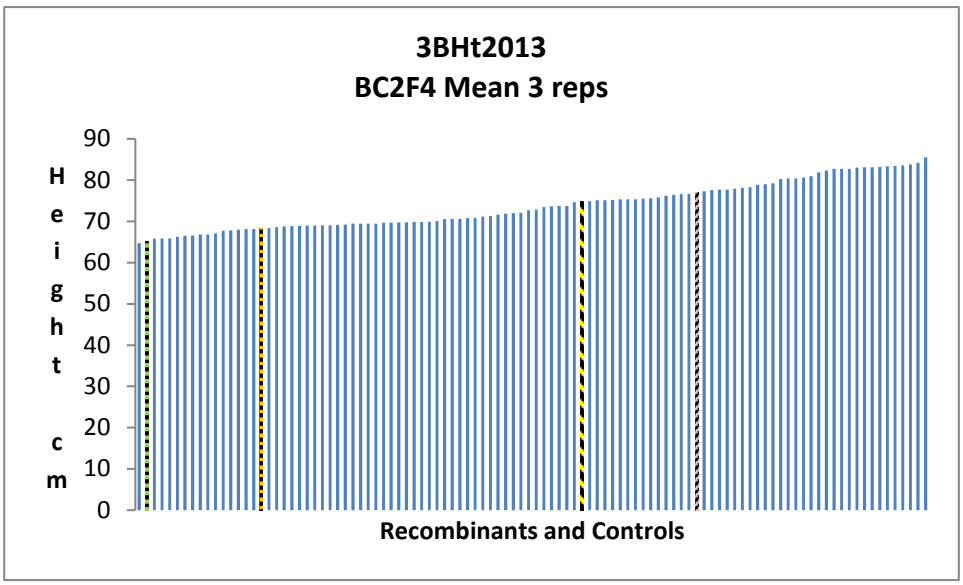


Figure 4.7. 3AHt2014H90 trial for recombinant lines around height QTL 3A

Figure 4.7a shows that the 3A NIL-A and NIL-C parents are at the extremes of the height spectrum, with NIL-C carrying the increasing allele. A spread of height is seen in the BC2F5 recombinants, from 80.7 to 94 cm, but essentially no transgression was observed. **Figure 4.7b** shows a weak bimodal distribution with the NIL parents, and the Avalon and Cadenza controls outside the peaks.

8a



8b

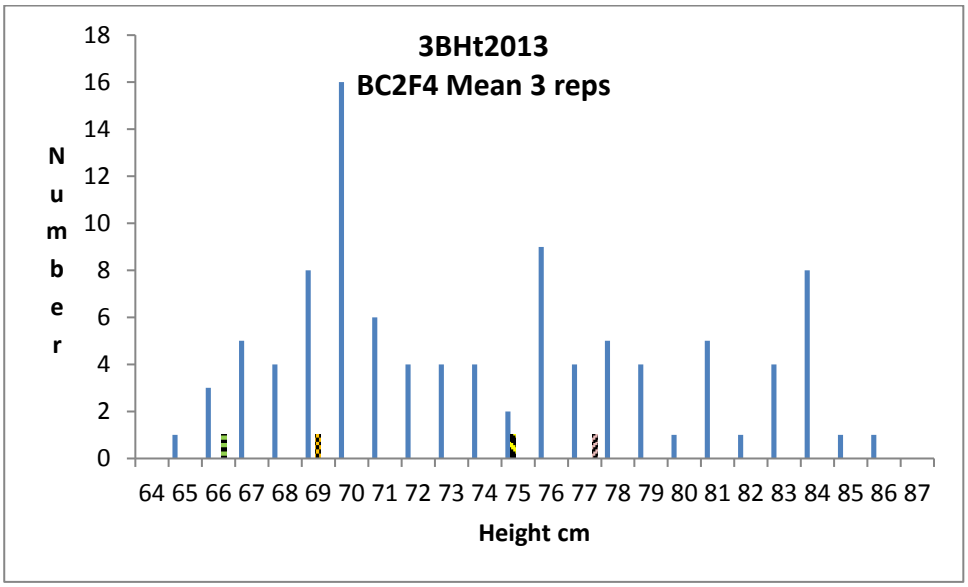
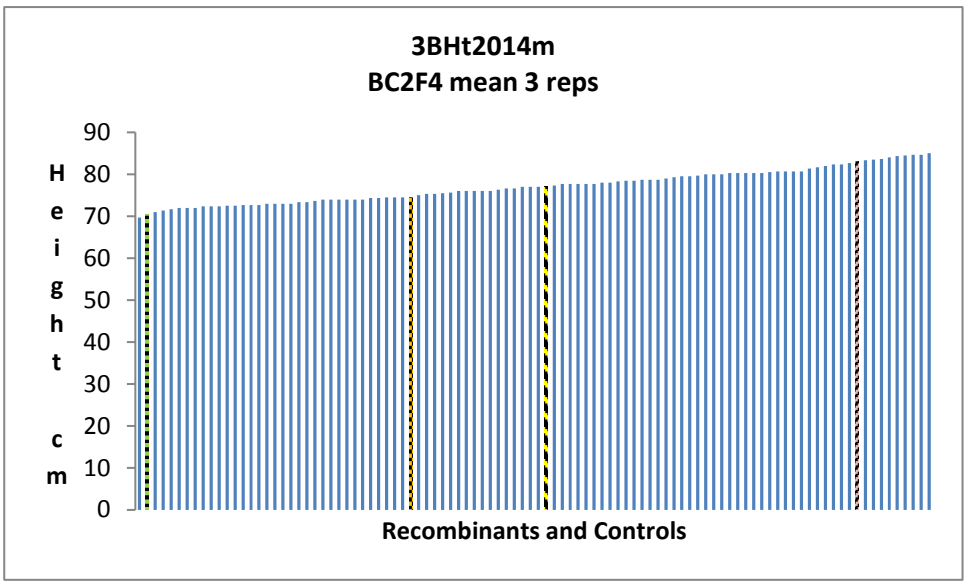


Figure 4.8. 3BHt2013 trial for recombinant lines around height QTL 3B

Figure 4.8a shows that the 3B NIL-A and NIL-C parents were fairly close in height, with NIL-A carrying the increasing allele. A spread of height was seen in the BC2F4 recombinants, from 79.2 to 95.7 cm, and considerable transgression was observed with some recombinants shorter than NIL-C and many taller than NIL-A. **Figure 4.8b** shows a reasonable bimodal distribution although the NIL parents are not clearly in separate peaks, while the Avalon and Cadenza controls are in different peaks.

9a



9b

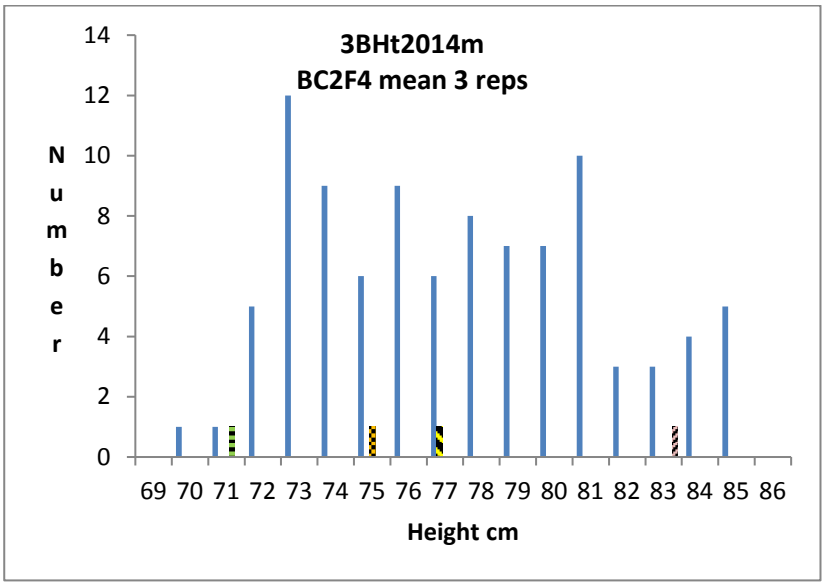
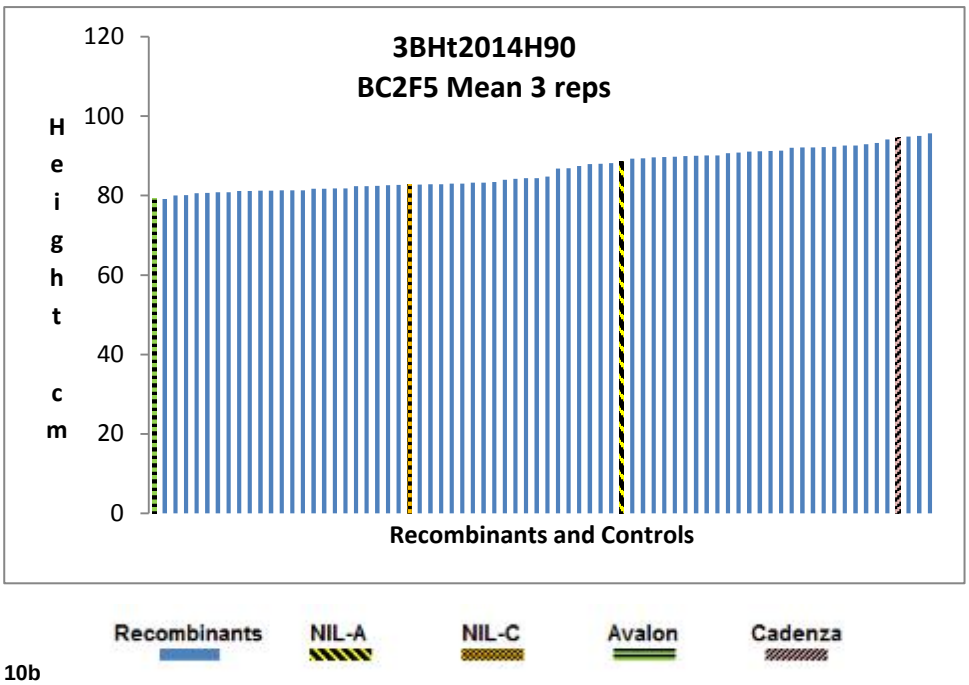


Figure 4.9. 3BHt2014m trial for recombinant lines around height QTL 3B

Figure 4.9a shows that the 3B NIL-A and NIL-C parents are close in height, with NIL-A carrying the increasing allele. A spread of height was seen in the BC2F4 recombinants, from 69.7 to 85.0 cm, and considerable transgression was observed with many recombinants shorter than NIL- C or taller than NIL-A. **Figure 4.9b** shows a poor bimodal distribution with the NIL parents not found in separate peaks, while the Avalon and Cadenza controls are in different peaks.

10a



10b

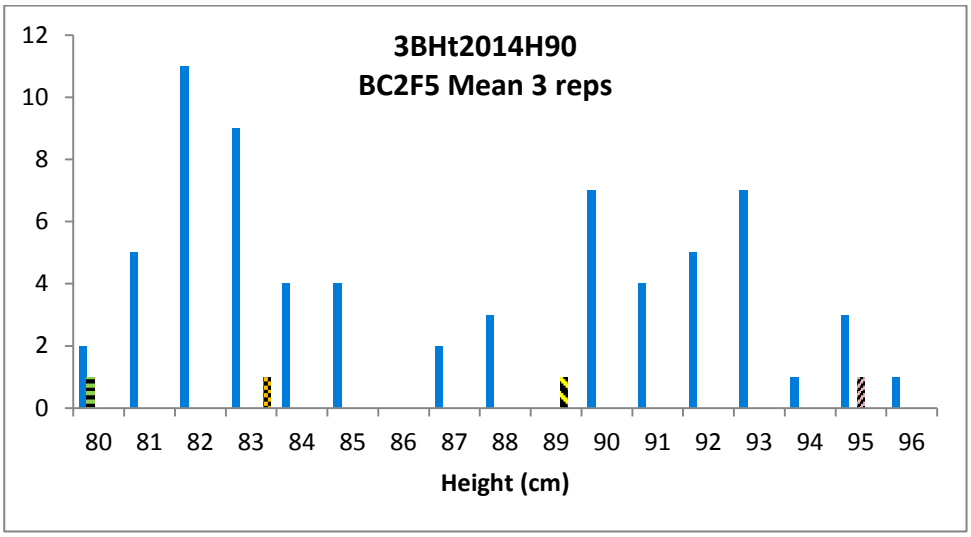
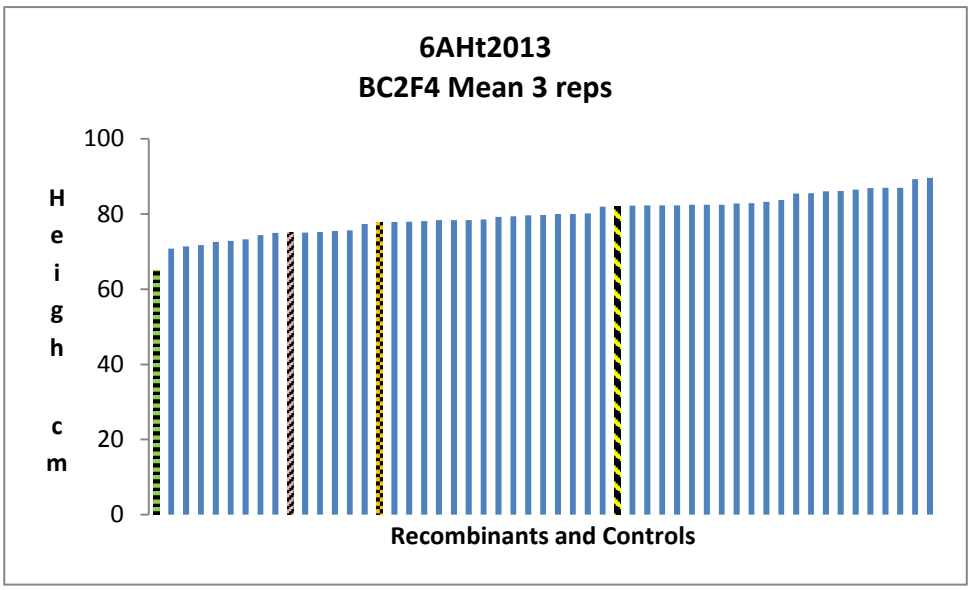


Figure 4.10. 3BHt2014H90 trial for recombinant lines around height QTL 3B

Data from all three reps of the **3BHt2014H90** trial were used in this analysis however as the seed used in this trial was harvested from the **3BHt2013** field trial there was concern about the data from approximately 30% of the lines and these were excluded from the analysis. **Figure 4.10a** shows that the 3B NIL-A and NIL-C parents are fairly close in height, with NIL-A carrying the increasing allele. A spread of height was seen in the BC2F5 recombinants, from 79.2 to 95.7 cm, and considerable transgression was observed with many recombinants either shorter than NIL-C or taller than NIL-A. **Figure 4.10b** shows a clear bimodal distribution with the NIL parents in separate peaks, as are the Avalon and Cadenza controls.

11a



11b

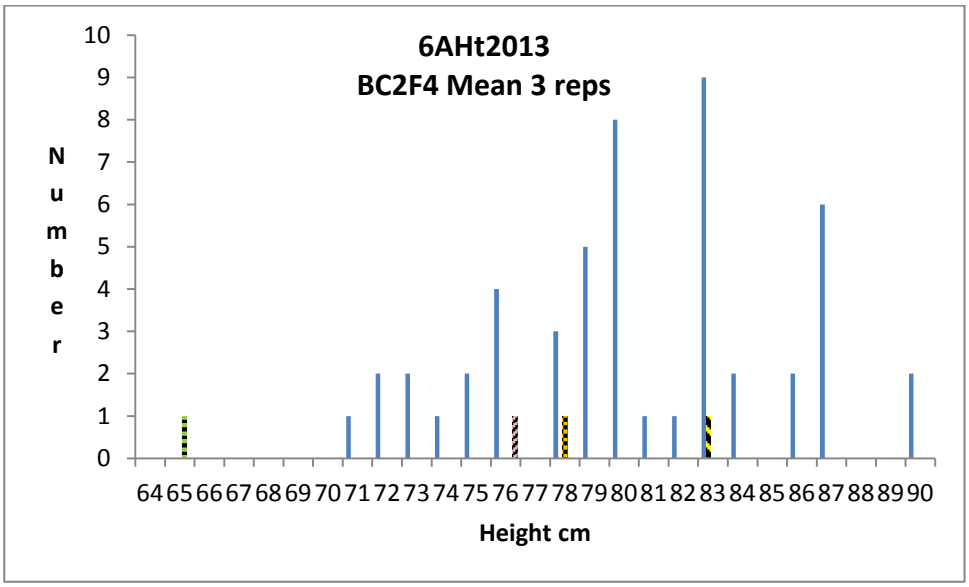
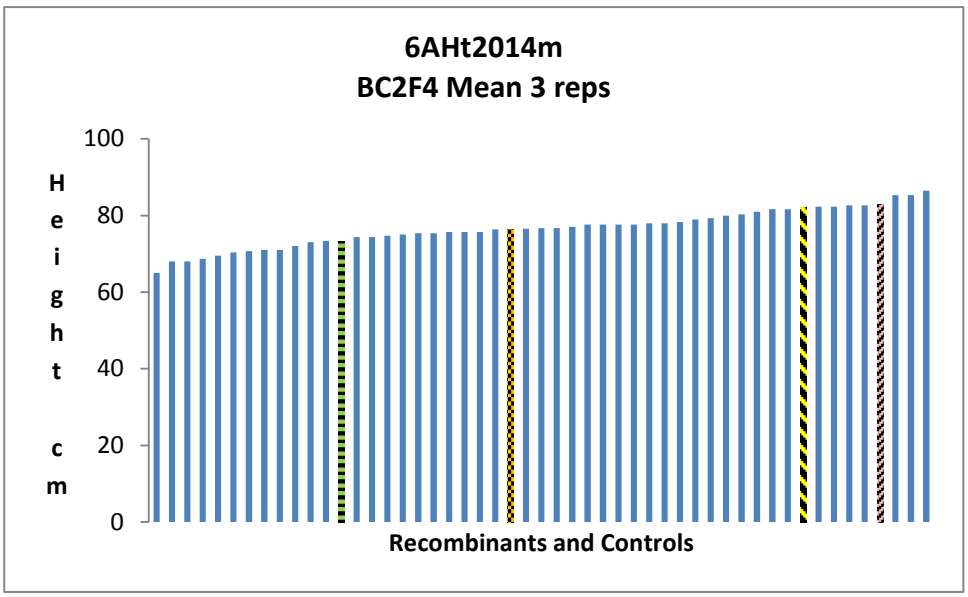


Figure 4.11. 6AHt2013 trial for recombinant lines around height QTL 6A

Figure 4.11a shows that the 6A NIL-A and NIL-C parents are fairly close in height, with NIL-A carrying the increasing allele. A spread of height was seen in the BC2F4 recombinants, from 70.8 to 89.7 cm, and considerable transgression was observed with many recombinants either shorter than NIL-C or taller than NIL-A. **Figure 4.11b** shows a weak bimodal distribution however the NIL parents are located in separate peaks.

12a



12b

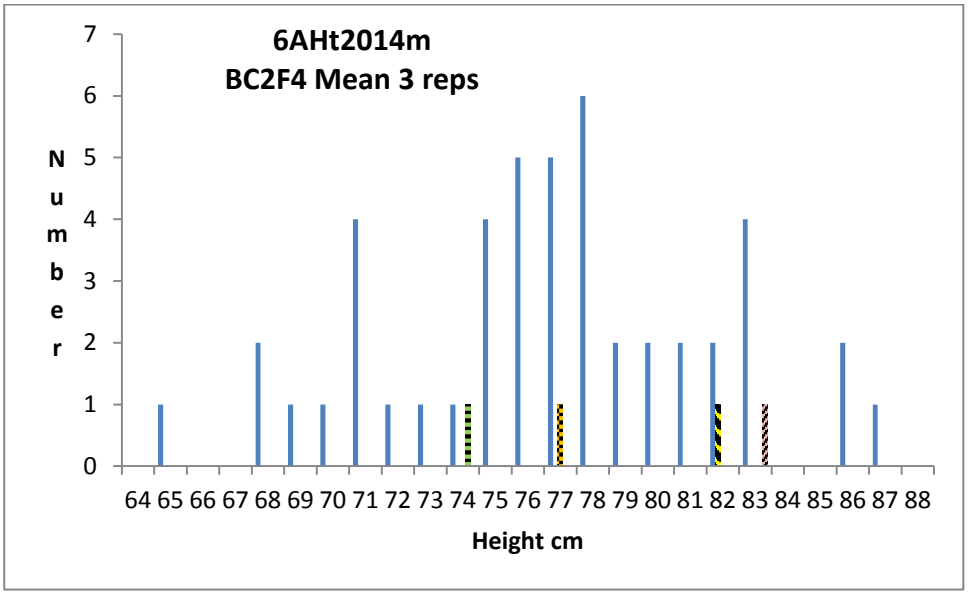
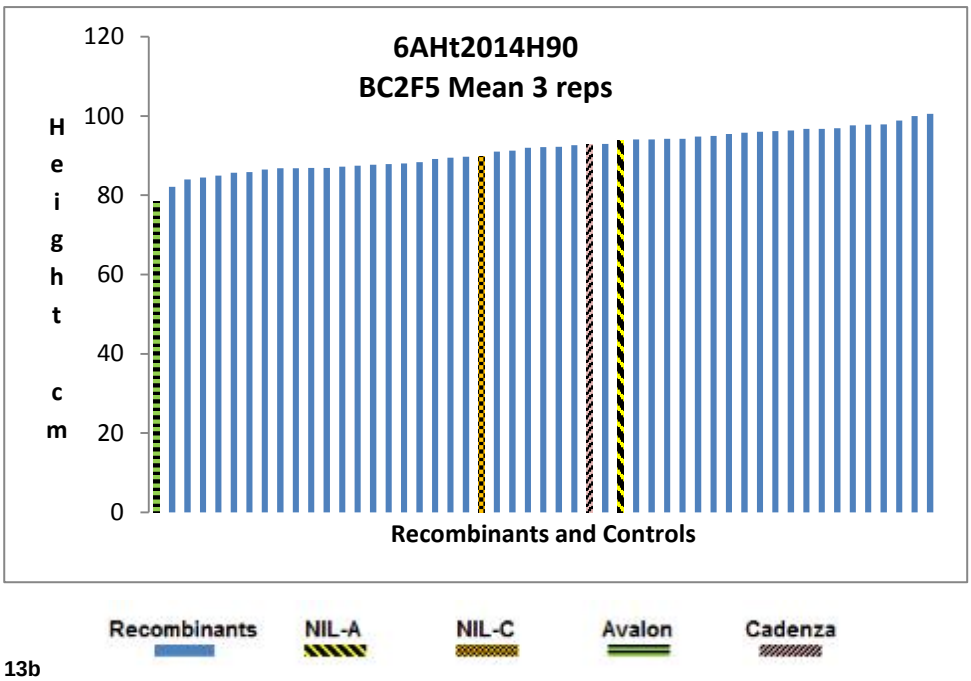


Figure 4.12. 6AHt2014m trial for recombinant lines around height QTL 6A

Figure 4.12a shows that the 6A NIL-A and NIL-C parents are fairly close in height, with NIL-A carrying the increasing allele. A spread of height was seen in the BC2F4 recombinants, from 65.0 to 86.5 cm, and considerable transgression was observed with many recombinants either shorter than NIL-C. **Figure 4.12b** shows a poor bimodal distribution however the NIL parents are located in separate peaks, as are the Avalon and Cadenza controls.

13a



13b

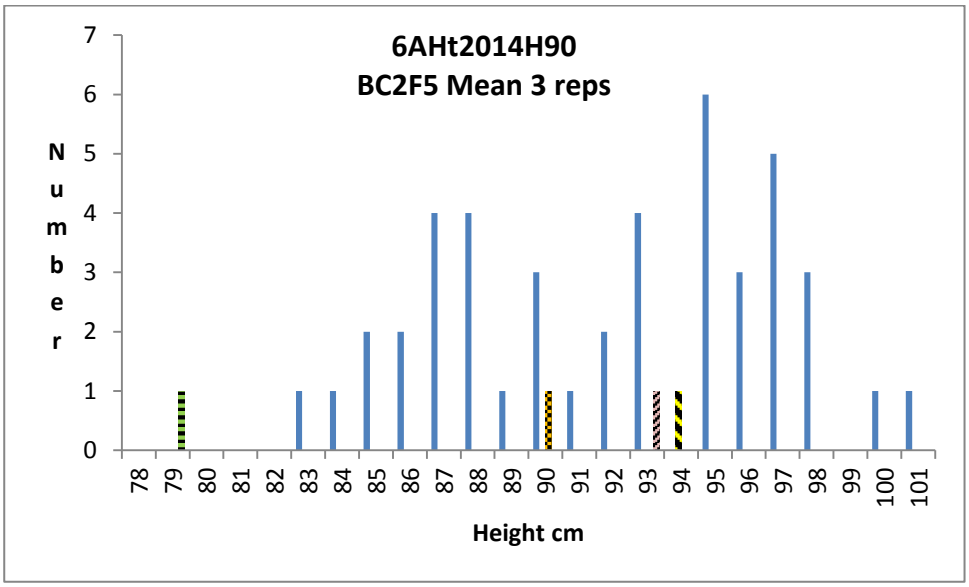
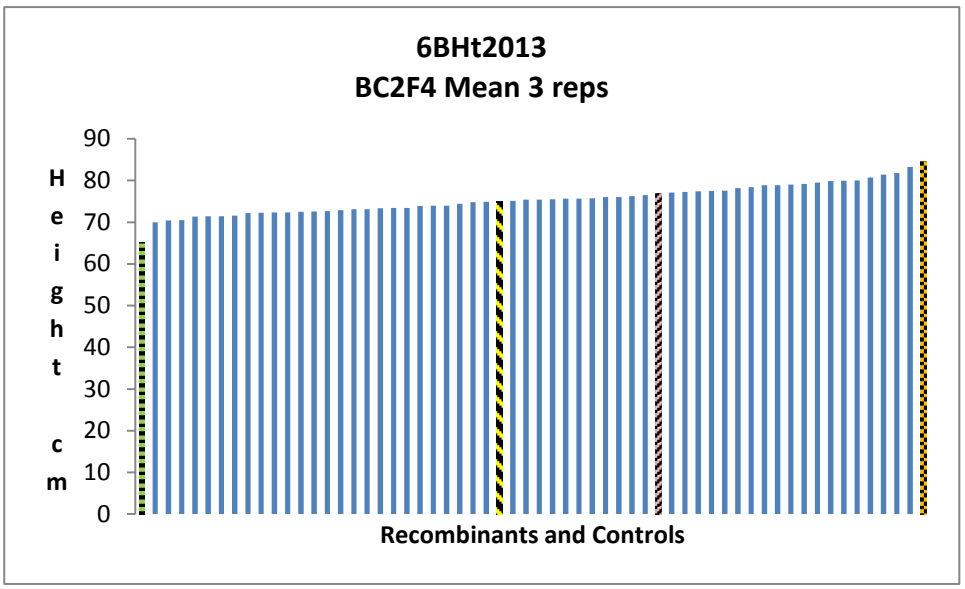


Figure 4.13. 6AHt2014H90 trial for recombinant lines around height QTL 6A

Data from all three reps of the **6AHt2014H90** trial were used in this analysis, however as the seed used in this trial was harvested from the **6AHt2013** field trial there was concern about the data from approximately 4% of the lines and these were excluded from the analysis.

Figure 4.13a shows that the 6A NIL-A and NIL-C parents are close in height, with NIL-A carrying the increasing allele. A spread of height was seen in the BC2F5 recombinants, from 82.1 to 100.5 cm, and considerable transgression was observed with many recombinants either shorter than NIL-C or taller than NIL-A. **Figure 4.13b** shows a weak bimodal distribution however the NIL parents are located in separate peaks as are the Avalon and Cadenza controls.

14a



14b

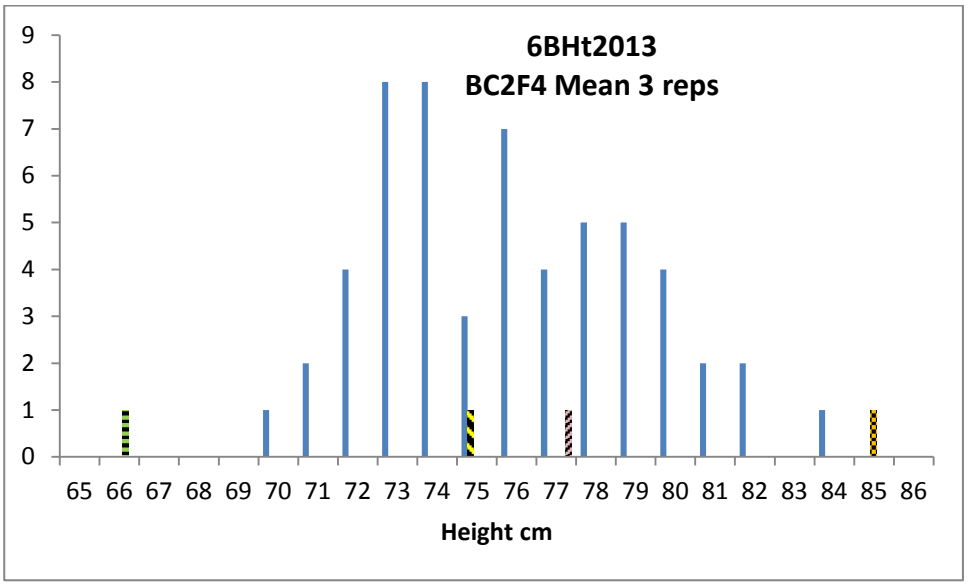
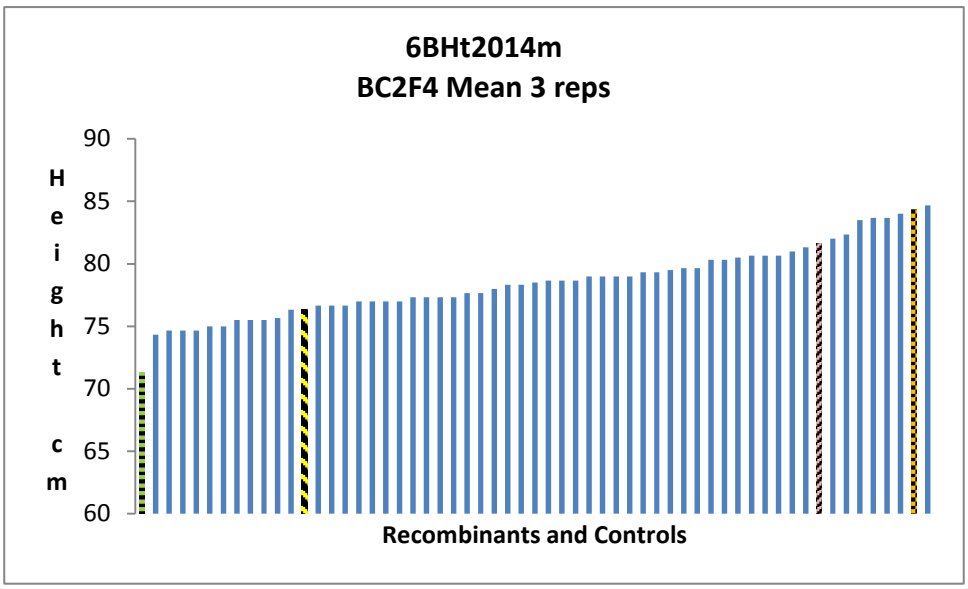


Figure 4.14. 6BHt2014 trial for recombinant lines around height QTL 6B

Figure 4.14a shows that the 6B NIL-C parent (increasing allele) is at the extreme of the height spectrum. A spread of height was seen in the BC2F4 recombinants, from 70.0 to 83.2 cm, and some transgression was observed with many recombinants shorter than NIL-A. **Figure 4.14b** shows a weak bimodal distribution but the NIL-C parent is outside the peaks.

15a



15b

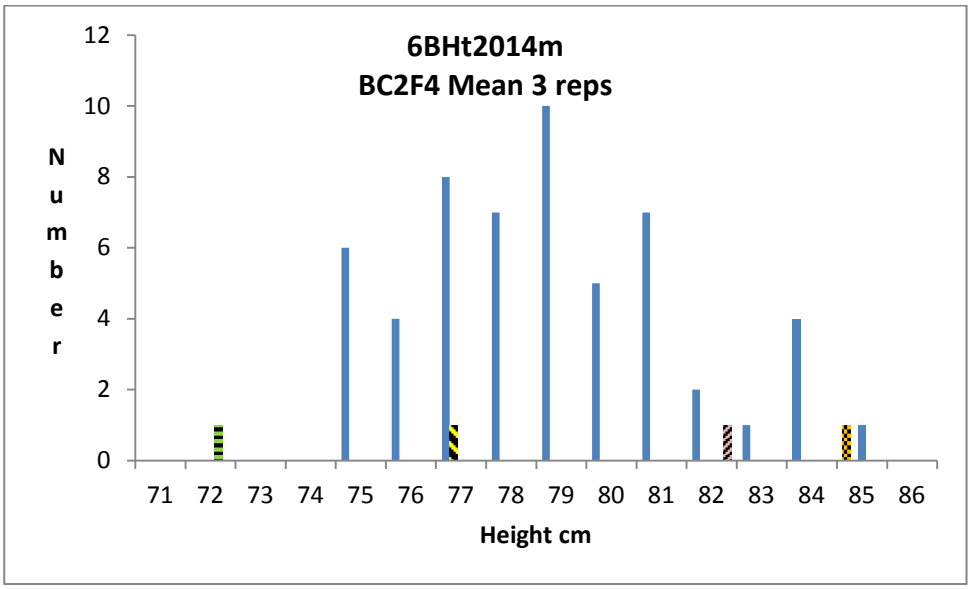
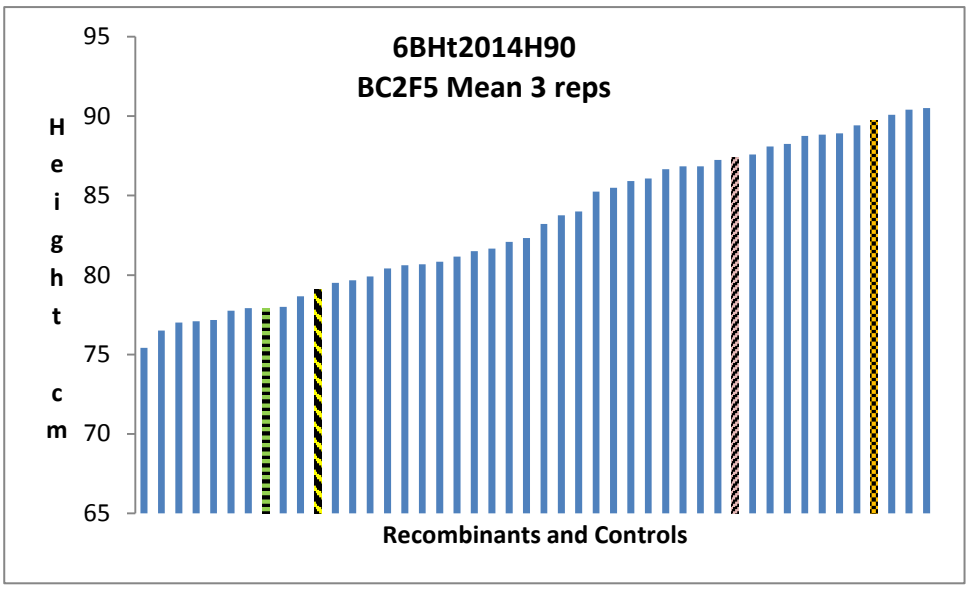


Figure 4.15. 6BHt2014m trial for recombinant lines around height QTL 6B

Figure 4.15a shows that the 6B NIL-A and NIL-C parents are quite close to the extremes of the height spectrum, with NIL-C carrying the increasing allele. A spread of height is seen in the BC2F4 recombinants, from 74.3 to 84.7 cm, and some transgression is observed with recombinants shorter than NIL-A. **Figure 4.15b** shows a poor bimodal distribution although the NIL parents are in different peaks.

16a



16b

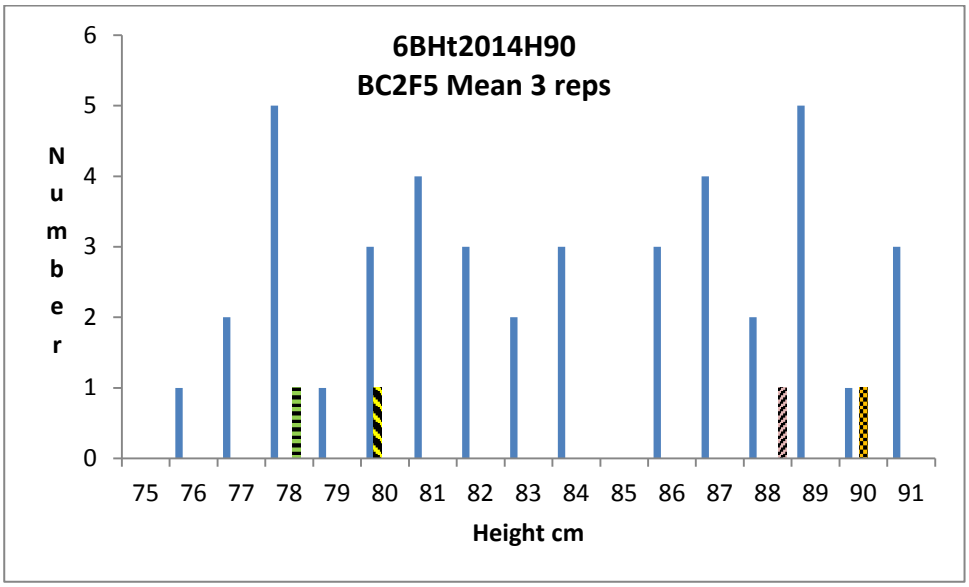


Figure 4.16. 6BHt2014H90 trial for recombinant lines around height QTL 6B

Data from all three reps of the **6BHt2014H90** trial were used in this analysis however as the seed used in this trial was harvested from the **6BHt2013** field trial there was concern about the data from approximately 20% of the lines and these were excluded from the analysis.

Figure 4.16a shows that the 6B NIL-A and NIL-C parents are quite close to the extremes of the height spectrum, with NIL-C carrying the increasing allele. A spread of height was seen in the BC2F5 recombinants, from 75.4 to 90.5 cm, and some transgression was observed with a few recombinants either shorter than NIL-A or taller than NIL-C. **Figure 4.16b** shows a good bimodal distribution with the NIL parents, and the Avalon and Cadenza controls, in separate peaks.

4.1.4. Summary

Near Isogenic Lines, derived from the A x C DH lines, and carrying alleles of genes for five different height QTLs have been crossed and backcrossed to generate a number of recombinant lines across the QTL intervals. Phenotyping of these recombinants in up to three field trials have confirmed that clear height differences are segregating in these populations. In most cases there was a bimodal distribution of height within each population indicating that the trait is due to a single Mendelian factor. A quantitative trait has been converted into a qualitative trait. The results suggest that these are very likely to be suitable materials in which to define and narrow the QTL regions controlling height.

4.2. Identify more diagnostic markers for the QTL that increase resource use efficiency

4.2.1. Introduction

The NILs described in section 4.1 each have an introgressed chromosomal segment containing a gene(s) (which are responsible for a QTL) which have an effect on crop height and potentially crop yield. The introgressed regions were defined using two flanking SSR markers but these may be far from the gene of interest. The aim of this part of Objective 1 was to define and narrow the QTL regions in these lines and identify the closest useful markers to these QTLs, this will allow plant breeders to easily screen new populations for the desired height traits. The ultimate goal was to clone and sequence the gene(s) responsible for the QTL. Functional analysis of the gene and the pathways in which it was likely to be involved should give an insight into its contribution to plant height.

4.2.2. Materials, Methods and Results

Genotyping of QTL lines

To further define the QTL regions additional markers were chosen between the flanking genetic markers (based on the integrated genetic maps from Cereals DB http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/indexNEW.php) to analyse the recombinant lines for each QTL.

Markers were mainly selected from Cereals DB (KASP markers) as this type of marker is favoured by plant breeders due to ease of use. Additional markers were selected from the IPK Gatersleben (gwm/gdm), Wheat Microsatellite Consortium (wmc), Beltsville Agricultural Research Station (barc) and INRA (cfd/cfa) collections; see GrainGenes (http://www.wheat.pw.usda.gov/) or were developed from the iSelect or Axiom resources (CerealsDB) or gene information in the Griffiths lab, **Table 4.5**. Methods for genotyping with SSRs and KASP markers from Wingen (2014) and Zikhali *et al.* (2014), with the precise conditions used dependant on the specific primer pairs. Limagrain in-

house KASP markers were used to genotype the additional 3A recombinants around barc19 (data not shown).

Table 4.5. SSR and KASP markers used to genotype recombinant lines associate with height QTL on chromosomes 2D, 3A, 3B, 6A, 6B

2D QTL	3A QTL	3B QTL	6A QTL	6B QTL
Marker name	Marker name	Marker name	Marker name	Marker name
cfd36	3A_M1	gwm285	barc171	wmc105
2D_M1	3A_M2	3B_M1	6A_M1	6B_M1
2D_M2	3A_M3	3B_M2	6A_M2	6B_M2
2D_M3	3A_M4	3B_M3	6A_M3	6B_M3
2D_M4	3A_M5	3B_M4	6A_M4	6B_M4
2D_M5	3A_M6	3B_M5	6A_M5	6B_M5
2D_M6	3A_M7	3B_M6	6A_M6	6B_M6
2D_M7	3A_M8	3B_M7	6A_M7	6B_M7
2D_M8	3A_M9	wmc326	6A_M8	6B_M8
2D_M9	3A_M10		6A_M9	6B_M9
2D_M10	3A_M11		6A_M10	6B_M10
2D_M11	3A_M12		6A_M11	6B_M11
2D_M12	3A_M13		6A_M12	6B_M12
2D_M13	barc19		6A_M13	6B_M13
2D_M14	3A_M14		6A_M14	6B_M14
2D_M15	3A_M15		6A_M15	6B_M15
2D_M16	3A_M16		6A_M16	gwm219
gwm261	3A_M17		6A_M17	
	3A_M18		6A_M18	
	3A_M19		6A_M19	
	wmc264		6A_M20	
			6A_M21	
			6A_M22	
			6A_M23	
			6A_M24	
			6A_M25	
			6A_M26	
			6A_M27	
			6A_M28	
			6A_M29	
			gwm570	

The genotypic data was combined with the phenotypic data to produce a ‘graphical genotype’ for each QTL. The order of the markers was determined partly through mapping using Joinmap 4 (see below), synteny of the markers in the barley genome, and finally through manual adjustment.

A colour coding system was devised to indicate the Avalon, Cadenza and heterozygous alleles and grade the phenotypic data (**Table 4.6**). This allowed a clear visualisation of the recombination events in the selected lines across each QTL region and their effect on height (**Figures 4.17, 19-24**). The SSR markers originally used to delimit the QTL regions and potentially unreliable genotyping data are shown in red.

Table 4.6. Genotype and phenotype colour coding

Genotype		**Height**	
Avalon	A	Short	
Heterozygote	H	Short- Medium	
Cadenza	C	Medium	
Unscored		Medium-Tall	
		Tall	

In addition genetic maps for each locus were generated in Joinmap 4 (data not shown) and this map data, along with the genotypic and phenotypic data, was used for QTL mapping (Single Trait Linkage Analysis (Single environment) in Genstat (16th Edition – VSN International). The closest markers are highlighted and positions of the QTLs are indicated below the graphical genotypes.

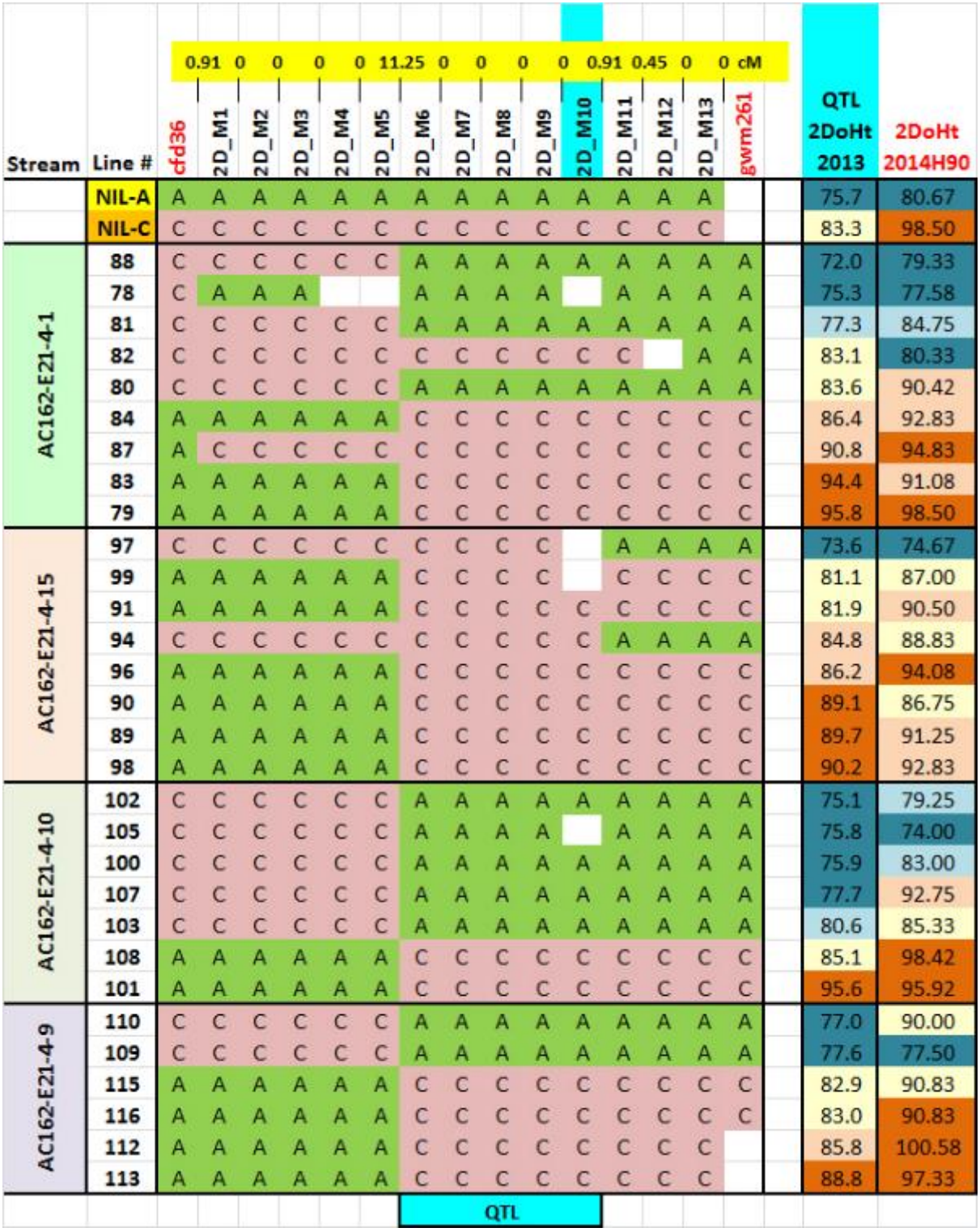


Figure 4.17. 2Do Graphical genotypes and phenotypic data for height QTL on chromosome 2D

Figure 4.17 shows the graphical genotypes of a subset of the 2Do lines, plus phenotypic data (height in cm, on right) from 2013 (**2DoHt2013**) and 2014 (**2Do2014H90**), the **2DoHt2014m** trial was lost due to poor drilling. QTL analysis in Genstat indicates the closest marker to be 2D_M10 (LOD = 6.14), however as there is no recombination between 2D_M10 and four adjacent markers the QTL region is extended to cover this larger region. The phenotypic data from 2014 (**2Do2014H90**) did not show a QTL.

As described above (**Table 4.3**) additional 2D lines were available (*2Da*) and these were phenotyped and genotyped with markers selected from the set shown in **Figure 4.17**. These spring-sown lines showed a very clear club-ear phenotype which co-segregated with crop height (**Figures 4.18** and **4.19**).

a. Avalon type: club ear **a. b.**
b. Cadenza type: wild type

Figure 4.18. Club ear phenotype of Avalon type 2Da lines.

The 2Da lines were also genotyped with a number of additional SSR and KASP markers (AK- prefix) by Ania Kowalski (post-graduate student, Griffiths lab) as part of an unrelated project, due to the convergence of fine mapping on the same region of 2D. A subset of the combined genotyping data are shown in **Figure 4.19**.

Without the AK- markers QTL, analysis of the 2Da data in Genstat indicates the closest marker to be 2D_M11 (LOD = 12.97, adjacent to 2D_M9 in the 2Do data, **Figure 4.19**). The addition of the AK- markers confirms the position of the QTL; the closest marker being 2D_M13 (LOD=16.0), however as there is no recombination between 2D_M13 and three other adjacent markers, including 2D_M11, (except for possible genotyping errors) the QTL region is extended to cover this larger region.

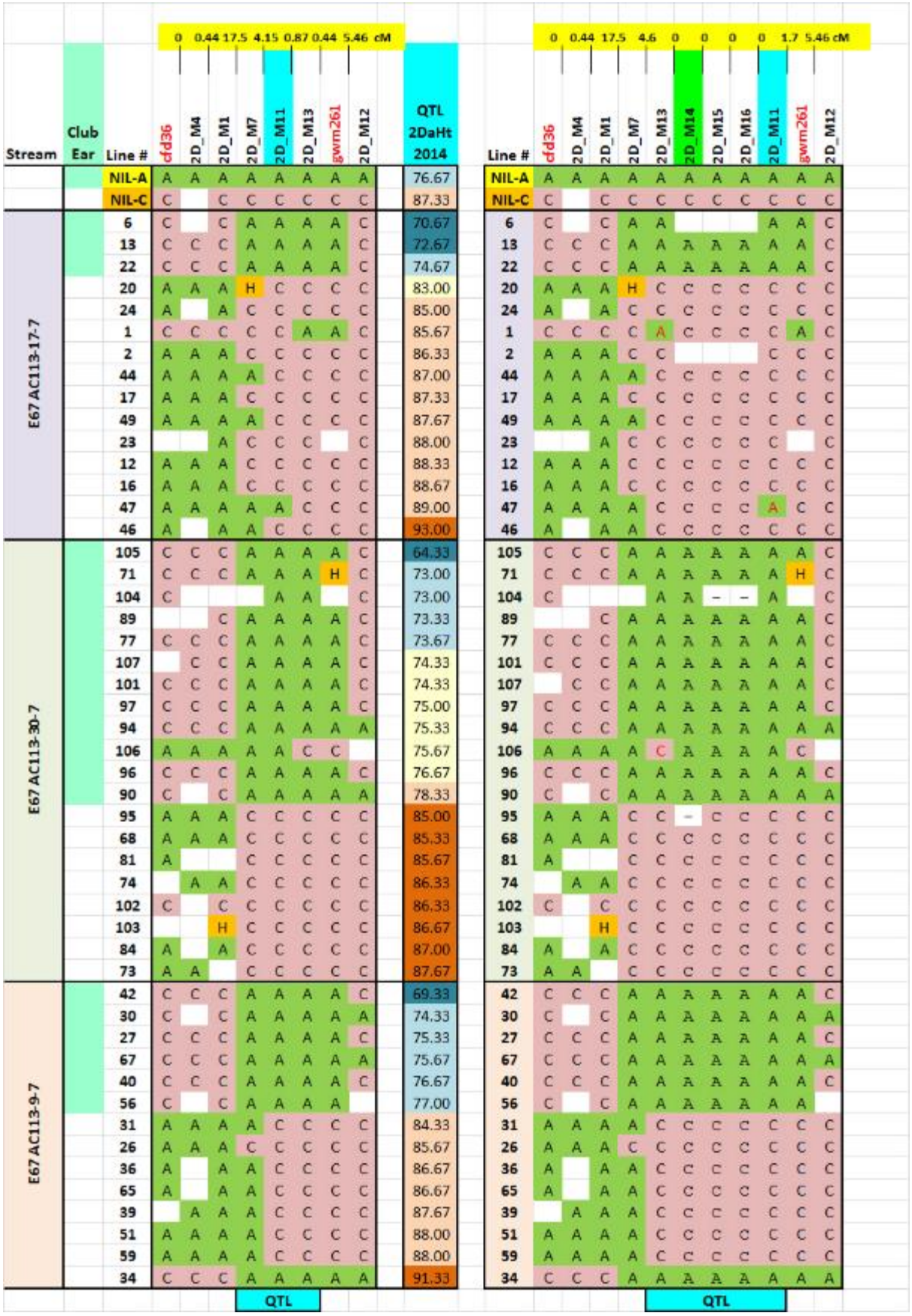


Figure 4.19. 2Da Graphical genotypes and phenotypic data for height QTL on chromosome 2A

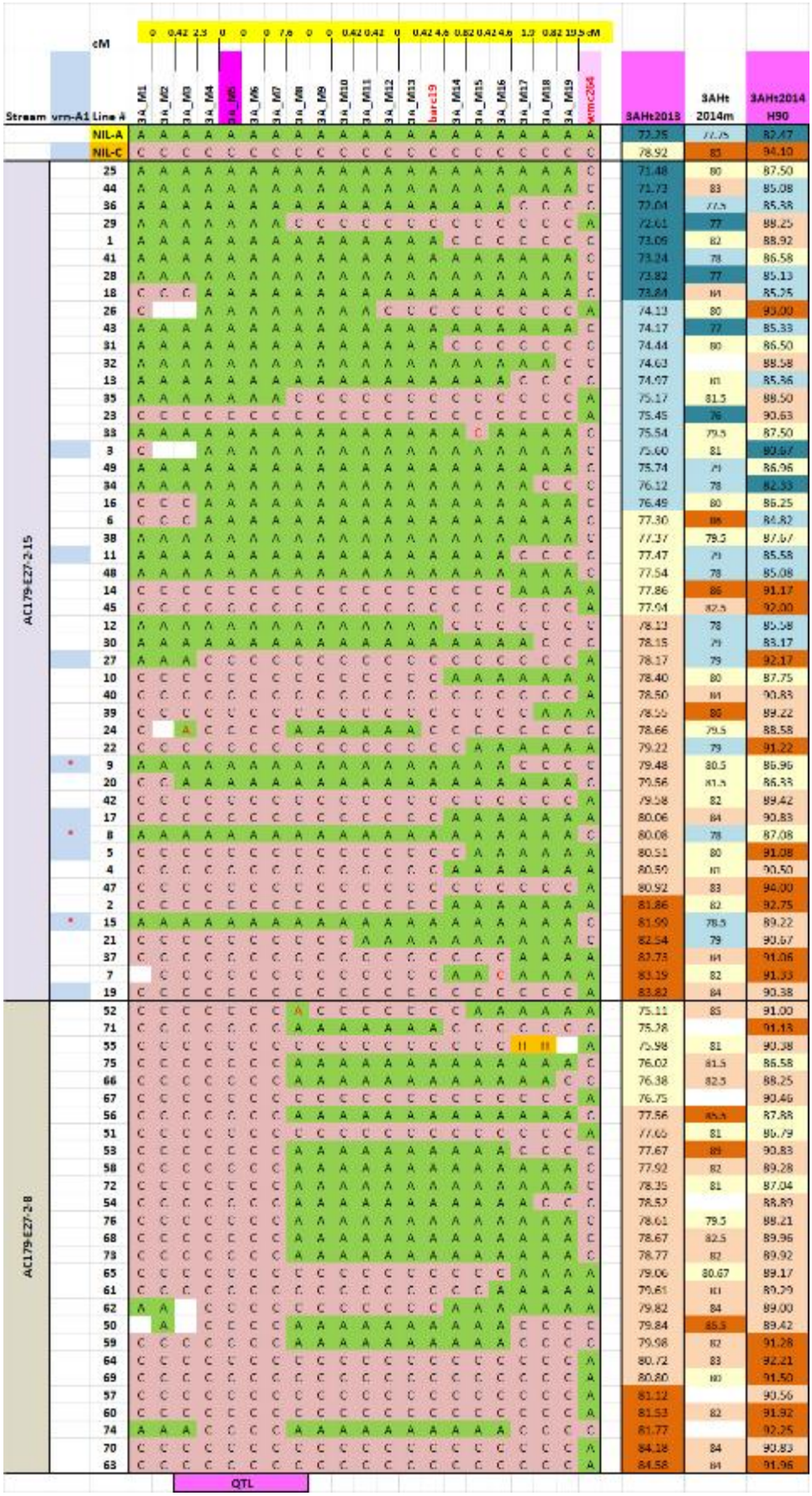


Figure 4.20. 3A Graphical genotypes and phenotypic data for height QTL on chromosome 3A

Figure 4.20 shows the graphical genotypes of the 3A lines, plus phenotypic data (height in cm, on right) from 2013 (**3AHt2013**) and 2014 (**3AHt2014m** and **3AHt2014H90**). It is clear from the height data that the AC179-E27-2-8 stream does not contain any short plants and this is confirmed by the presence of the Cadenza (increasing) allele across the QTL region.

QTL analysis in Genstat indicates the closest marker to be 3A_M5 (LOD = 7.6 for **3AHt2013** and LOD = 6.8 for **3AHt2014H90**). In addition, wmc264 is identified as a co-factor in **3AHt2014H90** (LOD = 3.6). As there is no recombination between 3A_M5 and three other adjacent markers the QTL region was extended to cover this larger region. QTL mapping was not carried out with the **3AHt2014m** data.

The *vrn-A1* gene was shown to be segregating in the AC179-E27-2-15 stream and three lines carrying *vrn-A1* (8, 9 and 15) show unexpectedly tall plants, particularly for **3AHt2013**, as they are Avalon over the QTL region and suggest the *vrn-A1* gene may have some influence on height as these spring-sown plants were not fully vernalised. In addition there is the suggestion of the involvement of a closely linked second gene (**Table 4.7**); if the Cadenza allele is also present at the second locus this enhances the increased height effect, conversely if the Avalon height allele is present, Cadenza at the second locus enhances the height decrease (**Table 4.7**).

Table 4.7. Effect of 3A height QTL and possible 2nd gene effect on height

3A_M4	3A_M5	3A_M6	3A_M7	3A_M8	3A_M9	3A_M10	3A_M11	3A_M12	3A_M13	barc19	No. of Lines	Mean Height 2013 data (cm)
A	A	A	A	A	A	A	A	A	A	A	21	75.21
A	A	A	A	C	C	C	C	C	C	C	2	73.89
C	C	C	C	A	A	A	A	A	A	A	14	78.24
C	C	C	C	C	C	C	C	C	C	C	27	79.7
Height QTL							2nd gene?					

One specific aim of this project was fine mapping of the 3A QTL. To facilitate this additional NIL-derived populations for the 3A QTL were generated. One population (sown in two batches as **3AHt2014F4B1** and **3AHt2014F4B2s** (**Table 4.4**)) either failed in the field or had incomplete phenotypic data, but genotyping has been carried out across the QTL region and a number of recombinants identified.

The second population were the “BC2F4 3A recombinants (near barc19)” lines, which were genotyped with additional markers by Limagrain (data not shown), the results of which are summarised in **Table 4.8** and indicate that the QTL is likely to be positioned between 3A_M5 and Limagrain marker 3A_LG1.

Table 4.8. Limagrain markers close to the height QTL on chromosome 3A

3A_M5	Closest marker to QTL
3A_LG1	Limagrain marker
3A_LG2	Limagrain marker
3A_M9	
(3A_M10)	Not tested on lines
3A_M11	
3A_M12	
(barc19)	Not tested on lines
3A_M16	
3A_LG3	Limagrain marker

Both these populations of recombinants are very close to the 3A height QTL and will provide useful future resources for even finer mapping of this region.

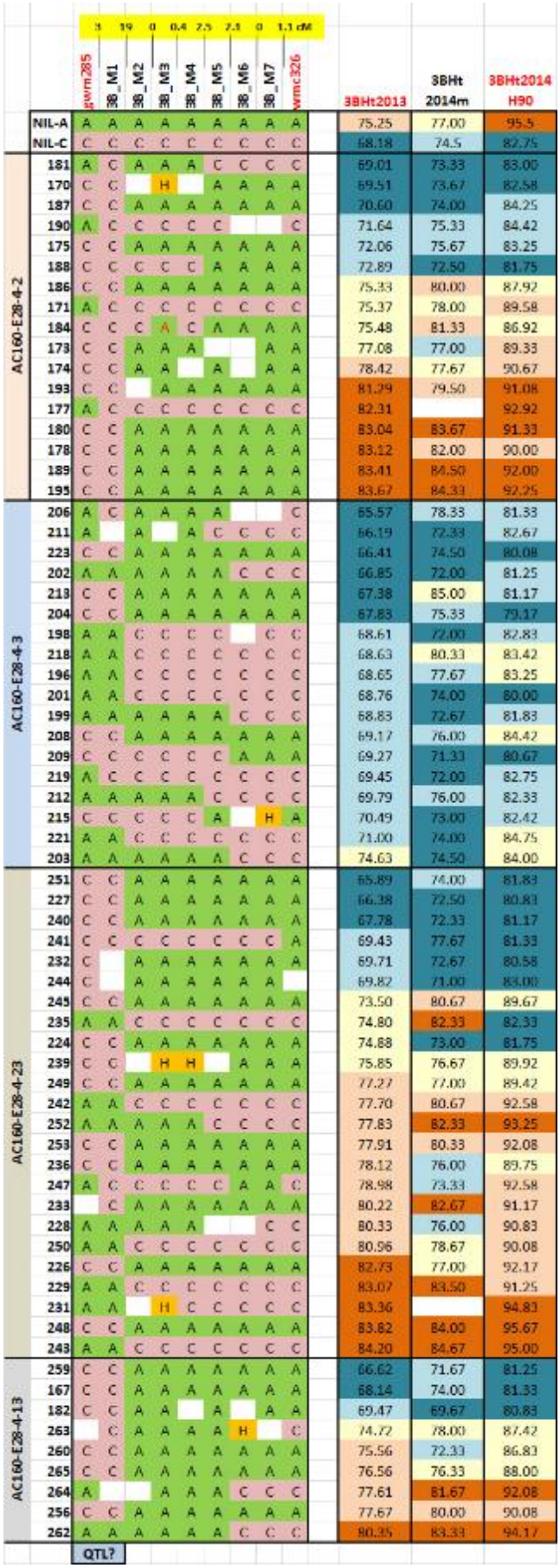


Figure 4.21. 3B Graphical genotypes and phenotypic data for height QTL on chromosome 3B

Figure 4.21 shows the graphical genotypes of the 3B lines, plus phenotypic data (height in cm, on right) from 2013 (**2DoHt2013**) and 2014 (**3BHt2014m** and **3BHt2014H90**). It is clear from the height data that the AC160-E28-4-3 stream does not contain any tall plants and this is confirmed by the presence of the Avalon (increasing) allele across most of the region likely to contain the QTL.

QTL analysis in Genstat did not identify the location of the QTL with either the **3Ht2013** or **3BHt2014H90** phenotypic dataset. A visual assessment of the graphical genotypes would suggest that the QTL is nearer to gwm285 and 3B_M1. The **3BHt2014m** data was not used for QTL mapping.

A large number of additional KASP markers, selected as mapping between gwm285 and wmc326 from CerealsDB, were shown *not* to be polymorphic between the NIL parents, which suggested that a chromosomal rearrangement must have occurred.

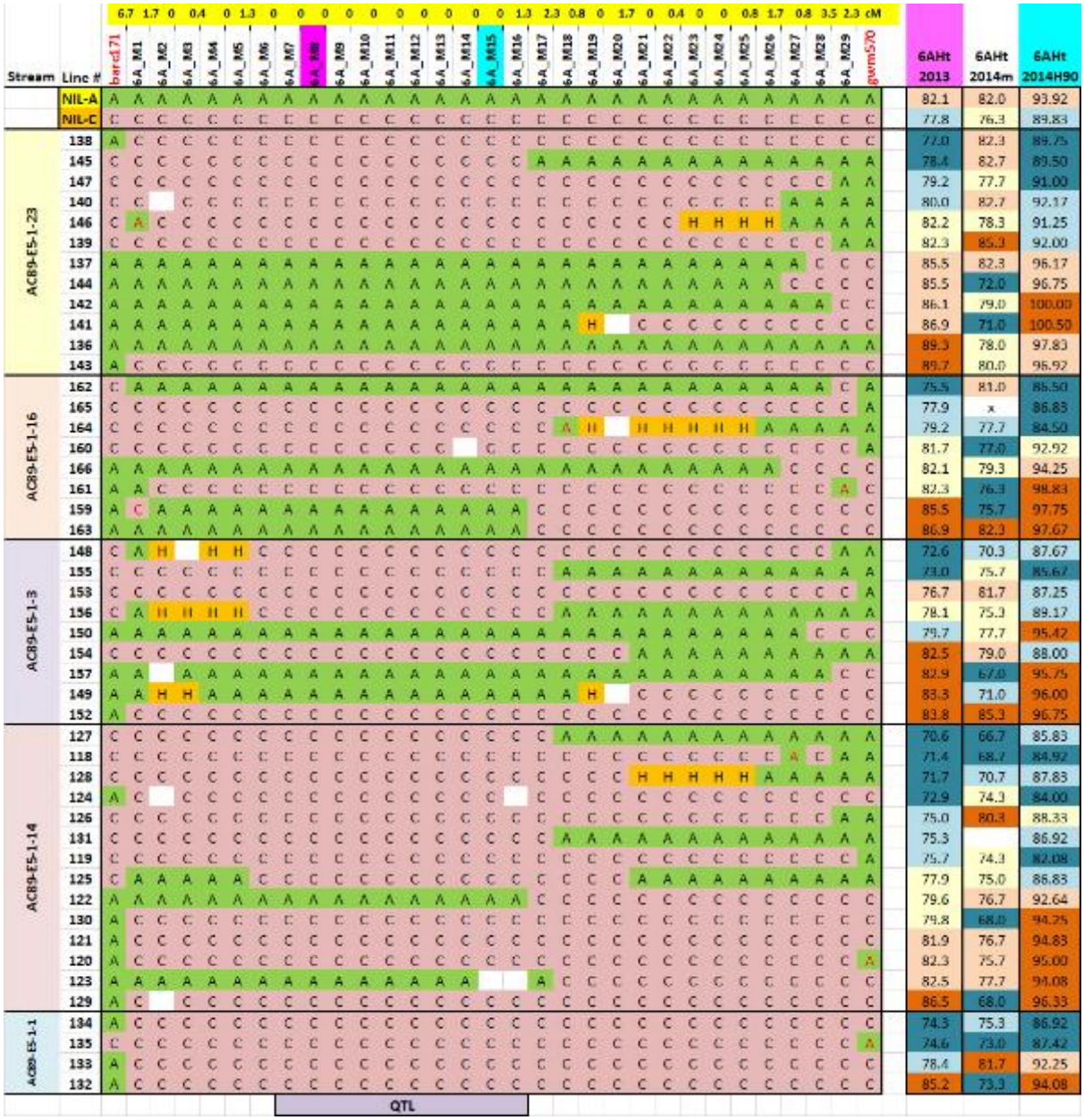


Figure 4.22. 6A Graphical genotypes and phenotypic data for height QTL on chromosome 6A

Figure 4.22 shows the graphical genotypes of the 6A lines, plus phenotypic data (height in cm, on right) from 2013 (**6AHt2013**) and 2014 (**6AHt2014m** and **6AHt2014H90**).

QTL analysis in Genstat indicates the closest marker to be 6A_M8 for **6AHt2013** (LOD = 4.7) and 6A_M15 for **6AHt2014H90** (LOD = 7.4). As there is no recombination between these two markers and nine other markers the QTL region is extended to cover this larger region. QTL analysis was not carried out with the **6AHt2014m** data.

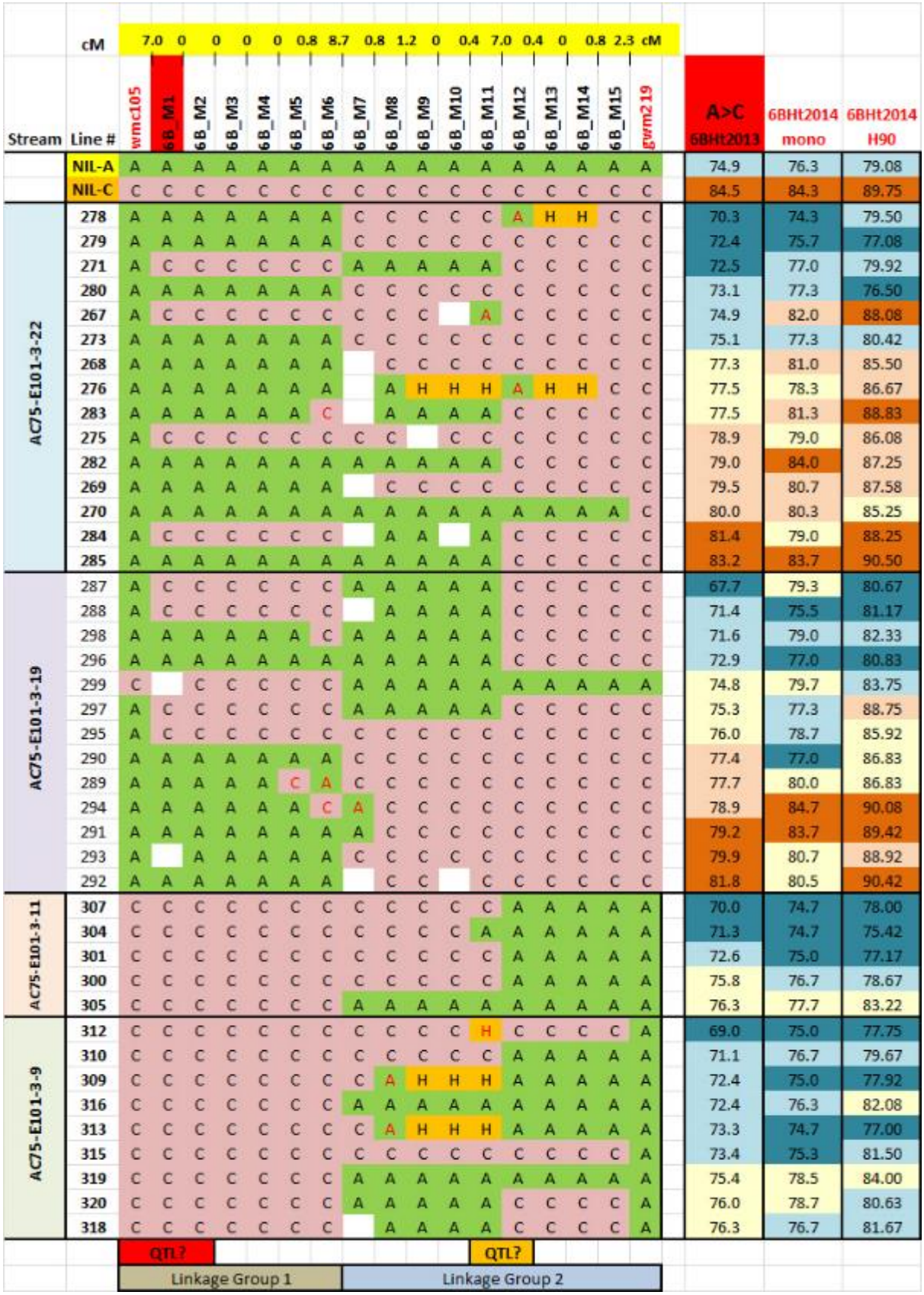


Figure 4.23. 6B Graphical genotypes and phenotypic data for height QTL on chromosome 6B

Figure 4.23 shows the graphical genotypes of the 6B lines, plus phenotypic data (height in cm, on right) from 2013 (**6BHt2013**) and 2014 (**6BHt2014m** and **6BHt2014H90**).

Joinmap 4 was unable to create a single linkage group with the 6B segregation data although this is not a problem for the QTL analysis in Genstat. In 6B, Cadenza is the increasing allele for the QTL, however the closest marker (6A_M1) to the only minor QTL identified (LOD = 2.45) has *Avalon* as the increasing allele. Additional QTL analysis using only the Linkage Group 2 data did not indicate the presence of a QTL. A visual assessment of the graphical genotypes suggest that a QTL might be located around 6B_M12. However as the 6B QTL was not detected in a number of the WGIN trials then this result is not unexpected.

The closest markers flanking the height QTL are summarised in **Table 4.9**. The project aimed to obtain additional molecular markers which reduce the genetic distance between markers flanking the QTLs to 5cM. This has been exceeded for the 2D, 3A and 6A QTLs. QTLs were not identified in the 3B and 6B lines. Previous field trials have indicated that the QTLs on chromosomes 3B and 6B are relatively small height effects, although clear differences are observed between the NIL parents and the recombinant progeny showed a good range of heights. While a QTL location could not be defined, the QTL interval on 6B has been substantially reduced, from 22.8 to 7.0 cM, however no additional polymorphic markers have been found in this interval, therefore further challenges remain for marker development at this locus. The genetic distance between the closest markers to the 3B QTL has been reduced from 44 to 22cM but, again, polymorphic markers in this interval could not be found. Unexpectedly, many KASP markers which were positioned between the flanking SSR markers on the A x C integrated map were *not* polymorphic between the NIL-parents, which suggests they are not within the introgressed QTL region. Analysis of the chromosome 3B sequence, once it became available, (Choulet *et al*., 2014) and an attempt to determine the position of all the KASP markers in this region in barley (http://plants.ensembl.org/Hordeum_vulgare/Info/Index) suggests that there is a rearrangement in the A x C NILs, compared to Chinese Spring, so that it is difficult to obtain useful marker information from the available maps.

Table 4.9. Markers closest to QTLs

Height QTL	Closest marker to QTL	5' KASP marker	3' KASP marker	Genetic distance
2D	2D_M14	2D_M13	2D_M16	<1 cM
3A	3A_M5	3A_M4	3A_M7	<1 cM
3B	**gwm285?**	3B_M1	3B_M2	22cM
6A	6A_M8/ 6A_M15	6A_M6	6A_M16	<1 cM
6B	no QTL detected			

Identification of candidate genes

For the three QTLs delimited to sub-centimorgan intervals (2D, 3A and 6A) it may be possible to identify gene candidates for the QTLs. This was partly facilitated by the provision by Limagrain UK of sequence spanning the QTL regions from their database. These sequences were mainly non-repetitive, transcribed sequences, which are broadly syntenic with the chromosomes. The positions of the KASP and SSR markers in the LG sequence were determined for ~80% of markers. BLAST searches at NCBI were performed with the LG sequence for each QTL region. BLASTX 2.2.30+ was used to search "All non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF excluding environmental samples from WGS projects" and searching against the database for "Flowering Plants". These searches have indicated promising gene candidates for the QTLs on 2D, 3A and 6A. The region where a QTL of 6B QTL might be located is outside the sequence provided by Limagrain. The availability of the chromosome 3B sequence has also suggested a number of 3B candidates, although further mapping on these NILs is required to define this region. The chromosome 3B sequence was also used to identify possible gene candidates for the 3A QTL. A number of gene candidates have been identified for the 2D, 3A and 6A QTLs (data not shown). The selection is based on likely genes involved in pathways which could affect height, but cannot be considered definitive. Finer mapping of the QTLs is required to define a shorter region in which *all* genes in the QTL intervals can be analysed and the likelihood of them being involved in plant height explored.

4.2.3. Summary

The project aimed to obtain markers which reduce the genetic distance between markers flanking the QTLs to a confidence interval of 5 cM. This has been exceeded for the 2D, 3A and 6A QTLs which have all been located between markers less than 1 cM apart. The interval between the QTL on 6B has been substantially reduced but further delimiting is hampered by marker availability. The QTL interval for the 3B QTL has been halved but a rearrangement of 3B in the A x C population means that current marker data is unhelpful. In addition to the specific objective it has also been possible to propose gene candidates for the height QTLs on chromosomes 2D, 3A and 6A.

5. Understand the physiological mechanisms by which the genes increase resource use efficiency and yield

5.1. Materials and Methods

Two types of field experiment were carried out to understand the physiological mechanisms by which the yield QTL have their effect: i) subsets of DH lines with and without specific yield QTL and ii) Near inbred lines (NILs). A summary of the experiments carried out are given in **Table 5.1**. The DH and NIL lines used are described in Appendices 1 to 6. The DH experiments at HM10 and HM11 included 128 Rialto x Savannah DH lines and 16 elite winter wheat varieties. A resolvable incomplete block design, known as an Alpha Design, was used for these two experiments, and a subset of 20 DH lines without PGR treatment were used for detailed growth analysis. The DH experiments at BX11 and TT11 included 30 Brigadier x Alcedo DH population lines and used a randomised block experiment with 2 replicates of each treatment. Experiments at HM12, BX12, HM13, GT13, HM14 and TT14 were split-split plot designs with N treatment (50% and 125% of RB209 recommendation) as main plots, NIL stream as sub plot, and +/- yield QTL randomised within the sub-sub plots. None of the experiments used for this objective received PGRs. A yield trial (LM14) was carried out at Woolpit in Suffolk and included all the 3A, 6A and 7D NILs. This experiment used an alpha design and height and yield measurements were made. A summary of the measurements taken in each experiment is given in **Table 5.2**.

Genstat 14th edition (VSN International, 2011) was used for all statistical analyses. Analysis of variance procedures for an alpha design, split-plot design and randomised block design were used to assess the effects of the germplasm treatments.

Table 5.1. Summary of experiments

Identifier	Year	Site	Soil type	Sow date	Seed rate seeds/m^2	Treatments	Experiment design	Nitrogen rate applied (kg/ha)	Plot size	QTL investigated
HM10	2009/10	High Mowthorpe North Yorks	Shallow silty clay loam over chalk	29 Sept	250	128 RiSa DH lines 3 PGRs	Alpha design 2 reps	200	12m x 2m	7D
HM11	2010/11	High Mowthorpe North Yorks	Sandy loam	12 Oct	250	128 RiSa DH lines 3 PGRs	Alpha design 2 reps	200	12m x 2m	7D
BX11	2010/11	Boxworth Cambs	Clay loam	11 Oct	250	30 AlBr DH lines	Randomised block, 2 reps	195	24m x 2m	7D
TT11	2010/11	Terrington Norfolk	Silty clay loam	2 Nov	250	30 AlBr DH lines	Randomised block, 2 reps	200	24m x 2m	7D
HM12	2011/12	High Mowthorpe North Yorks	Silty clay loam over chalk	18 Oct	265	4 AC 3A NILs + parents 4 SpR 6A NILs + parents	Split split plot, N rate main plot, NIL stream sub plot 3 reps	RB209: 240kg/ha (120/300)	12m x 2m	3A, 6A
HM13	2012/13	High Mowthorpe North Yorks	Silty clay loam over chalk	14 Nov	300	4 AC 3A NILs + parents 4 AC 6A NILs + parents 3 SR 7D NILs + parents	Split split plot, N rate main plot, NIL stream sub plot 3 reps		12m x 2m	3A, 6A, 7D
GT13	2012/13	Gleadthorpe Notts	Sand	12 Dec	250	4 AC 3A NILs + parents 4 SpR 6A NILs + parents 3 SR 7D NILs + parents	Split split plot, N rate main plot, NIL stream sub plot 3 reps	RB209: 250kg/ha (125/312)	12m x 2m	3A, 6A, 7D
HM14	2013/14	High Mowthorpe North Yorks	Silty clay loam over chalk	24 Oct	250	4 AC 3A NILs + parents 4 SpR 6A NILs + parents 3 SR 7D NILs + parents	Split split plot, N rate main plot, NIL stream sub plot 3 reps	RB209: 280kg/ha (140/350)	12m x 2m	3A, 6A, 7D
TT14	2013/14	Terrington Norfolk	Sand	27 Sept	250	4 AC 3A NILs + parents 4 AC 6A NILs + parents 3 SR 7D NILs + parents	Split split plot, N rate main plot, NIL stream sub plot 3 reps	RB209: 280kg/ha (140/350)	12m x 2m	3A, 6A, 7D

LM14	2013/14	Woolpit Suffolk	Clay Loam	3 Oct	250	4 AC 3A NILs + parents 4 SpR 6A NILs + parents 4 AC 6A NILs + parents 3 SR 7D NILs + parents	Alpha design 3 reps.	200kg/ha N	6m x 1m	3A, 6A, 7D

RiSa DH lines: Rialto x Savannah DH lines

AlBr DH lines: Alcedo x Brigadier DH lines

AC NILs: Avalon Cadenza

SpR NILs: Spark Rialto

SR NILs: Savannah Rialto

Table 5.2. Summary of measurements taken

	HM10	HM11	BX11	TT11	HM12	GT13	HM14	TT14	LM14
Plant establishment	✓	✓	✓	✓	✓	✓	✓	✓	
GS31 date	✓	✓	✓	✓	✓			✓	
GS31 light intercepted by canopy	✓	✓	✓	✓	✓			✓	
GS31 crop biomass	✓	✓	✓	✓	✓			✓	
GS31 shoots/m^2	✓	✓	✓	✓					
GS33 light intercepted by canopy	✓	✓	✓	✓	✓	✓	✓(39)		
GS33 crop biomass	✓	✓	✓	✓	✓	✓	✓(39)		
GS33 shoots/m^2	✓	✓	✓	✓			✓(39)		
GS59 date	✓	✓	✓	✓	✓		✓	✓	
GS59 light intercepted by canopy	✓	✓	✓	✓	✓	✓	✓	✓	
GS59 crop biomass	✓	✓	✓	✓	✓	✓		✓	
GS59 shoots/m^2	✓	✓	✓	✓	✓				
Height at flowering	✓	✓	✓	✓	✓	✓	✓	✓	✓
Date of maturity / green area duration	✓	✓	✓	✓	✓	✓	✓	✓	
Seeds/m^2	✓	✓	✓	✓	✓	✓	✓	✓	
Thousand grain weight	✓	✓	✓	✓	✓	✓	✓	✓	
Ears/m^2	✓	✓	✓	✓	✓		✓	✓	
Grain yield	✓	✓	✓	✓	✓	✓	✓	✓	✓
Straw/chaff yield	✓	✓	✓	✓	✓	✓	✓	✓	
Total biomass at harvest	✓	✓	✓	✓	✓	✓	✓	✓	
Grain N content	✓	✓	✓	✓	✓	✓	✓	✓	
Natural lodging	✓	✓	✓	✓	✓	✓	✓	✓	

5.2. Results

5.2.1. Effects from the 3A QTL

A summary of the effects of the height/yield QTL on chromosome 3A is given in **Table 5.3**. For the 3A QTL, yield for the Cadenza allele was increased by 0.49 t/ha on average over the five experiments carried out with the Avalon Cadenza NIL populations. Yield increases ranged from 0.27 t/ha to 1.05 t/ha and increases were statistically significant for four of the five experiments. A cross site analysis revealed that yield was significantly affected by the QTL background (P=0.026). In the four experiments where the effect of nitrogen on yield was investigated, the results show no evidence for a significant interaction between the QTL parent of the NIL and the nitrogen treatments. In all experiments, the yield difference between the Avalon and Cadenza alleles was numerically greater at the high nitrogen level than low nitrogen level, however this difference was much greater for the two experiments carried out in the 2013/14 season.

The effect of the 3A QTL on height was more variable than on yield, although positive height increases were obtained for the Cadenza allele in four of five experiments. The height differences were significant for four of the five experiments. It is notable that a positive yield increase of 0.37 t/ha, although not statistically significant, was obtained when the height of Cadenza was no greater than that of Avalon. When a cross-site analysis across the five trials was performed, height was significantly affected by the QTL background (P=0.053). Height differences ranged from -0.56 cm to +7.0 cm, with an average height difference of 4.19 cm.

Establishment was found to be similar for the NILs in both the Cadenza and Avalon backgrounds. An exception to this occurred in the TT14 experiment, where 3A NILs in the Cadenza background established significantly better than those in the Avalon background. Increased biomass at growth stages 31, 33 and 39 for the NILs containing the Cadenza allele in comparison to those containing the Avalon allele was observed in a number of experiments, although these differences were not significant. There appeared to be inconsistent effects of the QTL background on the date of growth stages 31, 33 and 59 and the amount of light interception at these growth stages. Green leaf area duration (GLA) was increased for the Cadenza allele in three of the four experiments where this was measured, and this effect was statistically significant in two experiments. Therefore the data suggests the 3A QTL may be having some growth effects which occur during stem extension and before the start of grain filling, but effects are greater after the start of grain filling.

Seeds rate (seed/m^2) was increased in NILs which contained the Cadenza allele, and this effect was statistically significant in the TT14 and HM14 experiments, whilst there was no consistent effect on thousand grain weight (TGW). This suggests that the main component of yield affected by the Cadenza allele is seeds/m^2. Given that ears/m^2 are often reduced, it is likely that the number of

grains/ear increases and evidence for this was obtained in the three experiments where this measurement was carried out.

The effect of the Cadenza allele on the straw and chaff yield and total biomass at harvest was inconsistent between years, although it is notable that there were some positive effects on straw yield (in the experiments carried out in the 2013/14 season). In contrast, harvest index appeared to be positively affected by the Cadenza allele, although this effect was non-significant. This suggests that there are effects of the 3A QTL before and after the start of grain fill, but the effects at the start of grain fill are greater.

Contrasting effects of the Cadenza allele on the nitrogen content of the grain were seen in the 2012/13 and 2013/14 season. In both the HM14 and TT14 experiments grain nitrogen was decreased for the Cadenza allele. Total nitrogen uptake was increased in three of the four experiments, although this difference was not significant.

The occurrence of natural lodging in these experiments was limited to two experiments: HM14 and TT14. In both experiments levels of lodging at harvest was low. In the HM14 experiment there was a small non-significant decrease in lodging for the NILs in the Cadenza background, whereas for the TT14 experiment, lodging was significantly increased for these NILs. Notably, height was significantly increased by the Cadenza QTL background in both of these experiments.

Table 5.3. Summary of effects from the 3A QTL. All effects in the direction of the allele for increasing yield (Cadenza or Rialto alleles). †, *, **, * to indicate level of significance (0.1, 0.05, 0.01, 0.001 respectively). # indicates single replicate. nc indicates no difference between the two alleles.**

	HM12	GT13	HM14	TT14	LM14
GS31 date	↓			↑	
GS31 light intercepted by canopy	↓			↑*	
GS31 crop biomass	↑			↑	
GS33 light intercepted by canopy	↓	↑	↑39		
GS33 crop biomass	↑	↑#	↑39		
GS33 shoots/m^2			↑39		
GS59 date	↑		↓*	↑	
GS59 light intercepted by canopy	↓	↑	↑***	↑	
GS59 crop biomass	↓	↓#	↑	↑	
GS59 shoots/m^2	↓		↑		
Height at flowering	↑*** 1.50cm	↓0.56cm	↑*** 7.00cm	↑*** 6.70cm	↑ 6.30cm***
Date of maturity / green area duration	↓	↑	↑**	↑***	
Seeds/m^2	↑	↑	↑*	↑**	
Thousand grain weight	↓†	↑†	↑	↓	
Ears/m^2	↓		↑	↓	
Grains/ear	↑		↑	↑*	
Grain yield	↑*0.31t/ha	↑0.37t/ha	↑**0.46t/ha	↑*0.27t/ha	↑1.05t/ha***
Grain yield (low N)	↑0.39t/ha	↑0.36t/ha	↑0.31t/ha	↑0.19t/ha	
Grain yield (high N)	↑0.37t/ha	↑0.38t/ha	↑0.62t/ha	↑0.33t/ha	
Straw/chaff yield	↓	↓	↑**	↑†	
Total biomass at harvest	↑	↓	↑**	↑	
Harvest Index	↑†	↑	↓	↑	
Grain N content	↑	↑	↓***	↓†	
Total N uptake	↑	↑	↑	nc	
Natural lodging			↓	↑**	

Data for GT13 represents a single Avalon Cadenza NIL pair (AC113-113).

5.2.2. Effects from the 6A QTL

A summary of the effects of the height/yield QTL on chromosome 6A is given in **Table 5.4**. For the 6A QTL, four experiments were carried out with the Spark Rialto NIL population and two experiments were carried out with the Avalon Cadenza NIL population. Yield was expected to be greater for the Rialto and Avalon alleles. Yield was increased for the Rialto allele by 0.25 t/ha on average over the four experiments, whilst the average yield difference for Avalon was 0.32 t/ha for the two experiments. In the TT14 experiment, the Cadenza allele out yielded the Avalon allele (0.15 t/ha). Yield increases ranged from 0.08 t/ha to 0.31 t/ha for Rialto and increases were statistically significant for two of the four experiments. When a cross-site analysis across the six trials was performed, yield was increased by 0.27t/ha for the increasing allele but this increase was not significantly affected by the QTL background (P=0.086). The impact of N treatment on the yield difference between the two QTL parents was investigated and the results highlighted that there was no effect at three of the four sites. At HM12, there was a statistically significant (at the 90% level) impact of the N treatment on the yield of the two QTL parents, with Rialto out yielding Spark more under low nitrogen conditions in comparison to high nitrogen conditions.

In three of the four experiments carried out with the Spark Rialto NIL population, the height of the Rialto allele was smaller than that of the Spark allele (1 cm reduction on average for these three experiments) and these were all statistically significant at the 90% or 95% level. In the LM14 experiment, height was increased by 2.8 cm for the Rialto allele. Height was increased for the Avalon allele in both experiments using the Avalon Cadenza NIL population and this effect was highly significant for the TT14 experiment. On average, height increased by 4.6 cm for the Avalon allele.

There was no evidence to suggest that the parental background of the NILs significantly affected establishment in any experiment. Light interception by the canopy at growth stages 31, 33 and 59 tended to increase for the yield increasing allele (Rialto or Avalon). A number of these effects were significant in the GT13 experiment and TT14 experiment. There were some examples of a decrease in light interception by the canopy but these differences were not significant for the yield increasing allele. Effects of the yield increasing allele on biomass were inconsistent between years, suggesting increased biomass was not contributing to the increase in yield observed. An increase in GLA duration was observed in three of the four experiments, although this didn't correlate with yield increases suggesting benefits from green leaf retention were not responsible for yield increases.

The effect of the Rialto or Avalon QTL background on seeds/m^2 or TGW was variable between years. The number of seeds/m^2 was significantly increased in the HM12 experiment, whilst TGW was significantly increased in the GT13, HM14 and TT14 experiments.

Grain N concentration was found to decrease and this suggests that while grain yield may be improved, whilst there were few improvements in straw yield, no additional N was available for remobilisation to the grain. It is notable that in the TT14 experiment, straw and chaff yield and total biomass at harvest were significantly higher for NILs in the Avalon background, whilst yield was reduced. This could be explained by the significant increase in natural lodging which occurred in this experiment. In the HM14 experiment, where lodging at harvest was also detected, lodging was reduced in the NILs in the Rialto background.

Table 5.4. Summary of effects from the 6A QTL. All effects in the direction of the allele for increasing yield (Rialto or Avalon). †, *, **, * indicate the level of significance (0.1, 0.05, 0.01, 0.001, respectively). nc indicates no difference between the two alleles.**

	HM12 (SpR)	GT13 (SpR)	HM14 (SpR)	TT14 (AC)	LM14 (SpR & AC)
GS31 date	↑			↓†	
GS31 light intercepted by canopy	↑			↑	
GS31 crop biomass	↓			↑	
GS33 light intercepted by canopy	↓	↑**		↑**	
GS33 crop biomass	↓	Nc			
GS59 date	↓		↓	↓	
GS59 light intercepted by canopy	↑	↑***	↓	↑	
GS59 crop biomass	↓	↑	↓	↑	
GS59 shoots/m^2	↑		↓**		
Height at flowering	↓*0.7cm	↓†0.7cm	↓† 1.6cm	↑*** 3.9cm	↑ 5.3 cm*** AC ↑ 2.8 cm SpR
Date of maturity / green area duration	↑	↑	↓†	↑†	
Seeds/m^2	↑**	↓	↑	↓	
Thousand grain weight	↓	↑*	↑*	↑***	
Ears/m^2	↓		↑†	↓	
Grains/ear	↑†		↓	↓	
Grain yield	↑***0.25t/ha	↑0.08t/ha	↑* 0.31t/ha	↓ 0.15t/ha	↑0.78t/ha AC ↑0.35t/ha SpR
Grain yield (low N)	↑0.19t/ha†	↑0.03t/ha	↑0.37t/ha	↓0.11t/ha	
Grain yield (high N)	↑0.30t/ha†	↑0.13t/ha	↑0.26t/ha	↓0.18t/ha	
Straw/chaff yield	↑	↓	↑	↑*	
Total biomass at harvest	↑*	↓	↑*	↑*	
Harvest Index	↓	↑***	↑	↓**	
Grain N content	↓*	↓	↓	↓†	
Total N uptake	↑	↓	↑*	↑	
Natural lodging			↓*	↑***	

5.2.3. Effects from the 7D QTL

A summary of the effects of the height/yield QTL on chromosome 7D is given in **Table 5.5**. For the 7D QTL, a total of nine experiments have been carried out to confirm yield effects and to understand a physiological basis for these effects. Two experiments were carried out using a Rialto Savannah DH population, two experiments were carried out using an Alecedo Brigadier DH population whilst the remaining experiments used a Rialto Savannah NIL population. Yield was expected to be greater for the Savannah and Brigadier alleles. Yield increases for the Savannah and Brigadier alleles were obtained in all experiments with the exception of GT13 and these increases were statistically significant in four of the experiments. The average yield difference was 0.25 t/ha, which ranged from -0.11 t/ha to +0.52 t/ha. This average yield increase was statistically significant (P=0.015). The impact of N treatment on the yield difference between the two QTL parents was investigated and the results highlighted that there was no consistent effect at the three sites.

The effect of the 7D QTL on height was more variable than on yield, which was not surprising given this QTL was not selected on a height basis. Positive and negative height effects for the Savannah allele were observed across the experiments. Height was increased in four experiments (the difference was statistically significant in two experiments), whilst there were no statistically significant negative effects of the Savannah/Brigadier allele on height. The average height difference across the eight experiments where height was measured was 0.80 cm, which was unsurprisingly not significantly affected by the parent background.

There was no evidence to suggest that the parental background of the 7D NILs significantly affected establishment in any experiment. Data to support an effect of the Brigadier or Savannah alleles on growth before flowering was inconsistent between experiments, therefore it could not be concluded whether yield effects were caused by improvements in early growth. Across the seven experiments where seed rate (seeds/m^2) was measured, the data supports both the Savannah and Brigadier alleles having a positive effect on seeds/m^2. The effect of the yield increasing allele on ears/m^2 was more variable.

An increase in the straw and chaff yield was observed in the earlier experiments carried out with the Alcedo Brigadier population. An increase in straw biomass was also observed in the HM14 experiment, whilst other experiments carried out using the Savannah Rialto NIL population did not support a similar increase. Grain nitrogen appeared to be significantly reduced by Brigadier, whilst the Savannah allele had little impact on the level of grain nitrogen.

Natural lodging at harvest was detected in the HM14 experiment, whereby the NILs in the Savannah background showed a small decrease in lodging which was not significantly different to the lodging levels of NILs in the Rialto background.

Table 5.5. Summary of effects from the 7D QTL. All effects in the direction of the allele for increasing yield (Savannah or Brigadier alleles). †, *, **, * to indicate level of significance (0.1, 0.05, 0.01, 0.001, respectively). nc indicates no difference between the two alleles.**

	HM10 (SR)	HM11 (SR)	BX11 (AB)	TT11 (AB)	GT13 (SR)	HM14 (SR)	TT14 (SR)	LM14 (SR)
GS31 date	↑	↓	↑				↑	
GS31 light intercepted by canopy	↑*	↓	-nc	↓			↓	
GS31 crop biomass	↑***	↓	↑	↓			↑	
GS33 light intercepted by canopy	↑*** 39	↑ 39	↓39	↓*39	↓			
GS33 crop biomass	↑ 39	nc 39	↑39	↓	↑			
GS33 shoots/m^2								
GS59 date	↓	↑	↑	nc		↑	↑	
GS59 light intercepted by canopy	↑	↑	↑	↓	↑***	↑*	↓	
GS59 crop biomass	↓	↓	↑	↑	↓	↑†	↓**	
GS59 shoots/m^2					↑	↑**		
Height at flowering	↑2.20cm	↓0.54cm	↑*3.37cm	↑*3.54cm	↑1.1cm	↓1.1cm	↓0.87cm	↓1.33cm
Height at flowering (115-040)					↓1.47cm	↓1.43cm	↓3.03cm	↓2.00cm
Date of maturity/green area duration	↓	↑			↑†	↓	↓	
Seeds/m^2	↑	↓	↑	↑	↑***	↑***	↑	
Thousand grain weight	↑	↓	↑		↓	↓**	↓**	
Ears/m^2	↓	↑	↑	↓†		↑**	↓*	
Grains/ear	↓	↑	↑	↑		↓	↑***	
Grain yield	↑* 0.3t/ha	↑*0.52t/ha	↑***0.38t/ha	↑0.22t/ha	↓0.09t/ha	↑*0.36t/ha	↑0.02t/ha	↑0.30t/ha
Grain yield (low N)					↓0.15t/ha	↑0.21t/ha	↑0.06t/ha	
Grain yield (high N)					↓0.04t/ha	↑0.51t/ha	↓0.02t/ha	
Straw/chaff yield	↑	↑	↑**	↑	↓	↑**	↓	
Total biomass at harvest	↑	↑	↑***	↑	↓	↑**	↓	
Harvest Index	nc	↑	↓	nc	↑†	↓	↑†	
Grain N content	↓	↓*	↓***	↓*	nc	nc	↓	
Total N uptake	nc	↓*	↓	↑	↓	↑**	↓	
Natural lodging						↓		

5.3. Discussion

Data collected from experiments using DH and NIL populations carried out over five experimental years has confirmed the yield effects of three QTL – 3A, 6A and 7D. Across these experiments, average yield increases due to inclusion of each QTL were 0.49 t/ha (P=0.026), 0.27t/ha (P=0.086) and 0.25 t/ha (P=0.015) for 3A, 6A and 7D, respectively.

Data obtained from the 3A, 6A and 7D NIL experiments carried out over three seasons has not provided any evidence to suggest that yield increases associated with the QTLs require more or less fertiliser (i.e. the same increase in yields can be obtained under reduced fertiliser conditions as for greater rates of fertiliser).

To further understand the impact that the three QTL may be having on resource use efficiency, N uptake efficiency (crop N uptake ÷ N supply from the soil and fertiliser), N utilisation efficiency (grain yield ÷ N taken up by the crop) and N use efficiency (NUE) (grain yield ÷ N supply from the soil and fertiliser) were calculated. These calculations enable hypotheses to be tested including: i) the yield improvements arise through greater sink size and ii) the yield increases do not necessitate an increase in fertiliser N requirement.

N utilisation efficiency was improved with inclusion of each of the three QTLs, but these differences were not statistically significant (at the 95% confidence level) (**Table 5.6**). NILs which contained the 3A height and yield QTL had a significantly greater NUE. For the NILs containing the 6A QTL, NUE was higher but the difference was not statistically significant. There was a significant increase in NUE for NILs containing the 7D QTL, with a 1.1% increase over the NILs without the 7D QTL.

N uptake efficiency (represented here by the total N uptake given that the soil mineral N and fertiliser rates were the same with and without the QTL) was significantly affected by the presence of the 3A QTL (**Table 5.6**). Across the four experiments, the total amount of N taken up by the NILs containing the 3A QTL was 6 kg/ha more than those without the 3A QTL. In contrast, for both the 6A and 7D QTLs, there was no effect whatsoever on the total amount of N which was taken up by the crop.

Table 5.6. N uptake (kg/ha), N utilisation efficiency (kg seed / kg of N uptake) and NUE (kg yield / kg of N supply) with and without the QTL.

	+3A	-3A	+6A	-6A	+7D	-7D
N uptake (kg/ha)	154*	148	158	158	148	148
N utilisation efficiency	33.2†	32.0	33.0	32.8	40.2	38.6
N use efficiency	27.9*	26.0	32.9	31.8	31.6*	30.5

Across the NIL experiments, the 3A QTL increased height by 4.19 cm and the 6A QTL increased height by 4.60 cm (Avalon Cadenza NILs). This would be expected to increase the risk of lodging because the increase in crop height will increase the wind-induced leverage that the shoot exerts on the stem base and anchorage system. A previous LINK project (LK0958) investigating QTL associated with lodging associated characters showed that the QTL associated with greater height on chromosome 6A was associated with weaker stem strength in Solstice x Xi19 DH mapping population, and was associated with greater stem strength in Rialto x Savannah DH mapping population. There were no associations for the 6A height QTL with anchorage strength, nor for the 3A height QTL with stem strength or anchorage strength. It therefore appears that there are no consistent linkages between the 3A and 6A height QTL with stem or anchorage strength, which means that the height effects can be used to estimate the impact of these QTL on lodging risk.

The wheat lodging model (Berry *et al*., 2003) can be used to estimate the effect of changing height on the seasonal lodging risk (**Figure 5.1**). **Figure 5.1** describes effects on stem lodging risk and a similar relationship also occurs for height against the risk of root lodging. The extent to which increasing crop height increases lodging risk depends on the initial risk of lodging. For a crop with a typical seasonal risk of lodging of 0.2, increasing height by 4.4 cm (the average effect of the 3A and 6A height QTL) increased the lodging risk to 0.27. This analysis indicates that incorporating either the 3A or 6A height QTL would result in a noticeable increase in lodging risk which would need to be mitigated against. This increased lodging risk could be mitigated by using varieties with 10% stronger stems and anchorage, by reducing plant population density by about 50 plants/m^2 (which may be sub-optimal in some situations), reducing N fertiliser to 50 kg N/ha below the optimum rate or by using a single PGR treatment (Berry *et al*., 2004).

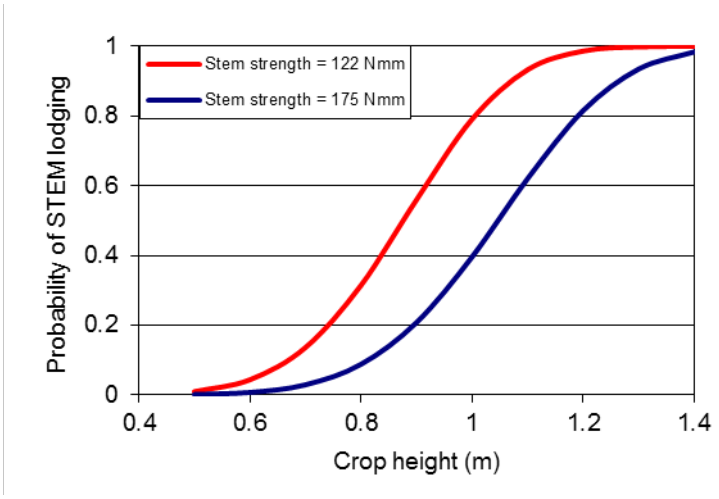


Figure 5.1. Effect of changing crop height on the seasonal probability of lodging for a crop with strong and weak stems, estimated using a model of lodging (Berry *et al*., 2003).

Greenhouse gas emissions associated with each QTL were calculated based on the method developed for wheat by Berry *et al.* (2010). This method includes direct and indirect N_2O emissions from the soil following the application of N fertiliser and from crop residues, and emissions associated with seed production, fertiliser manufacture, pesticides and field operations (fuel and machinery manufacture). Standard figures were used for seed, P, K and lime inputs (British Survey of Fertiliser Practice), pesticides (Pesticide Usage Survey), and energy associated with field operations (Williams *et al.* 2006), whilst crop yield and fertiliser rate were varied according to the experimental treatments.

GHG emissions were calculated per tonne of wheat and expressed as kg of CO_2 equivalent (CO_2e) after accounting for the different global warming potentials of the greenhouse gases. The global warming potential (GWP) used for N_2O was 296 kg CO_2e per kg N_2O. Baseline N_2O emissions equivalent to 613 kg CO_2e per ha were assumed to be incurred irrespective of N fertiliser rates. These were estimated from direct and indirect emissions from N in crop residues using IPCC (2006) Tier 1 methodology. Greenhouse gas emissions associated with the manufacture, packaging and transport of ammonium nitrate fertiliser were assumed to be 7.11 kg CO_2e per kg N (described in Berry *et al*., 2010). Further soil emissions of N_2O were assumed to be linearly related to N fertiliser rate as per IPCC Tier 1 methodology, with 0.0157 kg N_2O per kg N from direct emissions; plus 0.0036 kg N_2O per kg N indirect emissions from leaching and 0.0015 kg N_2O per kg N from volatilisation, giving a total of 0.0208 kg N_2O per kg N, or 6.16 kg CO_2e per kg N.

Calculating GHG emissions using the above methodology means that it is possible for incorporation of the height/yield QTLs to lead to a reduction in GHG emissions per tonne of grain if the yield increases are large enough. If these QTL were to be incorporated in an attempt to reduce GHG emissions associated with wheat production then this could lead to a reduced cropped area of wheat to maintain a given level of grain production to meet market demand. If previously uncultivated land is brought into production, to meet increasing wheat requirements, carbon will be released from vegetation and soil organic matter, leading to a large impact on GHG emissions. These potential indirect effects of land use change (LUC) have the potential to increase the GHG savings resulting from incorporating the height/yield QTL. It was assumed that, in order to maintain wheat productivity, all of the foregone yield (from not using yield enhancing QTL) must be produced on land converted from temperate grassland. The area of LUC (ha) to meet this production was obtained by dividing the foregone yield (t/ha) by the yield on the newly cropped land, taken as the yield of the particular QTL in question. Over a 30 year period the GHG emissions resulting from converting temperate grassland to arable crop land have been estimated at on average 6000 kg CO_2e per ha per year (Anon., 2006; Searchinger *et al*., 2008). It should be emphasised that estimating the potential indirect effects of LUC is hypothetical and assumes that the relationship between crop supply and demand is inelastic.

The yield increases conferred by the three QTLs of interest has been described in sections 5.2.1-5.2.3. For the 3A QTL, yield for the Cadenza allele was significantly increased by 0.49 t/ha on average over the five experiments carried out with the Avalon Cadenza NIL populations (P=0.026). In comparison to a 8 t/ha wheat crop, this additional 0.49t/ha increase in yield would reduce direct GHG emissions by 33 kg CO_2/t (**Table 5.7**) If LUC is also taken into account, the reduction in GHGs would increase to 79 kg CO_2/t. For the 6A QTL, yield was increased on average (across the Cadenza and Rialto alleles) by 0.27t/ha (P=0.086). This yield increase would reduce direct GHG emissions by 19 kg CO_2/t. A further reduction to 44 kg CO_2/t would be realised due to the reduction in LUC associated with the yield increase. Finally, for the 7D QTL, yield was significantly increased by 0.25 t/ha on average (P=0.015), equating to a 17 kg CO_2/t decrease in direct GHG emissions and a reduction of 40 kg CO_2/t when LUC is accounted for.

Table 5.7. Direct GHG emissions (kg CO_2) with and without the QTL. All QTL additions are in comparison to an 8t/ha wheat crop.

	+ 3A QTL	- 3A QTL	+ 6A QTL	- 6A QTL	+ 7D QTL	- 7D QTL
Direct GHGs/ha	4870	4870	4870	4870	4870	4870
Direct GHGs/ha (+ LUC)	4870	5238	4870	5073	4870	5058
Direct GHGs/t	560	593	574	593	576	593
Direct GHGs/t (+ LUC)	560	639	574	618	576	616

6. Investigate which yield and height genes are in current varieties and the scope for combining them to increase yield without increasing lodging risk

6.1. Introduction

Traditionally wheat breeding has been a numbers game, with large numbers of crosses being made each year and the ensuing progenies evaluated in the field in a two-step selection process: firstly on highly heritable traits (such as plant height and disease resistance) to reduce the number of lines down to a manageable size for trialling and secondly on quantitatively inherited traits such as grain yield and quality. The recent availability of informative DNA markers, linked to agronomic traits of interest (http://maswheat.ucdavis.edu/), allows the plant breeder to design his or her crossing programme more efficiently e.g. so that important genes/QTL do not segregate within a given cross. Markers can also be used in the screening of early generation breeding populations (e.g. F2's), which permit the stacking of desirable QTL combinations, which would not be possible to do phenotypically. In order to achieve efficient marker-assisted selection (MAS), the DNA markers must to be as close to the gene/QTL as possible (i.e. in linkage disequilibrium with the QTL alleles), they must be diagnostic for the presence of the gene/QTL in the elite germplasm pool being worked by the breeder and, ideally, markers must be available for all the major QTL controlling a given trait.

The aims of this work package were to:

a) understand if additional height QTL were present in elite UK winter wheat germplasm (other than those already identified on chromosomes 2D, 3A, 3B, 6A, 6B and 7D) by de-novo QTL mapping
b) combine the height QTL on chromosomes 3A, 6A and 7D, identified in the Savannah x Rialto DH population, in a double-dwarf background (*Rht-B1b* + *Rht-D1b*) in order to study the effect on height, lodging and yield
c) use the DNA markers developed during the course of this project to predict the presence of the height/yield QTL in current elite winter wheat varieties

6.2. Materials and Methods

6.2.1. De novo genetic mapping of height QTL

In the summer of 2008, preceding the start of the project, F1 derived, DH populations were measured for height to the ear tip. In total 13,769 lines derived from 162 segregating populations were measured and the data was stored in Excel and the phenotypic distributions plotted. The

parental lines of the DH populations were also screened for the presence of *Rht-B1b* and *Rht-D1b* semi-dwarf alleles using the markers described by Ellis *et al.* (2002). In addition to these DH populations, a panel of 335 UK winter varieties, all carrying *Rht-D1b*, were genotyped with the iSelect 90K single nucleotide polymorphism (SNP) chip (Wang et al, 2014). The SNP data was analysed using in-house software to remove monomorphic markers, those with rare alleles (MAF < 0.05) or SNPs with a call rate of less than 90%. The GWAS was conducted using a kinship matrix based on all the remaining SNP data (~20,000 markers) and the EMMA algorithm was used to detect significant associations.

6.2.2. Construction of a double-dwarf DH population fixed for the height increasing alleles for the QTL on chromosomes 3A, 6A and 7D

The existing phenotypic and genotypic data for the Savannah x Rialto (S x R) DH population generated in the Defra LINK project LK0958 was used to select a tall DH line (taller than 1 metre in all three site x seasons of LK0958) that carried all the height increasing alleles at the QTL on chromosomes 3A, 6A and 7D. The selected DH line (SR12) carrying *Rht-D1b* and was then crossed to the variety Robigus (*Rht-B1b*) and the F1 backcrossed to SR12 to generate a large BC1 population comprising 2068 individuals. The BC1 was screened with SSR markers at each height QTL peak, plus markers for the two dwarfing genes and the 1B/1R rye translocation. The marker-selected individuals were then used as donor plants for DH production. The double-dwarf population was grown in the field as small Hege 90 plots in 2010/11, plant heights were measured and the seed harvested for multiplication, followed by replicated yield trials. The DNA from the DHs was also genotyped with SNP markers in order to construct a genetic map of the population using MapDisto (http://mapdisto.free.fr/) and QTL analysis was performed using QTL Cartographer (http://statgen.ncsu.edu/qtlcart/).

6.2.3. The identification of a diagnostic markers for the height QTL on 3A, 6A and 7D

The publicly available SNP markers used at the John Innes Centre (http://www.cerealsdb.uk.net/) to find the 3A height QTL were aligned to the Limagrain SNP map in order to identify proprietary SNPs in the same region. These proprietary SNP markers had been used by Limagrain to genotype many thousands of wheat varieties and this database was searched to identify combinations of SNP markers that enabled the identification of a Cadenza-specific haplotype. The SNP database also contained data for the SNP marker in the promoter of the *TaGW2* gene (Yang *et al.,* 2012), which underlies the 6A QTL in the Spark x Rialto DH population (Simmonds *et al.,* 2014). For the 7D QTL from Savannah, wPt6263, one of the dominant DArT markers (http://www.diversityarrays.com/) close to the QTL peak in the original S x R mapping population was converted to a quantitative PCR marker and was used to screen a panel of UK varieties.

6.3. Results

6.3.1. De novo height QTL analysis

It was clear from the genotyping of the parents of the DH populations, measured for height in 2008 that many of the crosses were segregating for both the *Rht-B1b* and *Rht-D1b* alleles. A typical height distribution ranging from less than 60 cm (double dwarfs) to greater than 1 metre (wild type talls) is shown in **Figure 6.1**. Unfortunately, the segregation of these two major genes precludes the detection of any other height QTL in such populations. Prior to the introduction of the variety Robigus onto the AHDB Recommended List in 2003, all UK winter wheat varieties were *Rht-D1b*. Robigus was extensively crossed by all breeders and so by 2008 there were high yielding "sons of Robigus", such as Oakley, that had also been used as parents in the breeding programme. By this time, Limagrain had also listed the *Rht-B1b* varieties Lear and Gatsby. Out of the 162 DH populations measured for height in 2008, nearly 25% were segregating for both dwarfing genes, which accounted for ~3,000 of the DH lines.

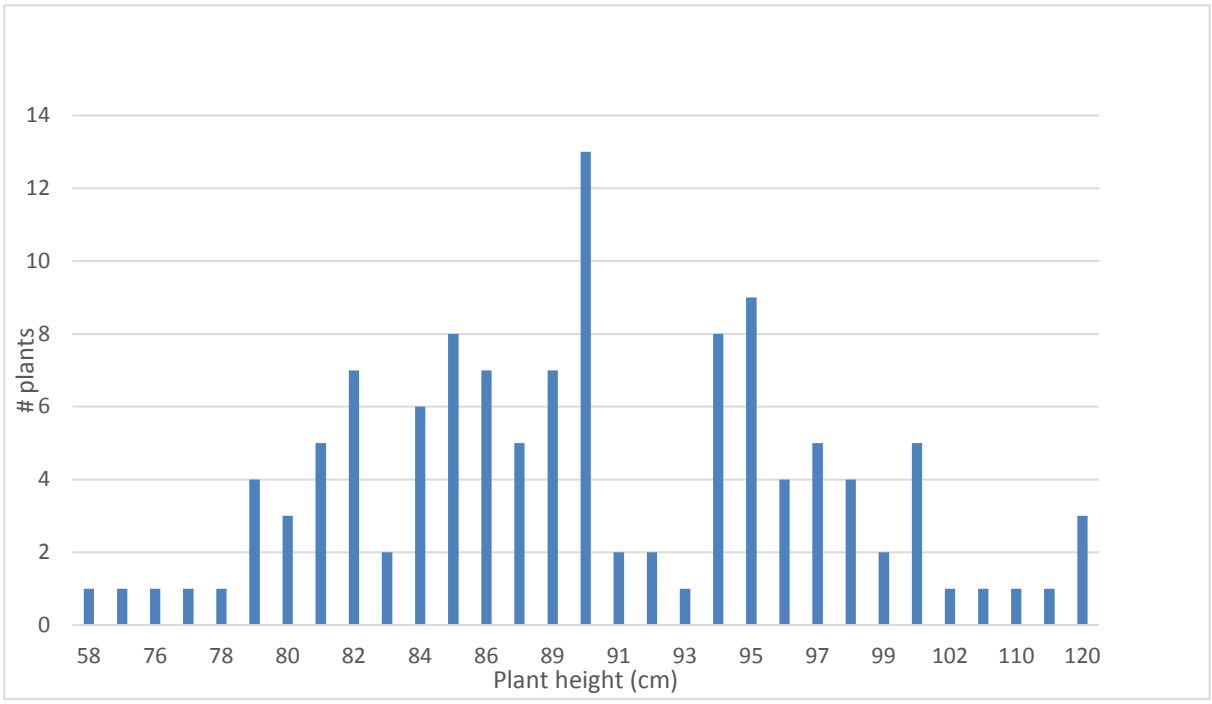


Figure 6.1. The height distribution of the DH population W063197

Twelve DH populations fixed for *Rht-D1b*, which showed a wide range of plants heights were selected for QTL mapping with SSRs (**Table 6.1**). However prior to this selection, the presence of the 6A QTL had been confirmed in a DH population with Claire and, in LK0958 Xi19, a Cadenza derivative, was found to have inherited the 3A QTL. In fact, all bar one of the populations listed in **Table 6.1** have either a Claire or Cadenza derivative in their pedigree. This fact, coupled with laborious nature of SSR mapping, meant that the molecular investigation of these DH populations was dropped in favour of genome-wide association analysis (GWAS) on a panel of semi-dwarf (all *Rht-D1b*) winter wheat varieties.

Table 6.1. The selected DH populations for QTL analysis and the putative height QTL sources.

Population	Pedigree parent 1	Pedigree parent 2	Min	Max	Min/Max Diff	QTL Source?	QTL Source?	No. of plants
NAWW9/ALCHEMY	A44-02 x (TORFRIDA x CONSORT)	CLAIRE x (CONSORT x WOODSTOCK)	58	120	62		Alchemy/Claire	121
A65-05/DUXFORD	ROBIGUS x CLAIRE	SOLSTICE x SCORPION 25	55	111	56	Alchemy/Claire	Cadenza QTL	132
CASSIUS/NSLWW78	WIZARD x (CLAIRE x NSLWW24)	(CONSORT x WOODSTOCK) x DEBEN	51	97	46	Alchemy/Claire	Alchemy/Claire	45
NAWW9/CEB01187	A44-02 x (TORFRIDA x CONSORT)	CLAIRE x AARDVARK	60	103	43		Cadenza QTL	91
ALCHEMY/A58-04	CLAIRE x (CONSORT x WOODSTOCK)	SOLSTICE x AARDVARK	72	113	41	Alchemy/Claire	Cadenza QTL	64
CONTENDER/A65-05	NELSON x WASMO	ROBIGUS x CLAIRE	61	102	41		Alchemy/Claire	110
HUMBER/CONTENDER	ANGLO x KRAKATOA	NELSON x WASMO	66	106	40			64
NSLWW80/A58-04	CLAIRE x PRIDE	SOLSTICE x AARDVARK	59	99	40	Alchemy/Claire	Cadenza QTL	68
A65-05/SJ03-3	ROBIGUS x CLAIRE	CORTEZ x BISCAY	56	95	39	Alchemy/Claire		59
A65-05/NSLWW80	ROBIGUS x CLAIRE	CLAIRE x PRIDE	71	110	39	Alchemy/Claire	Alchemy/Claire	116
A63-05/NSLWW77	XI19 x RIALTO	DEBEN x EINSTEIN	67	104	37	Cadenza QTL	Alchemy/Claire	81
CASSIUS/NSLWW80	WIZARD x (CLAIRE x NSLWW24)	CLAIRE x PRIDE	60	96	36	Alchemy/Claire	Alchemy/Claire	122

A GWAS analysis, using ~20,000 wheat SNPs on a panel of 335 elite UK varieties, failed to detect any of the QTL being investigated in this project. However, three new chromosomal locations were found to be significant for height effects on 6D, 5A and 7B (**Table 6.2**), but the size of the QTL effects at these loci is relatively small i.e. only 3 or 4 cm height difference (2 x additive effect).

Table 6.2 Results from the genome wide association analysis using ~20,000 SNP markers and the mean heights taken in 2012 on a panel of 335 elite UK winter wheat varieties.

Locus	Chrm	cM	R2.Locus	Pvalue.Locus	NumObs	# alleles	MAF	Bonferroni	Allele	Effect	Allele	Effect	2 x additive
wsnp_Ra_c13881_21836489	6D	79.0	0.1083	7.10E-10	334	2	0.47605	1.27E-05	A	1.7548	G	-1.7548	3.5096
Kukri_c31995_1948	6D	79.0	0.1041	1.48E-09	335	2	0.48657	2.65E-05	A	-1.7184	G	1.7184	3.4368
wsnp_Ex_c1690_3206784	6D	79.0	0.1023	2.10E-09	335	2	0.48955	3.77E-05	A	-1.7028	G	1.7028	3.4056
BS00074301_51	5A	113.8	0.0959	7.07E-09	335	2	0.40299	0.000126652	A	1.6801	C	-1.6801	3.3603
Tdurum_contig52695_388	5A	113.8	0.0959	7.07E-09	335	2	0.40299	0.000126652	A	1.6801	G	-1.6801	3.3603
Tdurum_contig86202_145	5A	113.8	0.0959	7.07E-09	335	2	0.40299	0.000126652	A	-1.6801	G	1.6801	3.3603
BobWhite_c23736_153	5A	113.8	0.0946	8.94E-09	335	2	0.40299	0.000160241	A	1.6672	G	-1.6672	3.3344
Kukri_rep_c71778_644	7B	1.9	0.0880	3.59E-08	332	2	0.15964	0.00064289	A	2.1380	G	-2.1380	4.2759
RAC875_c17182_600	7B	1.9	0.0864	4.21E-08	335	2	0.15821	0.0007555	A	2.1275	G	-2.1275	4.2550

6.3.2. Construction of a double-dwarf population

In order to study the effect of combining the height increasing QTL on 3A, 6A and 7D in the same variety, a specific DH population was constructed using marker-selected donor plants. The rationale behind this approach was to see if the double-dwarf background would compensate for the height increase caused by stacking the QTL together, but that the yield benefit of the QTL would remain. A three-way cross was made between the DH line SR12 with Robigus and the F1 backcrossed to SR12. The SR12 DH line was selected because it had inherited all the height increasing alleles from either Savannah (A) or Rialto (B) (**Table 6.3**). The markers shown in red in **Table 6.3** were those used to follow the three QTL with the largest effect on plant height and yield (i.e. those on chromosomes 3A, 6A and 7D). In addition to these SSR markers, the DH donor plants were also screened with the SNP markers for the 1B/1R rye translocation and the *Rht-B1b* and *Rht-D1b* alleles to select plants with 1RS (like Rialto and Savannah), homozygous for *Rht-D1b* and heterozygous for *Rht-B1b.* In total, 24 plants out of the 2048 that were screened, had the desired genotype based on the marker data. In the resultant DH progeny, the expected

segregation should be 50% double dwarfs to 50% *Rht-D1b* lines. A DH population, comprising 208 plants, was drilled in Hege 90 plots in the 2010/11 field season, the plant heights were measured and the genotypes at all the height QTL were validated with markers. All the plants were homozygous for the height increasing alleles at the QTL on chromosomes 3A, 6A and 7D (based on a single marker at the QTL peak), but only 50 were double-dwarfs rather than the expected 104. Seven DHs lacked either dwarfing gene (No *Rht* gene), which was an unexpected result, but they are the tallest individuals, which confirms the SNP data. The double dwarf DHs ranged in height from 57 to 83 cm and the *Rht-D1b* (*Rht2* only) lines ranged in height from 72 cm to 105 cm (**Figure 6.2**).

Table 6.3. Height increasing alleles of the SR12 DH line, and markers used to follow them (in red).

SSR locus	Chrm	cM	+ve QTL	SR12
Xgwm642	1D	95	B	B
Xwmc609	1D	143	B	B
Xcfd282	1D	148	B	B
				+
Xgwm715	2A	0	A	A
Xgdm093	2A	8	A	A
Xbcp3039	2A	30	A	A
				+
Xbarc045	3A	58	B	B
Xwmc264	3A	71	B	B
				+
wPt2573	6A	30	A	-
Xbarc146	6A	61	A	A
Xgwm1005	6A	90	B	B
				+
wPt6263	7D	0	A	A
Xgwm1007	7D	21	A	A
Xbarc184	7D	25	A	A
Xgwm044	7D	34	A	A
				+
HT_05				1013
HT_06				1059
HT_07				1076
Mean_Ht				1049

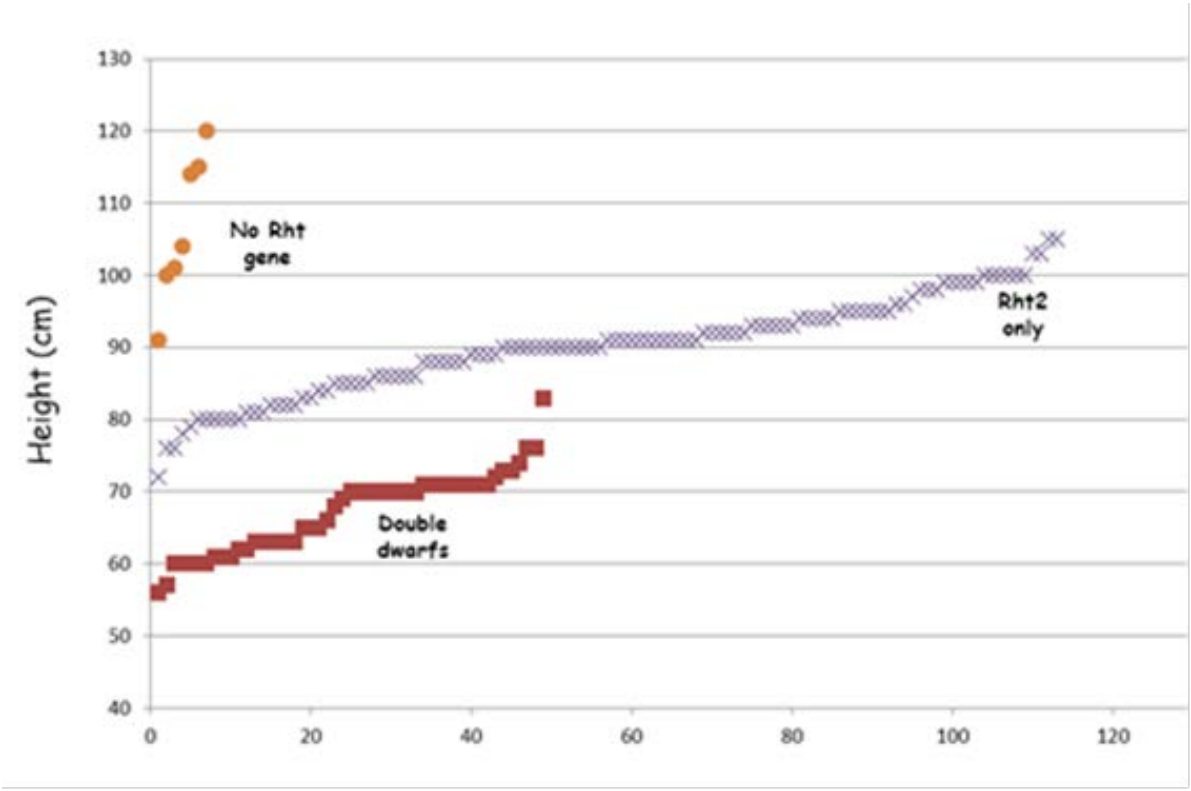


Figure 6.2. The range of heights achieved in the DH's inheriting both the *Rht-B1b* and *Rht-D1b* dwarfing alleles (the double dwarfs) compared to those with just *Rht-D1b* (*Rht2*) or no *Rht* gene.

Due to the large range in heights observed in the *Rht-D1b* DH lines, the decision was taken to genotype the entire population with SNPs and to run a QTL analysis. Only one SNP marker, on chromosome 6D, was found to be significant, with an additive effect of 3.5 cm. The height increasing SNP allele is derived from the SR12 DH line, but this chromosomal region was not found to be significant in the original S x R mapping population – presumably because its effect was masked by the segregation of the other major height QTL on 3A, 6A and 7D. Also this is a different region on chromosome 6D to the one identified in the GWAS experiment on the panel of 335 *Rht-D1b* varieties.

In order to test the yield performance of the double dwarf lines, the entire DH population was drilled at the JIC in autumn of 2012, but due to the appalling weather conditions that season the multiplication plots were lost. Fortunately, there was sufficient remnant seed to run a single rep yield trial at the Limagrain site at Wolferton in 2013/14, but the trial had to be written off due to black grass.

6.3.3. The identification of a diagnostic markers for the height QTL

The height QTL on chromosome 3A from the variety Cadenza has been mapped more finely than any other QTL region in this project. Therefore there was a far greater chance that the tightly-linked SNP markers to the 3A QTL would be more diagnostic than for the other QTL regions being

investigated. Unfortunately, the in-house Limagrain wheat genotyping database comprises almost entirely of proprietary SNP markers, but it was possible to identify markers that mapped into the fine mapped QTL region of 3A. SNP markers are biallelic markers and, in general, it's not possible to identify a single SNP that is 100% diagnostic, unless it detects a causal mutation within a gene. The best way to describe a genomic region is by combining the genotypic data of a number of tightly linked SNPs in order to form a haplotype. To unequivocally distinguish the Cadenza allele in the 3A QTL region, seven different SNP markers were required to build the haplotype. In total, these 7 SNPs identified 62 different haplotypes, 13 of which predominated and were found to occur in 94% of the lines in the SNP database. The Cadenza haplotype was rare and only occurred in 0.6% of the lines – all of which were Cadenza derivatives. The named wheat varieties found to have the Cadenza QTL allele on 3A based on this SNP haplotype analysis were: Tonic (one of the parents of Cadenza), Cadenza itself, Bellagio, Cocoon, Duxford, Phlebas, Scorpion25, Spark, Warlock24 and Xi19. Unfortunately seven SNPs is far too many to use for routine MAS, but it's possible to speculate that the some of the other SNP haplotypes may help to detect an allelic series at this height QTL.

Analysis of the *TaGW2* SNP data revealed that virtually all current elite UK varieties are fixed for the favourable grain width increasing allele on chromosome 6A, apart from some Cadenza derivatives like Gallant and Cordiale. The other elite source of the unfavourable grain width allele was Claire, via its parent Flame. No current recommended varieties have inherited the smaller grain allele from Claire - the last one to do so was Exsept. **Table 5.4** clearly shows the yield advantage offered by this QTL (0.25 to 0.32 t/ha on average), which may or may not be associated with a small increase in height depending on the genetic background. It's interesting to note that a large height QTL was detected on chromosome 6A in the Savannah x Rialto DH population studied in LK0958. This population is fixed for the favourable allele at *TaGW2* indicating that the height effect caused by this region on 6A is probably not a pleiotropic effect of the *TaGW2* locus.

Using the quantitative PCR assay derived from wPt6263 to screen varieties, it was found that very few were positive for this marker on 7D. Interestingly the positive lines were mainly high yielding varieties such as Brigadier and Oakley and the inheritance of the marker could be traced back through pedigrees to cv. Rendezvous (**Figure 6.3**). The positive yield effect of this QTL region has been verified in both the Brigadier x Alcedo and Savannah x Rialto DH populations and also in the 7D NILs created in the Rialto genetic background (**Table 5.5**). The average yield increase provided by the Savannah allele was found to be 0.25 t/ha and is due, in part, to more seed/m^2. However using this marker to follow the QTL region in Oakley derivatives within the commercial UK breeding programme no such yield increase is seen. Presumably this marker is not close enough to the gene underlying the 7D yield QTL and so this region also needs to be fine mapped in a future study.

Figure 6.3. The pedigree trees for the varieties Savannah and Oakley. Varieties shown in *red* were positive for the wPt6263 marker on chromosome 7D.

6.4. Discussion

Plant height is under strong selection pressure in a wheat breeding programme since this is the easiest and most efficient way to avoid releasing lodging prone varieties. Unfortunately breeding material is generally selected, prior to yield trials, in small untreated plots (i.e. no PGR treatment) and so potentially beneficial height/yield increasing alleles are lost. This is the case for the 3A QTL, which increases height by 6 to 7 cm and so can be easily selected against – as demonstrated by the scarcity of the Cadenza 3A QTL SNP haplotype in current recommended list varieties. In contrast, the 6A TGW QTL has rapidly become fixed in elite UK germplasm since it is associated with a smaller or no height (e.g. Spark x Rialto) increase depending on the genetic background. The QTL region can be followed using a SNP marker in the promotor of the *TaGW2* gene (*Yang et al.,* 2012) and now the smaller grain size allele can only be found in older varieties like Claire or in spring/alternative types like Cadenza and its derivatives. Unfortunately the marker developed to follow the 7D QTL was found not to be close enough to the underlying yield gene in current high yielding varieties such as Oakley.

The failure of the GWAS experiment to identify any of the height QTL region studied in this report could indicate again the success of traditional phenotypic selection either for (e.g. 6A) or against (e.g. 3A) these height associated yield effects. However, 20,000 SNPs do not provide adequate saturation of the wheat genome to guarantee that all ~100,000 wheat genes are in linkage disequilibrium with a SNP marker. This is clearly shown by GWAS analysis of panels of wheat

varieties with and without the *Rht-D1b* allele, in that no significant association is found on 4DS with the 90K iSelect data unless the specific SNP marker, identifying the causal mutation (Ellis *et al.,* 2002), is used (James Cockram, personal communication).

It is clear that efficient MAS for plant height will only be possible once the genes, underlying the major QTL, have been cloned. This will enable the causal mutations responsible for the phenotype to be identified, thus allowing the development of perfect markers, as in the case of the *Rht-B1* and *Rht-D1* (Ellis *et al.,* 2002). This project has made substantial progress in that direction, but even the smallest genetic interval on chromosome 3A still requires a large number of SNP markers to unequivocally identify it. Once perfect markers are available, then beneficial height and yield increasing alleles can be readily selected in the breeding populations and combined in different combinations, as was attempted by the construction of the double dwarf DH population in this project.

7. Quantify the responsiveness of the different height genes to different PGR active ingredients

7.1. Materials and Methods

7.1.1. Height QTL analysis with and without PGRs

Experiments

The Rialto x Savannah DH population (128 lines) was grown with and without PGRs at Boxworth (Cambridgeshire) in 2006/07 (BX07), at High Mowthorpe (North Yorkshire) in 2009/10 (HM10) and at High Mowthorpe (North Yorkshire) in 2010/11 (HM11). The 128 DH lines that were investigated are described in Appendices1 and 2. The experiment in 2006/07 was carried out as part of LK0958 'Identification of genetic markers for lodging resistance in wheat' and PGR treatments were 1) untreated and 2) 3C chlormequat 720 (chlormequat chloride (720 g/l) applied at 2.25 l/ha at GS30/31 followed by Terpal (2-chloroethylphosphonic acid (155 g/l) plus mepiquat chloride (305 g/l)) applied at 0.75 l/ha at GS37/39. The PGR treatments used in HM10 were 1) untreated, 2) 3C chlormequat 720 applied at GS30 (1.55 l/ha) and GS31/32 (0.70 l/ha), 3) 3C chlormequat 720 and Canopy (prohexadione calcium + mepiquat chloride) applied at GS30 (0.6 l/ha and 0.4 l/ha, respectively) and at GS31/32 (0.6 l/ha and 0.4 l/ha, respectively). The PGR treatments used in HM11 were 1) untreated, 2) 3C chlormequat 720 applied at GS30 (1.125 l/ha) and GS312 (1.125 l/ha), 3) Canopy (prohexadione calcium + mepiquat chloride) applied at GS30 (0.75 l/ha) and at GS31 (0.75 l/ha).

QTL analysis

Prior to QTL analysis, a sub-set of evenly-spaced marker loci was selected (~15-20 cM intervals), which provided good map coverage, a minimal amount of missing data and that, where possible, were in common between the Rialto x Savannah population to facilitate alignment of QTL. The QTL detection framework comprised 161 loci for Rialto x Savannah. The marker data and the three years of phenotypic data, plus the three year mean for all the traits were entered into the software package QTL Cartographer Version 2.0 (http://statgen.ncsu.edu/qtlcart/) and also MapDisto. MapDisto was used to calculate single marker analysis of variance (ANOVA) and QTL Cartographer was used to predict the size and position of each QTL using the composite interval mapping (CIM) procedure with the standard defaults. QTL were declared if the LOD score was greater than 2.5 according to CIM and if there was a significant difference ($p<0.05$) between the means of the two allelic classes at the marker loci flanking the QTL.

Height QTL were identified on chromosomes 1D, 2A, 2D, 3A, 4D, 6A and 7D. The closest genetic marker to each height QTL was then used to subdivide each of the 128 DH lines into subsets of

lines with and without the genetic markers closest to each of the height QTL. The height response to PGR treatments for sub-groups of DH lines with the genetic markers associated with height was then calculated.

7.1.2. Analysis of historic AHDB Recommended List data

AHDB Cereals & Oilseeds supplied plant height data (measured at, or soon after, anthesis) from AHDB Recommended List variety experiments which were treated with and without a PGR. Data from harvest years 2006 to 2010 were provided. In total, there were 39 experiments that were treated with and without PGRs. From this dataset 19 experiments were chosen that only used chlormequat as the PGR treatment and were fungicide treated. The chlormequat PGR treatment was applied between GS30 and GS32. A subset of 8 varieties were chosen which were included in most of the experiments. Analysis of variance was carried out on the data to investigate whether there were any significant variety effects on the amount of shortening caused by the PGR treatment.

7.1.3. Field experiments

Five field experiments were carried out to investigate the extent to which the effect of a PGR on crop height interacted with genotype. Details of the experiments are given in **Table 7.1**. The genotypes investigated in experiments HM10 and HM11 are described in Appendices 1 and 2. The genotypes investigated in BX12P, BX13 and GT14 are described in **Table 7.2** and **Table 7.3**.

The PGR treatments used in HM10 and HM11 are described in section 7.1.1. The PGR treatments used in BX12P, BX13 and GT13 are described in **Table 7.4** and **Table 7.5**.

Measurements in all the PGR experiments described above included height to the ear tip between GS65 and GS75, and grain yield was additionally measured in the HM10 and HM11 experiments.

A split plot analysis of variance was used to test for significant differences between the PGR and genotypic treatments and whether these two treatments interacted. In all experiments the PGR treatment was the main plot.

Table 7.1. Summary of experiments

Identifier	Year	Site	Soil type	Sow date	Seed rate seeds/m^2	Treatments	Experiment design	Plot size
HM10P	2009/10	High Mowthorpe North Yorks	Shallow silty clay loam over chalk	29 Sept	250	128 RiSa DH lines & 16 elite varieties 3 PGRs	Split plot, PGR main plot, 2 reps	12m x 2m
HM11P	2010/11	High Mowthorpe North Yorks	Sandy loam	12 Oct	250	128 RiSa DH lines & 16 elite varieties 3 PGRs	Split plot, PGR main plot, 2 reps	12m x 2m
BX12P	2011/12	Boxworth Cambs	Clay loam	23 Sep	300	20 elite varieties 8 PGRs	Split plot, PGR main plot, 3 reps	2m x 2m
BX13	2012/13	Boxworth Cambs	Clay loam	19 Nov	250	20 elite varieties & NILs 8 PGRs	Split plot, PGR main plot, 3 reps	2m x 2m
GT14	2013/14	Gleadthorpe Notts	Sandy loam	4 Nov	250	20 elite varieties & NILs 8 PGRs	Split plot, PGR main plot, 3 reps	2m x 2m

RiSa DH lines: Rialto x Savannah DH lines

AlBr DH lines: Alcedo x Brigadier DH lines

Table 7.2. Genotypes used in BX12P PGR experiments

	Genotype	Predicted responsiveness to PGR	Rationale
1	Solstice	High	Analysis of RL height data
2	Oakley	High	Analysis of RL height data
3	Gladiator	High	Historic analysis of RL
4	Denman	High	Analysis of RL height data
5	KWS Sterling	High	2011 RL standing Powers
6	Stigg	High	2011 RL standing Powers
7	Claire	Medium	Analysis of RL height data
8	Cordiale	Medium	Analysis of RL height data
9	Alchemy	Medium	Historic analysis of RL
10	KWS Santiago	Medium	2011 RL standing Powers
11	Gallant	Medium	2011 RL standing Powers
12	JB Diego	Medium	2011 RL standing Powers
13	Invicta	Medium	2011 RL standing Powers
14	Gravitas	Medium	2011 PGR expt
15	Coronation	Medium	2011 PGR expt
16	Humber	Low	Analysis of RL height data
17	Zebedee	Low	Historic analysis of RL
18	Viscount	Low	2011 RL standing Powers
19	KWS Target	Low	2011 RL standing Powers
20	Crusoe	Low	2011 PGR expt

Table 7.3. Genotypes used in BX13 and GT14 PGR experiments

	Genotype	Height QTL or predicted responsiveness to PGR
1	7D 115-040B Savannah	7D
2	7D 115-040 A Rialto	7D
3	7D 352-043 B Savannah	7D
4	7D 352-043 A Rialto	7D
5	2D-height AC113-67-7 Cadenza	2D
6	2D-height AC113-67-7 Avalon	2D
7	2D-height AC162-21-8 Cadenza	2D
8	2D-height AC162-21-8 Avalon	2D
9	6A-height-AC89-5-1 Cadenza	6A
10	6A-height-AC895-1 Avalon	6A
11*	6A-height-AC43 - E55 -6 Cadenza	6A
12*	6A-height-AC43 - E55 -6 Avalon	6A
13*	3A AC113-113-10 Cadenza	3A
14*	3A AC113-113-10 Avalon	3A
15	AC144-32-1 Cadenza	3A
16	AC144-32-1 Avalon	3A
17†	AC179-27-8	3A
18†	AC179-27-8	3A
19	3B-height AC160-28-4 Avalon	3B
20	3B-height AC160-28-4 Cadenza	3B
21	6B-hs and ht AC75-101-3 Avalon	6B
22	6B-hs and ht AC75-101-3 Cadenza	6B
23	Denman	
24	Solstice	
25	Stigg	
26	Target	
27	Viscount	
28	Zebedee	

* GT14 only †BX13 only

Table 7.4. PGR treatments used in BX12P and BX13 (product rates in l/ha)

Treatment	GS30	GS31	GS37	GS39-45
1 (NIL)	Nil	Nil		
2 (CCC)	CCC 1.125	CCC 1.125		
3 (Can)	Canopy 0.8 + CCC 1.0	Canopy 0.6 + CCC 1.0		
4 (Mod)	Moddus 0.2	Moddus 0.2		
5 (Cer)			Cerone 0.7	Cerone 0.3
6 (Ter)			Terpal 1.0	Terpal 0.5
7 (Can+T)	Canopy 0.8 + CCC 1.0	Canopy 0.6 + CCC 1.0	Terpal 1.0	
8 (Can+2T)	Canopy 0.8 + CCC 1.0	Canopy 0.6 + CCC 1.0	Terpal 1.0	Terpal 0.5

Table 7.5. PGR treatments used in GT14 (product rates in l/ha)

Treatment	GS30	GS31	GS37	GS39-45
1 (Nil)	Nil	Nil		
2 (CCC)	CCC 1.125	CCC 1.125		
3 (Can)	Canopy 0.8 + CCC 1.0	Canopy 0.6 + CCC 1.0		
4 (Mod)	Moddus 0.2	Moddus 0.2		
5 (Cer)			Cerone 0.7	Cerone 0.3
6 (Ter)			Terpal 1.0	Terpal 0.5
7 (BASF PGR1)		BASF PGR1	BAScode	
8 (Can + CCC + 2T)	Canopy 0.8 + CCC 1.0	Canopy 0.6 + CCC 1.0	Terpal 1.0	Terpal 0.5

7.2. Results

7.2.1. Height QTL analysis with and without PGRs

Six height QTL were found with average height effects ranging from about 3 cm to more than 6 cm (**Figure 7.1**). Height QTL on chromosomes 3A and 6A had the greatest effect of up to 8 cm.

A summary of the PGR effects on absolute and percentage height reduction for groups of DH lines with or without the genetic markers associated with various height QTL is shown in **Table 7.6** (absolute height reduction) and **Table 7.7** (percentage height reduction). An illustration of the absolute height reductions caused by the PGRs are shown in **Figure 7.2**. On average, the PGRs reduced height by about 80 mm.

1D height QTL: The short allele of this height QTL was more sensitive to the PGR (chlormequat followed by Canopy) and across all PGR treatments both in terms of absolute and percentage height reduction (**Table 7.6, Table 7.7** and **Figure 7.2**). On average the short allele of this QTL was shortened by an additional 8 mm, or the percentage shortening increased from 9.8% to 11.2% ($P<0.01$).

2A height QTL: The tall allele of this QTL was significantly shortened 14 mm more by the chlormequat followed by Terpal treatment in 2007 (**Table 7.1**) which resulted in an increase in the percentage height reduction from 8.8% to 10.2% (**Table 7.2**). However no significant differences in PGR sensitivity were detected in the other experiments.

2D height QTL: The short allele underwent more shortening and this was significant in absolute and percentage terms in 2007 when chlormequat followed by Terpal were used. Across all the experiments the short allele was shortened by an additional 6 mm, or by 11% compared with 10% ($P<0.1$; **Table 7.2**).

3A height QTL: The tall allele was shortened more in absolute terms in response to both chlormequat and chlormequat + Canopy in 2010. However, there was little evidence of significant effects on the percentage shortening.

6A height QTL: The tall allele was shortened more in absolute terms in 2007 (chlormequat followed by Terpal), 2010 (chlormequat) and 2011 (chlormequat + Canopy). On average across all experiments the tall allele was shortened by an additional 7 mm. In terms of percentage shortening the effects were less strong with effects observed in the 2007 and 2010 experiments ($P<0.1$) and no statistical effect across all experiments.

7D height QTL: The tall allele was generally shortened more by PGRs, but effects were not significant either in terms of absolute height reductions or percentage height reductions.

1D, 2D height QTL: Height QTL on chromosomes 1D and 2D tended to show the greatest difference in PGR sensitivity. When the genetic markers associated with greater PGR sensitivity were grouped together for these height QTL, the sensitive group had an average height reduction across experiments of 87 mm compared with 73 mm for the insensitive group and a percentage reduction of 11.7% compared with 9.2% ($P<0.01$; **Table 7.2**).

2D, 3A, 6A height QTL: When the genetic markers associated with greater PGR sensitivity were grouped together for height QTL 2D, 3A and 6A, the sensitive group had an average height

reduction across experiments of 103 mm compared with 69 mm for the insensitive group (P<0.001; **Table 7.1**) and a percentage reduction of 12.7% compared with 9.7% (P<0.01; **Table 7.2**).

2D, 3A, 6A, 7D height QTL: When the genetic markers associated with greater PGR sensitivity were grouped together for height QTL 2D, 3A, 6A and 7D the sensitive group had an average height reduction across experiments of 116 mm compared with 70 mm for the unresponsive group (P<0.001; **Table 7.1**) and a percentage reduction of 13.7% compared with 9.7% (P<0.05; **Table 7.2**).

The genetic marker associated with shorter height resulted in greater PGR sensitivity for the 1D and 2D markers. The genetic marker associated with greater height resulted in greater PGR sensitivity for the 3A and 6A markers. This illustrates that differential sensitivity to PGRs is not strongly affected by the overall height of the genotype. This observation is supported by a weak correlation between PGR induced height reduction and overall plant height (**Figure 7.4**).

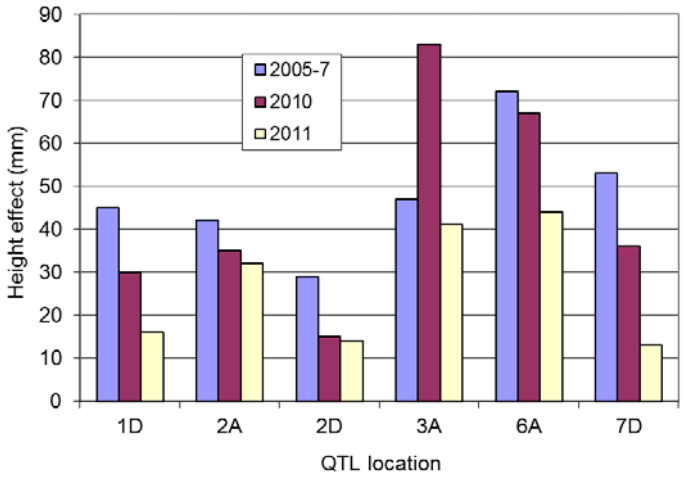


Figure 7.1. Summary of height QRL for Rialto x Savannah DH population grown in 3 seasons between 2005 and 2007 (LK0958) and in 2009/10 and 2010/11.

Table 7.6. Effect of PGRs on absolute height reduction (mm) for groups of DH lines with or without genetic markers for different height QTL. CCC – chlormequat, Ter – Terpal, Can – Canopy.

		2007	2010	2010	2011	2011	Mean
		CCC+Ter	CCC	CCC+Can	CCC	CCC+Can	
1D	Short type	84.4	69.2	92.9	76.3	104.7	85.8
1D	Tall type	90.1	70.7	78.0**	68.6	82.4*	77.8†
2A	Short type	82.6	69.7	87.2	73.9	92.3	81.4
2A	Tall type	96.7†	71.2	83.0	71.1	93.5	83.1
2D	Short type	100.6	75.8	88.3	74.6	89.2	85.7
2D	Tall type	80.2**	66.9	83.3	70.7	95.4	79.4
3A	Short type	82.1	58.1	77.9	69.9	92.5	76.5
3A	Tall type	91.4	78.4*	90.2*	73.3	93.2	85.1
6A	Short type	79.7*	59.6***	81.9	65.9	88.8*	76.3
6A	Tall type	98.6	82.8	88.3	79.0	96.7	89.1
7D	Short type	85.9	66.8	80.7	69.0	95.5	79.6
7D	Tall type	91.9	73.7	89.2	76.8	91.3	84.7
1D 2D	Insensitive	82.0	65.9	74.3	61.1	81.9	72.7
1D 2D	Sensitive	100.3†	73.0	92.3*	70.9	98.1	86.9*
2D 3A 6A	Insensitive	61.5	41.9	74.6	69.8	95.4	69.5
2D 3A 6A	Sensitive	116.6***	82.5*	100.3*	100.8*	116.4	103.3***
2D 3A 6A 7D	Insensitive	74.2	45.0	61.8	69.6	98.9	69.9
2D 3A 6A 7D	Sensitive	137.0***	114.4*	122.0***	94.2	111.2	115.8***

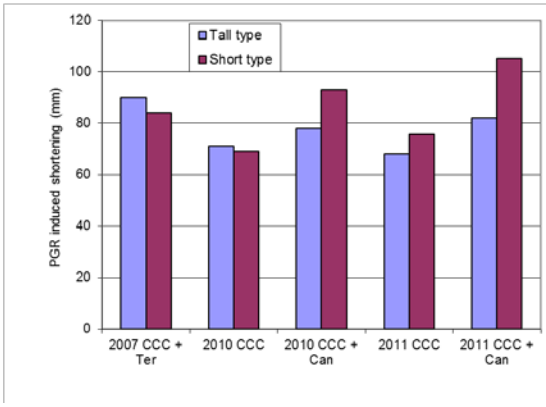
Differential sensitivity to PGRs tested by t-test: † (P<0.1), * (P<0.05), ** (P<0.01), *** (P<0.001).

Table 7.7. Effect of PGRs on percentage height reduction for groups of DH lines with or without genetic markers for different height QTL. CCC – chlormequat, Ter – Terpal, Can – Canopy.

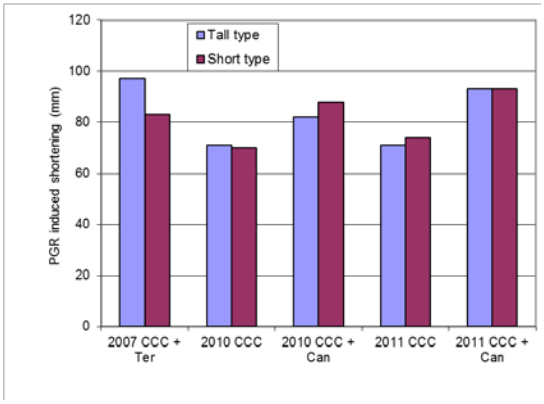
		2007	2010	2010	2011	2011	Mean
		CCC+Ter	CCC	CCC+Can	CCC	CCC+Can	
1D	Short type	9.02	7.96	11.00	11.71	16.21	11.22
1D	Tall type	9.41	8.02	8.91***	10.20	12.30***	9.76**
2A	Short type	8.83	8.09	10.33	11.46	14.40	10.65
2A	Tall type	10.03	7.96	9.41	10.37	13.76	10.30
2D	Short type	10.69	8.85	10.37	11.39	13.76	11.01
2D	Tall type	8.33***	7.44	9.56	10.47	14.25	10.02†
3A	Short type	9.05	6.97	9.64	11.00	14.66	10.30
3A	Tall type	9.37	8.71†	10.08	10.74	13.74	10.52
6A	Short type	8.68	7.08	9.95	10.33	14.01	10.09
6A	Tall type	10.10†	9.16†	9.83	11.51	14.19	10.97
7D	Short type	9.50	7.87	9.61	10.47	14.70	10.39
7D	Tall type	9.27	8.18	10.16	11.53	13.70	10.62
1D 2D	Insensitive	8.58	7.49	8.59	9.06	12.28	9.18
1D 2D	Sensitive	11.01*	8.91	11.39**	11.36	15.92	11.72**
2D 3A 6A	Insensitive	6.95	4.85	9.44	11.27	15.52	9.70
2D 3A 6A	Sensitive	11.92**	9.02†	11.07	14.63	17.09	12.75**
2D 3A 6A 7D	Insensitive	8.38	5.38	7.84	11.03	16.07	9.74
2D 3A 6A 7D	Sensitive	13.57*	12.17	13.00*	13.58	16.06	13.67*

Differential sensitivity to PGRs tested by t-test: † (P<0.1), * (P<0.05), ** (P<0.01), *** (P<0.001).

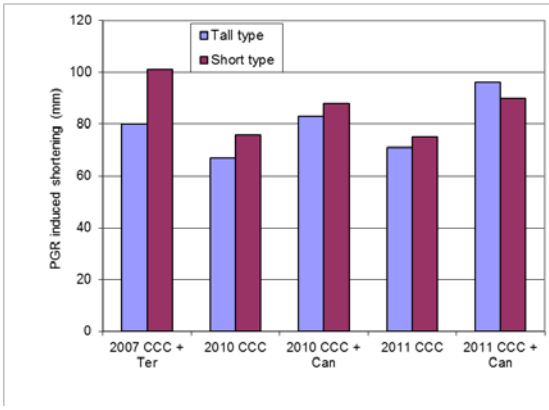
Height QTL on chromosome 1D



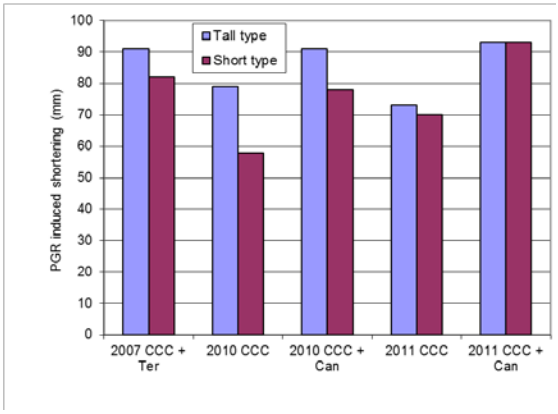
Height QTL on chromosome 2A



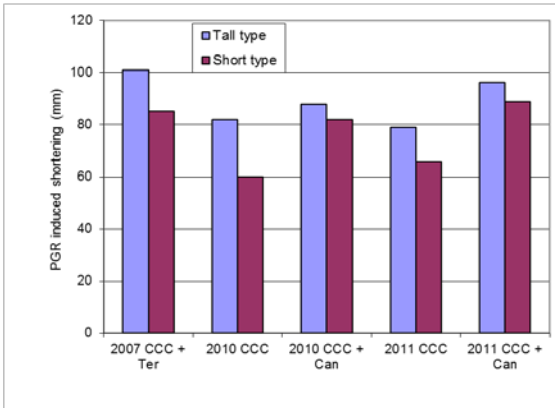
Height QTL on chromosome 2D



Height QTL on chromosome 3A



Height QTL on chromosome 6A



Height QTL on chromosome 7D

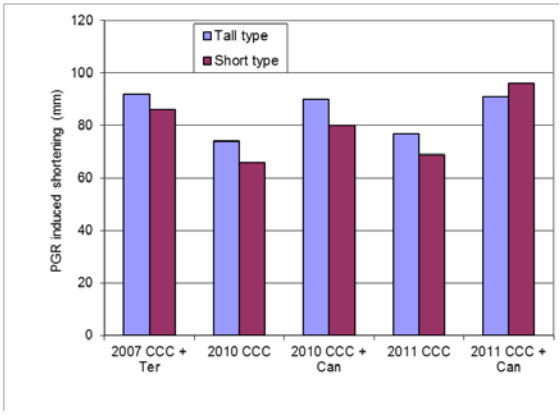
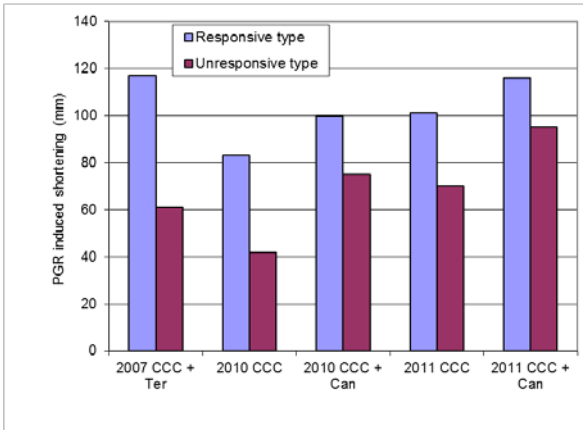


Figure 7.2. Effect of PGRs on height reduction for groups of DH lines with or without genetic markers for different height QTL. CCC – chlormequat, Ter – Terpal, Can – Canopy.

Height QTL on chromosome 2A, 3A & 6A



Height QTL on chromosome 2A, 3A, 6A, 7D

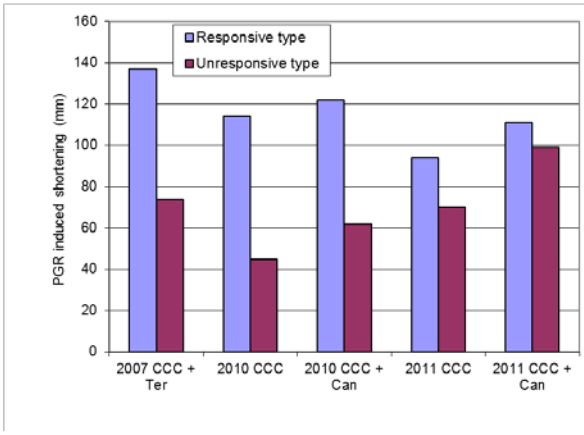


Figure 7.3. Effect of PGRs on height reduction for groups of DH lines with or without multiple genetic markers for different height QTL. DH lines were grouped according to which marker was more responsive to a PGR (responsive type) or less responsive (unresponsive type). CCC – chlormequat, Ter – Terpal, Can – Canopy.

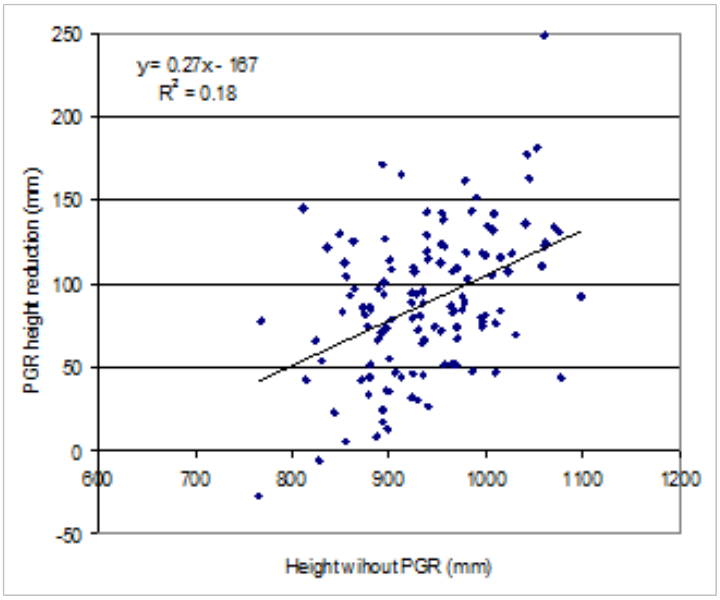


Figure 7.4. Relationship between overall crop height and the amount of height reduction caused by chlormequat (2.25 l/ha) at GS31 followed by Terpal (0.75 l/ha) at GS37, for Rialto x Savannah DH lines

7.2.2. Analysis of historic AHDB Recommended List data

The AHDB Recommended Lists (RL) include an estimate of the lodging resistance score for each variety. This is scored on a 1 to 9 scale where 9 is very resistant. This score is estimated from observations of natural lodging in experiments. Varietal differences in the degree of PGR induced increase in the lodging resistance score are likely to relate primarily to differences in the degree of PGR induced shortening, but it is also possible that PGR effects on stem and anchorage strength may come into play. A summary of the Lodging resistance scores between 2006 and 2010 shows that specific variety Lodging resistance scores can change by as little as 0.2 points to as much as 1.5 points (**Table 7.8**), which may be evidence of differing varietal sensitivity to PGRs.

Analysis of chlormequat induced height reductions from 10 to 12 RL experiments carried out in 2006, 2007 and 2008 showed that Cordiale underwent an average height reduction of 3.3 cm compared with 7.5 cm for Solstice (**Table 7.9**). However, these differences were not statistically significant. This was because of large variation in PGR effect between experiments, e.g. Solstice in 2006 had an overall mean of 8.3 cm, but the four values which made up the mean were 15.0,13.7, 6.0 and -1.5 cm, and Alchemy with a mean of 8.4 cm had individual values of 11.8,10.3, 9.7 and 1.8 cm.

It should be recognised that Solstice is about 13 cm taller than Cordiale which may explain some of the possible difference in shortening. On a percentage of total height basis, Solstice was shortened by 8.1% and Cordiale by 4.1%. There was a weak positive trend between variety height and the degree of PGR induced shortening for which a linear regression had an R^2 value of 0.33. However, there were several tall varieties which did not respond strongly to PGRs, e.g. Invicta had low response to PGRs but was relatively tall (**Figure 7.5**).

Table 7.8. Summary of resistance to lodging scores for the common varieties published in Recommended List in years 2006 to 2010. Varieties with lodging resistances scores of about 7 chosen to provide a fair comparison.

Variety	Resistance to lodging score **without PGR**	Resistance to lodging score **with PGR**	Increase in lodging resistance score
Zebedee	6.3	6.5	0.2
Claire	6.4	7.0	0.6
Soissons	6.5	7.2	0.7
Malacca	7.0	7.8	0.8
Robigus	7.0	7.8	0.8
Einstein	6.6	7.6	1.0
Istabraq	6.2	7.2	1.0
Oakley	6.5	7.5	1.0
Battallion	7.0	8.0	1.0
Alchemy	7.0	8.4	1.4
Gladiator	6.2	7.6	1.4
Gatsby	7.0	8.5	1.5

Table 7.9. Summary of chlormequat induced height reductions from AHDB Recommended List fungicide treated experiments with and without PGR.

	2006	**2007**	**2008**	**2009**	**2006-08**
Alchemy	8.4	1.3	6.3	9.8	4.9
Claire	8.0	2.9	5.1		5.2
Cordiale	2.0	3.2	5.0		3.3
Einstein	7.3	3.0	5.4	8.0	5.0
Humber	3.6	4.1	6.6		4.4
Oakley	3.8	2.1	6.2	8.5	3.6
Robigus	6.3	3.7	3.3	3.3	4.5
Solstice	8.3	6.8	7.2	10.5	7.5
No expts	4	4 to 5	2 to 3	0 to 3	10 to 12
L.S.D	7.8	9.2	11.2	6.0	4.9
P Value	NS	NS	NS	NS	NS

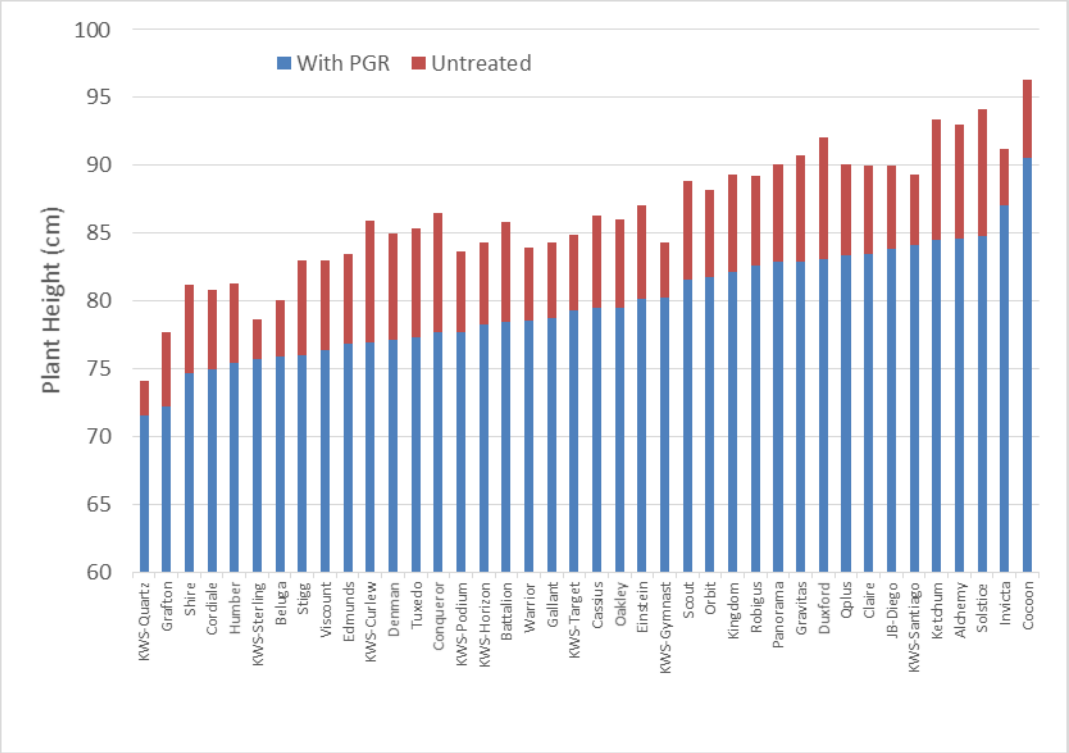


Figure 7.5. Average heights for untreated and PGR treated varieties between 2006 and 2010 from AHDB Recommend List data.

7.2.3. Field experiments

A summary of the results of the PGR and genotype interaction experiments is described in **Table 7.10**. Statistically significant interactions between PGR and genotype treatments were detected in three experiments (HM10, HM11, BXP12, BX13), with the interaction significant at the 90% level of significance (P=0.076) in the GT14 experiment. It must be recognised that 40% of the plots were lost from the BX13 experiment due to poor establishment. The data was analysed by ANOVA with missing plots and values estimated by Genstat were used in **Table 7.10**. The large number of missing plots will reduce the level of confidence for the treatment differences.

Correlations between PGR treatment effects

PGR treatments with different modes of action were investigated to test the hypothesis that if a genotype was found to be insensitive to a specific PGR active substance then other PGR active substances could be used to which the genotype was more sensitive. In particular it was expected that if differential genotype sensitivity was found then these genotypes would be unlikely to respond in the same way to gibberellic acid (GA) biosynthesis inhibitors (e.g. chlormequat) compared with substances that stimulate ethylene production (e.g. Cerone). This hypothesis was tested by calculating correlation coefficients between pairs of PGR treatments for their PGR induced height reductions.

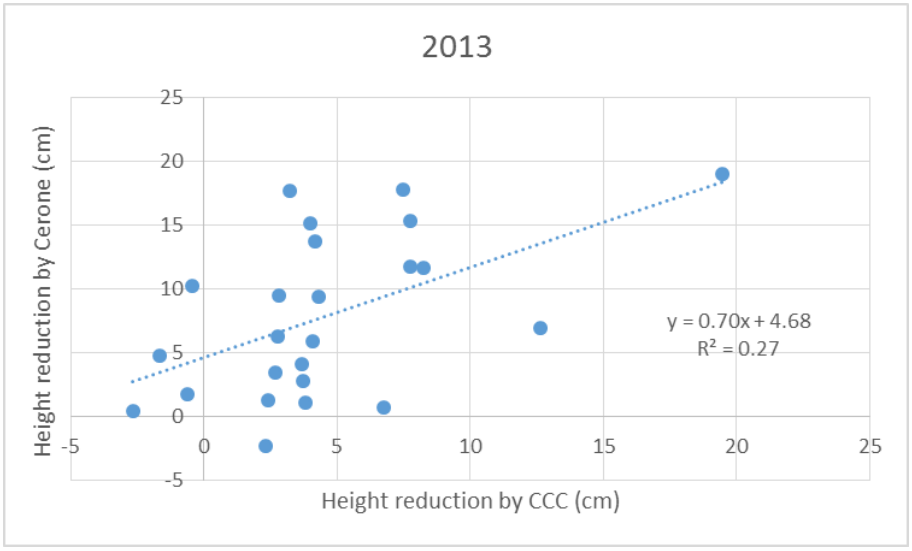
For the BX12P elite variety experiment, there were high correlation coefficients of between 0.75 and 0.94 between the PGR treatments causing significant height reductions. PGR treatments with different GA inhibition mode of action (Canopy) and a mixture of GA inhibition and ethylene production (Terpal) had a correlation coefficient of 0.75. This correlation may have been driven by mepiquat chloride which is present in both Canopy and Terpal. It was not possible to conclude about the effects of the single active PGRs (CCC, Moddus and Cerone) as these treatments did not significantly affect height.

For the experiments involving elite varieties and NILs, there were generally higher correlations between the PGR treatments in the GT14 experiment compared with the BX13 experiment. The size of the chlormequat induced height reductions correlated significantly with the height reductions of all the other PGR treatments. In the BX13 experiment, the range of correlation coefficients with chlormequat was 0.52 to 0.73 (correlation coefficient between chlormequat and Cerone was 0.52). In the GT14 experiment, the range of correlation coefficients with chlormequat was 0.75 to 0.90 (correlation coefficient between chlormequat and Cerone was 0.82).

A similar effect was found for Moddus. In the BX13 experiment, the range of correlation coefficients with Moddus was 0.31 to 0.66 (correlation coefficient with Cerone was 0.66). In the GT14 experiment, the range of correlation coefficients with Moddus was 0.67 to 0.90 (correlation coefficient with Cerone was 0.84).

A similar effect was found for Canopy. In the BX13 experiment, the range of correlation coefficients with Canopy was 0.37 to 0.68 (correlation coefficient with Cerone was 0.45). In the GT14 experiment, the range of correlation coefficients with Canopy was 0.49 to 0.90 (correlation coefficient with Cerone was 0.49).

It therefore appears that if a variety is insensitive to a PGR then it is likely to be insensitive to a wide range of PGR active substances, including gibberellic acid biosynthesis inhibitors and ethylene produces (**Figure 7.6**). On average across the BX13 and GT14 experiments, Canopy showed the poorest correlation coefficient with Cerone (0.47) compared with chlormequat (0.67) and Moddus (0.75).



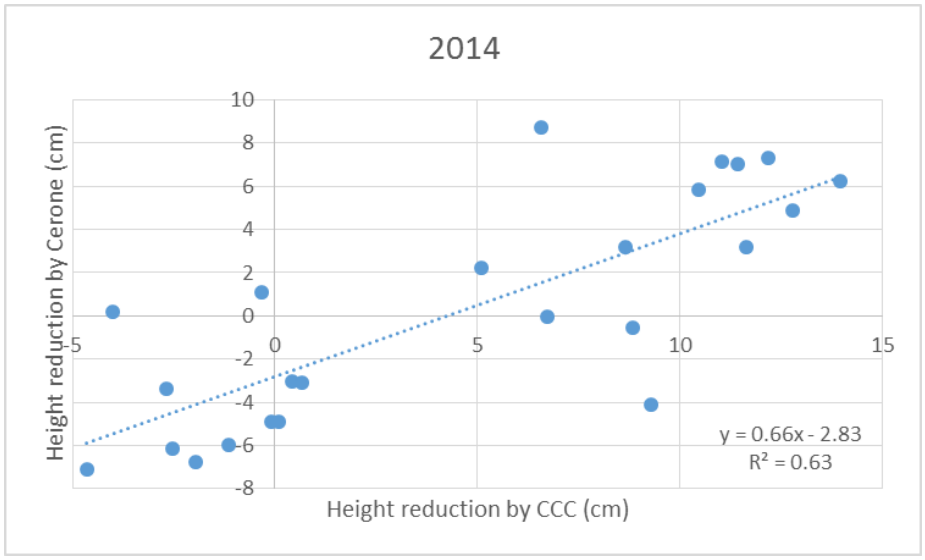


Figure 7.6. Correlation between chlromequat (CCC) and Cerone induced height reductions in 2013 (BX13) and in 2014 (GT14).

Table 7.10. Treatment effect on height (cm). Genotype range given in parenthesis

	HM10[1]	HM11[1]	BX12P[2]	BX13[3]	GT14[3]
Untreated	85.9 (63.9 – 107.1)	64.3 (50.1 – 84.0)	83.3 (75.9 – 93.8)	83.4 (69.9 – 106.4)	83.3 (70.5 – 99.2)
CCC	79.0 (58.3 – 95.6)	53.0 (39.0 – 68.5)	82.7 (74.5 – 92.3)	78.2 (66.1 – 98.1)	78.7 (65.7 – 88.9)
Moddus			82.8 (74.9 – 91.9)	77.4 (65.9 – 99.2)	83.0 (71.2 – 91.0)
Cerone			83.3 (75.2 – 91.9)	81.4 (64.3 – 104.5)	82.8 (68.6 – 93.4)
Canopy	77.4 (55.6 – 99.4)	57.0 (41.1 – 68.8)	78.2 (70.0 – 86.6)	75.5 (63.4 – 94.7)	68.9 (61.2 – 78.4)
Terpal			79.9 (72.5 – 88.4)	74.2 (65.1 – 94.2)	75.4 (66.0 – 85.9)
Canopy & Terpal			75.0 (67.7 – 83.1)	70.2 (59.4 – 89.1)	
Canopy & Terpal (x2)			77.2 (69.3 – 85.1)	71.1 (62.6 – 87.6)	
BASF PGR1					67.0 (54.6 – 92.4)
CCC, Canopy & Terpal					69.4 (58.2 – 77.8)
PGR P value	0.007	0.721	0.001	<0.001	<0.001
PGR LSD	2.306	11.00	3.72	4.44	1.884
Variety P value	<0.001	<0.001	<0.001	<0.001	<0.001
Variety LSD	3.658	5.62	1.02	1.37	2.828
PGR x Variety P value	0.017	0.008	<0.001	<0.001	0.076
PGR x Variety LSD	6.40	5.12	4.54	7.56	9.402

[1] Rialto x Savannah DH lines

[2] Elite varieties

[3] Elite varieties and NILs

Elite variety and PGR interactions

The elite variety and PGR interaction experiment carried out at BX12P showed the greatest variety-PGR interactions for the PGR treatments with multiple active substances (Canopy, Terpal, Canopy + Terpal, Canopy + CCC + Terpal). This was mainly because these PGR treatments caused average height reductions (across varieties) of 5 to 8 cm, whereas, CCC, Moddus and Cerone only achieved average height reductions of less than 1 cm (**Table 7.11**). Across all the PGR treatments, Solstice underwent the greatest shortening to PGRs at 6.5 cm and KWS Target was least sensitive with an average height reduction of just 1.2 cm. Across the PGR treatments that caused significant height reductions, the range of height reductions was greater with Solstice reducing height by 10 cm on average and KWS Target reducing height by 2.5 cm. There was a significant interaction between these varieties for each of the individual PGR treatments that significantly reduced height. Solstice was 14 cm taller than KWS Target, however the percentage by which Solstice shortened was still substantially greater than KWS Target at 11% compared with 3%. Across all varieties, there was no significant correlation between the untreated crop height and the average shortening caused by the PGR treatments. On average, the varieties which were predicted to have high responsiveness to PGRs were shortened by 6.0 cm, medium responsive varieties were shortened by 6.2 cm and low responsive varieties were shortening by 4.9 cm (averaged across the PGR treatments that significantly reduced height).

A summary of the PGR induced height reductions for the six common elite varieties grown in the BX12P, BX13 and GT14 PGR experiments are shown in **Table 7.12**. This shows that across all the PGR treatments in all experiments (21 site x PGR treatment combinations) Stigg underwent the greatest height reduction (7.2 cm) and KWS Target underwent the smallest height reduction (3.4 cm). A paired t-test using the treatment mean height reduction in each of the 21 experiment x PGR treatment combinations showed that there was a significant difference in the shortening effect of the PGRs ($P<0.01$). Stigg and KWS Target had significantly different height reductions in 4 of the 21 experiment x PGR treatment combinations. It was clear that the single active substance PGR products often did not cause a significant shortening effect, with significant effects observed in only two of the nine experiment x PGR treatment combinations. This contrasted with PGR treatments containing more than one active substance for which 10 of the 12 experiment x PGR treatment combinations had a significant effect. When the t-test is restricted to the PGR treatments containing more than one active substance the average shortening effect for Stigg was 9.2 cm compared with 4.9 cm for KWS Target ($P<0.01$). Zebedee also underwent more shortening than KWS Target ($P<0.05$), but none of the other variety differences were significant. Stigg and KWS Target had similar crop heights without PGR in these experiments which demonstrates that the difference in PGR response was not related to overall height. In fact, Stigg, Zebedee, KWS Target, Denman and Viscount all had similar untreated heights, with Solstice several centimetres taller.

Table 7.11. PGR induced height reductions (cm) in the BX12P PGR experiment

	Genotype	Predicted responsive-ness to PGR	Untreated height (cm)	CCC	Can	Mod	Cer	Ter	Can + Ter	Can + CCC + 2Ter	Mean
1	Solstice	High	93.8	1.50	8.61	1.89	1.84	6.17	15.28	9.95	**6.46**
2	Oakley	High	80.6	0.12	4.62	0.95	1.50	4.45	4.28	4.34	**2.89**
3	Gladiator	High	76.5	-0.17	2.11	-0.44	-1.50	2.72	6.44	2.56	**1.67**
4	Denman	High	82.4	0.56	6.33	1.56	-0.05	5.28	9.89	8.95	**4.65**
5	KWS Sterling	High	76.1	1.56	3.17	-3.94	-0.61	2.00	6.06	4.12	**1.77**
6	Stigg	High	79.6	0.62	6.50	2.23	-0.83	3.00	9.39	8.17	**4.15**
7	Claire	Medium	86.8	-0.34	5.11	0.89	-1.06	1.00	7.72	6.27	**2.80**
8	Cordiale	Medium	75.9	-0.61	5.89	1.00	0.67	3.39	8.22	6.61	**3.60**
9	Alchemy	Medium	91.2	0.22	8.44	1.33	0.00	6.89	12.33	9.00	**5.46**
10	KWS Santiago	Medium	88.4	0.67	5.56	2.89	0.72	3.89	8.95	6.95	**4.23**
11	Gallant	Medium									
12	JB Diego	Medium	90.3	1.27	6.16	-0.78	0.66	4.11	9.50	5.55	**3.78**
13	Invicta	Medium	88.3	-1.17	1.72	0.27	-0.17	0.89	5.27	3.27	**1.44**
14	Gravitas	Medium	89.8	-1.06	4.44	-1.11	-1.23	1.44	7.72	5.77	**2.28**
15	Coronation	Medium	84.7	3.39	7.50	0.28	0.50	5.78	12.17	10.17	**5.68**
16	Humber	Low	78.4	2.50	5.50	0.61	0.28	2.22	7.22	4.89	**3.32**
17	Zebedee	Low	81.2	1.17	3.50	-0.16	-2.77	2.95	8.45	3.84	**2.43**
18	Viscount	Low	79.4	0.00	4.11	-0.06	0.27	4.50	5.88	5.22	**2.85**
19	KWS Target	Low	80.1	0.11	2.44	-1.28	-0.50	-0.17	5.17	2.55	**1.19**
20	Crusoe	Low	80.1	1.67	6.28	3.72	3.83	5.61	9.39	8.33	**5.55**
	Mean		**85.5**	**0.63**	**5.16**	**0.52**	**0.08**	**3.48**	**8.39**	**6.13**	**3.48**

LSD = 4.54 cm

Table 7.12. PGR induced height reductions (cm) for the common elite varieties in the BX12P, BX13 and GT14 PGR experiments.

Genotype	Predicted responsive-ness to PGR	Mean untreated height (cm)	CCC			MOD			CER			CAN		
			2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014
Solstice	High	86.7	1.50 a	4.11 a	-4.64 a	8.61 bc	6.33 a	5.43 a	1.89 a	-0.78 a	-9.33 a	1.84 a	5.89 ab	-7.10 a
Denman	High	75.0	0.56 a	2.78 a	0.10 ab	6.33 ab	1.00 a	10.72 ab	1.56 a	1.83 ab	-5.24 ab	-0.05 a	6.33 ab	-4.93 a
Stigg	High	76.8	0.62 a	3.75 a	13.98 c	6.50 ab	2.58 a	17.75 b	2.23 a	5.58 ab	6.60 c	-0.83 a	2.83 ab	6.26 b
Zebedee	Low	77.1	1.17 a	3.83 a	5.10 bc	3.50 a	4.41 a	10.98 ab	-0.16 a	0.16 ab	2.73 bc	-2.77 a	1.08 a	2.22 ab
Viscount	Low	75.2	0.00 a	3.67 a	-0.33 ab	4.11 ab	4.33 a	9.49 ab	-0.06 a	-1.83 a	-6.97 ab	0.27 a	4.17 ab	1.12 ab
KWS Target	Low	78.4	0.11 a	-0.42 a	0.44 ab	2.44 a	3.66 a	6.55 a	-1.28 a	7.00 b	-1.30 abc	-0.50 a	10.25 b	-3.06 ab

Genotype	Predicted responsive-ness to PGR	TER			CAN + Ter		BASF PGR1	CAN + CCC + 2Ter			Mean (all PGRs)	Mean (mixtures of PGRs)
		2012	2013	2014	2012	2013	2014	2012	2013	2014		
Solstice	High	6.17 bc	6.39 a	1.85 a	15.28 d	7.72 a	2.29 a	9.95 de	9.66 a	1.89 ab	3.57	6.80
Denman	High	5.28 bc	0.66 a	4.98 a	9.89 bc	10.83 a	9.35 ab	8.95 de	8.39 a	7.69 bc	4.14	7.01
Stigg	High	3.00 ab	4.75 a	9.93 a	9.39 abc	9.08 a	14.82 b	8.17 bcd	6.66 a	17.83 d	7.21	9.21
Zebedee	Low	2.95 ab	4.33 a	7.85 a	8.45 abc	6.83 a	12.43 b	3.84 ab	6.25 a	12.94 cd	4.67	7.06
Viscount	Low	4.50 c	1.67 a	3.05 a	5.88 ab	12.00 a	12.53 b	5.22 abc	8.50 a	3.06 ab	3.54	6.20
KWS Target	Low	-0.17 a	5.66 a	5.01 a	5.17 a	8.33 a	8.17 ab	2.55 a	6.58 a	5.37 abc	3.36	4.94

A significantly different PGR response (P<0.05) between two varieties is denoted with the two varieties have no common letters following the height reduction figure.

NIL and PGR interactions

The NIL heights without PGR are summarised in **Table 7.13**. There were generally consistent differences in height between the NIL pairs for the 2013 and 2014 experiments. It was expected that the 7D NIL with the Savannah QTL would be slightly taller, but this was not the case with the Rialto NIL being a few centimetres taller. However, this effect was consistent with the fertiliser NIL experiments (see section 5.2). The 2D NIL with the Cadenza QTL was consistently taller, as expected. The largest NIL effect for any of the NILs was for the 2D NIL AC162-21-8, which showed an average height difference of 18 cm across the two experiments. The 6A NIL AC895-1 results were as expected with the NIL containing the Avalon QTL taller in both years. The 6A NIL AC43-E55-6 was only grown in 2014 and did not perform as expected with the NIL containing the Avalon QTL shorter. In 2014, the 3A NIL AC113-113-10 with the Avalon height QTL was 18 cm taller. It was expected that the NIL containing the Cadenza QTL would be taller in this NIL pair. In 2013 there was no difference in height observed between the AC179-27-8 Avalon and Cadenza NILs. There were no significant height differences for the 3A NIL AC144-32-1. The 3B NIL performed consistently with the Avalon QTL taller as expected. The 6B NIL performed consistently with the Cadenza QTL taller as expected. In summary, most of the NILs appeared to perform as expected apart from the 3A NILs and 6A NIL AC43-E55-6.

Linear regression analysis between the untreated crop height against the average reduction in height across all PGR treatments showed a positive relationship with an R^2 value of 0.40 (**Figure 7.7**).

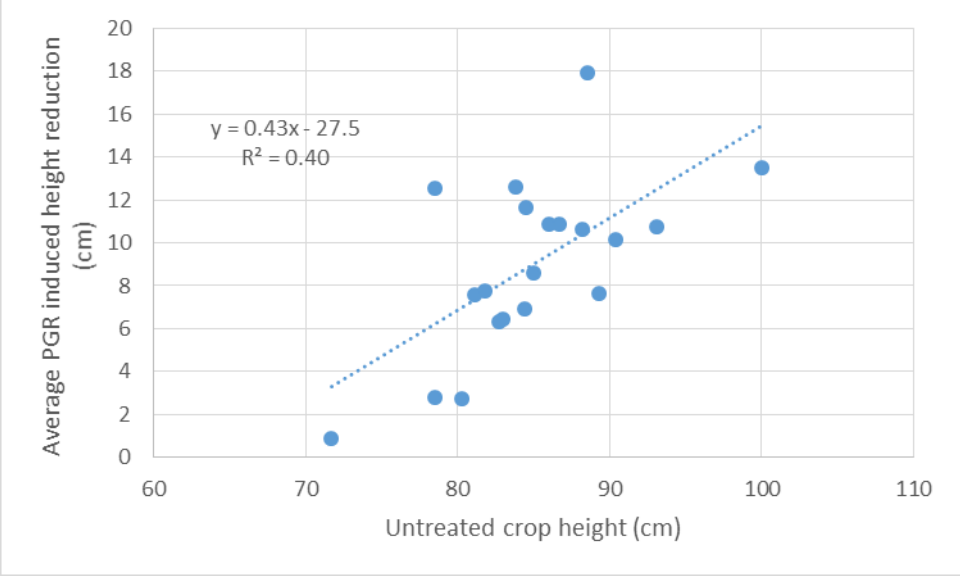


Figure 7.7. Untreated crop height against the average PGR induced height reduction for the NIL x PGR experiments in 2013 and 2014.

2D NILs

There were no consistent differential PGR effects for 2D NIL AC113-67-7, nor for AC162-21-8 in 2013 (**Table 7.14** and **Table 7.15**). However in 2014, the Cadenza parent of AC162-21-8 underwent significantly more shortening in six of the seven PGR treatments than the NIL with the Avalon parent. On average the NIL with the Cadenza parent was shortened by 16.0 cm compared with 0.7 cm for the NIL with the Avalon parent (paired t-test; P<0.001).

3A NILs

In 2014, the 3A NIL AC113-113-10 with the Avalon parent underwent significantly more shortening in two of the seven experiment x PGR combinations (**Table 7.14** and **Table 7.15**). Across all PGR treatments the NIL with the Avalon parent was shortened by 7.6 cm compared with 2.8 cm for the NIL with the Cadenza parent (P<0.05). In 2013, the AC179-27-8 NIL showed significantly more shortening for the NIL with the Cadenza parent in one of the seven PGR treatments. Note that 3A NILs did not perform as expected in 2013 with no height difference displayed by the AC179-27-8 pair and the NIL with the Avalon parent taller in the AC144-32-1 pair. Differential PGR effects were much greater in 2014.

The 3A NIL AC144-32-1 with the Avalon QTL underwent significantly more shortening in three of the fourteen experiment x PGR combinations. Across all PGR treatments the NIL with the Avalon parent was shortened by 11.6 cm compared with 6.9 cm for the NIL with the Cadenza parent (P<0.01). Note that the NIL with the Avalon parent had a similar height to the NIL with the Cadenza parent.

3B NILs

The 3B NIL AC160-28-4 had contrasting effects in 2013 compared with 2014. In 2013, the NIL with the Cadenza parent underwent significantly greater PGR responses in three of the seven PGR treatments (**Table 7.14** and **Table 7.15**). On average the NIL with the Cadenza parent was shortened by 13.2 cm compared with 6.8 cm for the NIL with the Avalon parent (P<0.05). However, in 2014, the NIL with the Avalon parent underwent significantly greater PGR responses in five of the seven PGR treatments. On average, the NIL with the Avalon parent was shortened by 13.5 cm compared with 2.1 cm for the NIL with the Cadenza parent (P<0.001).

6A NILs

There were consistent differential PGR effects on 6A NIL AC895-1 across both experiments, the NIL with the Avalon parent underwent significantly more shortening in 10 of the 14 experiment x PGR treatment combinations. On average, the NIL with the Avalon parent was shortened by 17.4 cm compared with 5 cm for the NIL with the Cadenza parent (P<0.001).

The 6A NIL AC43-E55-6 was only grown in 2014 and showed the opposite effect to 6A NIL AC895-1, with the NIL with the Cadenza parent undergoing significantly more shortening than the NIL with the Avalon parent. But note that this NIL behaved strangely because the NIL with the Cadenza QTL was taller.

6B NILs

There were no significant differential effects on shortening for any of the PGR treatments. Across all PGR treatments from both experiments the NIL with the Avalon parent was shortened by 12.6 cm compared with 10.7 cm for the NIL with the Cadenza parent. A paired t-test showed that this effect was significant.

7D NILs

7D NIL 115-040 had consistent interactions between with the PGR treatments. In seven of the 14 experiment x PGR treatment combinations, the NIL with the Rialto parent underwent significantly more shortening ($P<0.05$). The significant effects were across a range of active substances including GA inhibitors and ethylene producers. On average, across all 14 experiment x PGR treatment combinations the NIL with the Rialto parent was shortened by 10.9 cm compared with 2.8 cm for the NIL with the Savannah parent (paired test; $P<0.001$).

PGR effects for the 7D NIL 352-043 were similar to 7D NIL 115-040 in 2014 in that the NIL with the Rialto parent underwent significantly more shortening (11.4 cm) compared with the Savannah parent (2.7 cm) ($P<0.001$). However, in 2013 the opposite effect was found with the Savannah parent undergoing greater shortening (12.7 cm) compared with the Rialto parent (5.8 cm) ($P<0.01$).

Leaf wax investigation

An investigation of whether leaf waxiness explained any variation between genotypes in responsiveness to PGRs was carried out in the HM11 experiment. This was done by selecting 20 DH lines from the Rialto x Savannah DH population with a wide range of height responses to PGR in previous experiments. Samples were taken of two flag leaves per DH line at anthesis and analysed for their wax concentration. No correlation was found between PGR sensitivity and wax content (**Figure 7.8**).

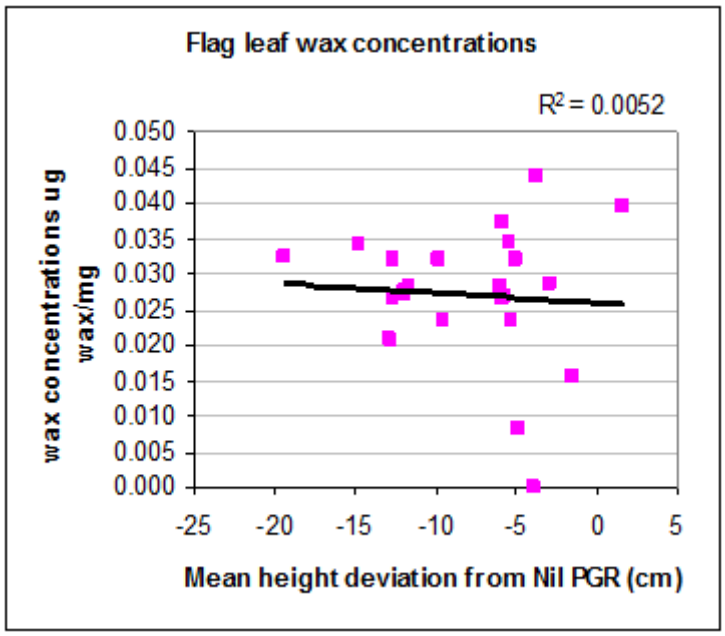


Figure 7.8. Comparison between PGR induced height reduction for Rialto x Savannah DH lines against the leaf wax concentration.

Table 7.13 NIL crop heights to ear tip (cm) without PGR treatment

Genotype	Height QTL			
		2013	2014	Average
115-040B Savannah	7D	76.9	80.0	78.5
115-040 A Rialto	7D	84.0	89.3	86.7
352-043 B Savannah	7D	83.9	79.7	81.8
352-043 A Rialto	7D	82.4	87.5	85.0
AC113-67-7 Cadenza	2D	86.9	99.2	93.1
AC113-67-7 Avalon	2D	79.1	92.9	86.0
AC162-21-8 Cadenza	2D	106.4	93.5	100.0
AC162-21-8 Avalon	2D	92.9	72.4	82.7
AC895-1 Cadenza	6A	89.7	80.7	85.2
AC895-1 Avalon	6A	95.7	98.7	97.2
AC43 - E55 -6 Cadenza	6A		78.5	78.5
AC43 - E55 -6 Avalon	6A		71.7	71.7
AC113-113-10 Cadenza	3A		84.9	84.9
AC113-113-10 Avalon	3A		83.6	83.6
AC179-27-8 Cadenza	3A	79.8		79.8
AC179-27-8 Avalon	3A	79.8		79.8
144-32-1 Cadenza	3A	83.8	84.9	84.4
144-32-1 Avalon	3A	86.6	82.3	84.5
AC160-28-4 Cadenza	3B	82.4	79.8	81.1
AC160-28-4 Avalon	3B	86.7	94.1	90.4
AC75-101-3 Cadenza	6B	89.8	86.5	88.2
AC75-101-3 Avalon	6B	87.1	80.5	83.8
Genotype x PGR LSD		7.56	9.40	

Table 7.14 Height reductions of NILs treated with single PGR actives

Genotype	Height QTL	CCC		Mod		Cer	
		2013	2014	2013	2014	2013	2014
115-040B Savannah	7D	-2.67 a	-2.53a	-8.66 a	-8.43 a	0.49 a	-6.15 a
115-040 A Rialto	7D	7.75 b	9.30b	3.34 b	-1.74 a	11.75 b	-4.14 a
352-043 B Savannah	7D	12.65 a	-1.13 a	2.50 a	-5.53 a	6.99 a	-6.00 a
352-043 A Rialto	7D	2.67 b	8.84 b	-1.49 a	1.33 a	3.50 a	-0.56 a
AC113-67-7 Cadenza	2D	-0.59 a	11.45 a	-2.42 a	9.09 a	1.75 a	7.04 a
AC113-67-7 Avalon	2D	2.42 a	12.79 a	6.92 b	14.81 a	1.33 a	4.88 a
AC162-21-8 Cadenza	2D	8.25 a	11.65 a	1.92 a	5.59 a	11.67 a	3.17 a
AC162-21-8 Avalon	2D	3.25 a	-2.68 b	3.33 a	-7.48 b	17.75 a	-3.39 a
AC895-1 Cadenza	6A	2.83 a	0.68 a	-0.50 a	-2.71 a	9.49 a	-3.11 a
AC895-1 Avalon	6A	19.48 b	11.05 b	9.16 b	8.79 b	18.99 b	7.15 b
AC43 - E55 -6 Cadenza	6A		12.81 a		7.29 a		9.79 a
AC43 - E55 -6 Avalon	6A		-5.69 b		-3.54 b		-5.47 b
AC113-113-10 Cadenza	3A		-0.09 a		-6.90 a		-4.90 a
AC113-113-10 Avalon	3A		6.73 a		3.69 b		-0.06 a
AC179-27-8 Cadenza	3A	2.33 a		-3.83 a		-2.33 a	
AC179-27-8 Avalon	3A	-1.67 a		-5.45 a		4.83 a	
144-32-1 Cadenza	3A	7.75 a	-4.01 a	5.50 a	-5.92 a	15.34 a	0.17 a
144-32-1 Avalon	3A	4.00 a	6.58 b	5.17 a	4.84 b	15.16 a	8.75 a
AC160-28-4 Cadenza	3B	7.49 a	-1.96 a	8.99 a	-5.01 a	17.82 a	-6.76 a
AC160-28-4 Avalon	3B	6.75 a	10.48 b	1.25 b	4.34 a	0.75 b	5.82 b
AC75-101-3 Cadenza	6B	4.33 a	8.65 a	6.11 a	2.11 a	9.39 a	3.16 a
AC75-101-3 Avalon	6B	4.17 a	12.18 a	4.05 a	4.75 a	13.72 a	7.31 a

A significantly different PGR response (P<0.05) between two varieties is denoted with the two varieties have no common letters following the height reduction figure.

Table 7.15 Height reductions of NILs treated with mixtures of PGR actives

Genotype	Height QTL	Can		Ter		Can + Ter	BASF PGR1	Can + CCC + 2Ter	
		2013	2014	2013	2014	2013	2014	2013	2014
115-040B Savannah	7D	3.16 a	18.85 a	4.33 a	6.10 a	9.65 a	9.59 a	6.16 a	9.14 a
115-040 A Rialto	7D	11.25 b	21.11 a	9.42 a	11.54 a	24.59 b	13.18 a	10.59 a	24.43 b
352-043 B Savannah	7D	14.49 a	14.09 a	16.48 a	4.24 a	23.98 a	4.00 a	12.15 a	9.42 a
352-043 A Rialto	7D	4.00 b	20.02 a	6.50 b	13.24 a	12.00 b	13.84 b	13.33 a	23.25 b
AC113-67-7 Cadenza	2D	4.08 a	28.68 a	4.91 a	14.53 a	7.83 a	29.67 a	8.41 a	26.43 a
AC113-67-7 Avalon	2D	2.92 a	21.42 a	5.83 a	12.03 a	10.33 a	24.52 a	9.92 a	21.96 a
AC162-21-8 Cadenza	2D	7.17 a	22.36 a	12.17 a	12.72 a	17.25 a	27.66 a	18.75 a	28.60 a
AC162-21-8 Avalon	2D	9.50 a	5.49 b	12.58 a	-0.06 b	20.08 a	8.12 b	17.00 a	4.79 b
AC89-5-1 Cadenza	6A	3.66 a	11.66 a	11.99 a	6.16 a	14.65 a	14.56 a	12.82 a	7.71 a
AC895-1 Avalon	6A	10.49 a	23.57 b	20.98 b	12.78 a	18.32 a	33.80 b	30.98 b	24.95 b
AC43 - E55 - 6 Cadenza	6A		13.03 a		12.49 a		17.61 a		14.77 a
AC43 - E55 - 6 Avalon	6A		6.15 a		3.09 a		10.13 a		1.56 b
AC113-113-10 Cadenza	3A		9.55 a		0.38 a		7.55 a		9.11 a
AC113-113-10 Avalon	3A		16.80 a		9.73 a		26.25 b		14.36 a
AC179-27-8 Cadenza	3A	6.33 a		1.34 a		8.33 a		11.83 a	
AC179-27-8 Avalon	3A	0.61 a		9.44 b		11.44 a		10.22 a	
144-32-1 Cadenza	3A	3.84 a	6.48 a	12.09 a	5.02 a	8.75 a	18.33 a	16.50 a	7.06 a
144-32-1 Avalon	3A	10.83 a	8.99 a	17.16 a	11.37 a	19.32 b	20.67 a	15.66 a	14.13 a
AC160-28-4 Cadenza	3B	9.16 a	7.21 a	16.65 a	3.81 a	15.32 a	8.62 a	16.65 a	8.50 a
AC160-28-4 Avalon	3B	8.16 a	19.27 b	8.75 b	9.77 a	12.16 a	26.55 b	9.83 a	18.15 b
AC75-101-3 Cadenza	6B	7.77 a	19.57 a	9.61 a	11.01 a	12.22 a	23.61 a	10.55 a	21.06 a
AC75-101-3 Avalon	6B	3.72 a	17.28 a	15.28 a	12.48 a	15.33 a	25.91 a	18.05 a	22.33 a

A significantly different PGR response ($P<0.05$) between two varieties is denoted with the two varieties have no common letters following the height reduction figure.

7.3. Conclusions

- Genotypes can undergo significantly different amounts of shortening (both in absolute and proportional terms) in response to PGRs. More than two-fold differences in shortening are possible. However PGR effects commonly interact strongly with the environment which makes it more difficult to identify genotypic differences.

- There is often a weak positive relationship between the untreated final crop height and the amount of PGR induced shortening, but this does not explain varietal differences in PGR sensitivity (e.g. some tall varieties, or QTL for increasing height, are relatively insensitive to PGRs).
- If a genotype is sensitive to a PGR then it tends to be sensitive to a wide range of active substances including GAI inhibitors and ethylene producers. This means that non-PGR solutions for improving lodging resistance may need to be developed for varieties that are insensitive to PGRs.
- There was evidence for some specific genetic markers associated with height to also be significantly associated with differences in PGR sensitivity. The best candidates were height QTL on chromosomes 1D and 2D identified within a Rialto x Savannah mapping population (for which the QTL for reduced height were more sensitive to PGRs). However, 2D NILs from an Avalon x Cadenza cross either showed no differential sensitivity to PGRs or that the taller NIL was more sensitive to PGRs.
- In the majority of cases, the taller NIL responded more to PGRs. On average the tall NILs were 88.4 cm and were shortened by 11.4 cm (12.9%); the short NILs were 73.9 cm and were shortened by 6.5 cm (8.8%). However there were two examples where the short NIL was shortened by significantly more.
- There was no evidence that genotypic variation in PGR sensitivity was related to differences in leaf waxiness which would may have affected uptake of the PGR into the plant.
- For two NIL pairs (3B NIL AC160-28-4, 7D NIL 352-043), the direction of significant differential responses to PGRs were reversed between seasons. This is evidence of very strong GxE interactions for varietal sensitivity to PGRs.
- There were examples of contrasting NIL sensitivity to PGRs between different streams of the same NIL within the same season (e.g. 6A NIL AC43-E55-6 vs 6A NIL AC895-1). Different NIL streams have different amounts of inserted DNA (because they were developed from a modest number of backcrosses), so this could be evidence that specific genes do determine PGR sensitivity, but not the genes that control height.
- Overall conclusion: There are genotypic differences in PGR sensitivity, which were consistent across different modes of PGR action. GxE for PGR sensitivity means that only differences between the most and least sensitive genotypes are often identified. No consistent evidence that genotypic differences in PGR sensitivity are related to overall height or with specific height genes.

8. Conclusions

Across 21 field experiments, this project has demonstrated that three yield QTL on chromosomes 3A, 6A and 7D each increase yield by 0.25 to 0.49 t/ha, with the potential for a combined yield improvement of about 1 t/ha. It is likely that the QTL on chromosome 3A which gives the greatest yield increase is not commonly present in elite germplasm and it is unlikely that any elite varieties have all three yield enhancing QTL. This project therefore illustrates that there is substantial scope to continue to increase yield potential of new wheat varieties by manipulating relatively few genes. Nitrogen (N) fertiliser rate treatments showed there was no evidence that the yield effects would necessitate more N fertiliser. However it was shown that yield QTL on chromosomes 3A and 6A were associated with greater height which means that breeding strategies and crop management will be required to reduce the greater lodging risk arising from increased height. It was calculated that the increase in yield would result in fewer GHGs per tonne of grain. If all three yield QTL were combined to increase yield by 1 t/ha, then the reduction in direct GHGs is estimated at 64 kg CO_2/t, and the reduction in direct and indirect (including land use change) GHGs is estimated at 159 kg CO_2/t. PGRs have a negligible GHG cost per tonne of grain (< 2 kg CO_2/t) (including application costs).

The yield QTL were generally associated with increases in total biomass, increases in grain number or grain number and grain size, and often greater growth increases after flowering than before flowering. Understanding how the yield QTL increase yield will help target crop management to realise the greater yield potential of new varieties that contain these yield QTL. For example, the yield QTL on chromosome 3A was shown to increase grains/m^2, this is therefore likely to require crop management that focuses on increasing photosynthesis during grain filling in order to fill the greater number of grains.

One of the drawbacks of combining the QTL for greater yield using existing genetic markers is that this will increase crop height by several centimetres. To investigate whether the increase in height could be mitigated a specific DH population was made to stack the three yield QTL identified on chromosomes 3A, 6A and 7D within a double-dwarf background (*Rht1* and *Rht2*; NB current recommended varieties possess one of these dwarfing genes, but not both). The resulting genetic lines were 60 to 80 cm tall, therefore showing that the yield QTL could be combined into one variety without causing a high lodging risk. Unfortunately, the yield trial in 2013/14 was abandoned due to black grass infestation, so the combined effects on yield are not known.

Near inbred lines (NILs) are pairs of lines differing only for the region of the chromosome containing the yield or height QTL of interest. This project focussed on NILs for three yield QTL on chromosomes 3A, 6A and 7D and three height QTL on chromosomes 2D, 3B and 6B. The project developed new NILs for the yield QTL on chromosome 7D and further refined and multiplied up

existing NILs. This precision germplasm will provide pre-breeding materials valuable for introgression of the yield and height QTL into elite germplasm, and to quantify the breeding value of combining these QTL by intercrossing NILs and recombinants.

This project has developed new genetic markers for yield and height that are amenable for high throughput use in commercial breeding programmes. These genetic markers have been mapped at high resolution to maximise the reliability with which they predict effects on yield and height. The project aimed to develop more reliable genetic markers which reduce the genetic distance between genetic markers flanking the yield and height QTL to 5 centimorgans (cM). This has been exceeded for the 2D, 3A and 6A QTL which have all been located between genetic markers less than 1 cM apart. The interval between the QTL on 6B has been substantially reduced but further delimiting is hampered by marker availability. The QTL interval for 3B has been halved but a rearrangement of 3B in the mapping population means that current marker data is unhelpful. It has also been possible to propose gene candidates for the QTLs on 2D, 3A and 6A which will help to find even more reliable genetic markers and understand the mechanism by which the QTL affects yield / height. This will be important for understanding whether the same gene affects yield and height or whether these effects can be uncoupled.

Currently, early generation selection for height is done phenotypically and this is skewing breeding populations towards, what the plant breeder considers, to be an optimal height. The results of Objective iii clearly indicate that the some of the height QTL studied in this project are being selected against, despite their positive effect on yield. Having diagnostic genetic markers for these key QTL will enable breeders to design crosses and efficiently select and combine these QTL in order increase yield, whilst minimising lodging risk. The ultimate goal will be to clone each of the genes underlying each of these QTL to know if increased yield is a pleiotropic effect of increased height. If it isn’t, then cloning the tightly-linked yield genes will be a huge advance for increasing yield without increasing height and the risk of lodging.

Varieties can undergo significantly different amounts of shortening in response to PGRs. More than two-fold differences in shortening were observed between genotypes. However PGR effects commonly interacted strongly with the environment which made it difficult to identify consistent genotypic differences unless they were large. There was often a weak positive relationship between the untreated final crop height and the amount of PGR induced shortening, but this did not fully explain varietal differences in PGR sensitivity (some tall varieties were relatively insensitive to PGRs). There was no consistent evidence that varietal differences in PGR sensitivity were related to the presence or absence of specific height genes, or to differences in leaf waxiness. If a genotype was sensitive to a PGR then it tended to be sensitive to a wide range of PGR active substances including gibberellic acid (GA) inhibitors (e.g. chlormequat) and ethylene

producers (e.g. Cerone). This means that non-PGR solutions for improving lodging resistance may also be required for varieties that are insensitive to PGRs (e.g. lower seed rate, delayed N fertiliser etc…). This research opens up the possibility of bespoke lodging control methods for specific varieties.

9. Recommendations for further work

Future R&D is required to develop fully diagnostic genetic markers which will enable plant breeders to combine multiple genes. Ultimately the aim is to identify the DNA sequence of each gene in question to provide a perfect genetic marker. This can be achieved by re-sequencing alleles of candidate genes, analysing close recombinants, validating candidate SNPs in germplasm collections, and through functional genomics approaches. In particular, this will answer the question about whether the genes for yield and height are pleiotropic effects or can be unlinked. Unlinking yield and height effects will be a great advantage to plant breeders as it will help them to simultaneously increase yield and reduce lodging risk. It will be important to carry this out for yield QTL identified on chromosomes 3A and 7D.

The development of a breeding population which combined the three yield QTL in a double dwarf gene background (*Rht1* and *Rht2*) successfully showed that the 3 yield QTL could be combined into varieties with typical crop heights of 60–80 cm. However effects on yield could not be tested due to black grass infestation. The germplasm is being re-multiplied in 2014/15 and funding for new experiments is required to test effects on yield.

Specific crop management methods to realise the genetic potential of the new varietal types must be developed. This project identified that crop management to maximise grain filling is required to fill the larger number of grains set, but the type of crop management must now be specified (e.g. can it be achieved through the use of fungicides or later N fertiliser applications?).

New research is required to understand the environmental and genetic factors which control variation in PGR sensitivity to allow PGRs to be targeted more precisely. If a variety was insensitive to PGRs, then it tended to be insensitive to all of the main PGR active ingredients currently available. Research is required to identify more precisely which varieties are insensitive to PGRs. The current lodging resistance scores with and without PGRs does not adequately differentiate between varieties because it is reliant on lodging in trials (which does not always happen). Differences in PGR height reduction would be a much more reliable indicator. Research is also required to identify new PGR modes of action to effectively shorten varieties which are less sensitive to current PGRs. It is the ultimate aim to develop bespoke lodging control methods for specific varieties.

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Appendix 1. Rialto x Savannah DH lines & elite varieties used in HM10

Variety no.	Variety name
1	RS1
2	RS2
3	RS3
4	RS5
5	RS6
6	RS7
7	RS8
8	RS9
9	RS10
10	RS11
11	RS12
12	RS13
13	RS14
14	RS16
15	RS17
16	RS18
17	RS19
18	RS20
19	RS21
20	RS23
21	RS24
22	RS25
23	RS26
24	RS27
25	RS28
26	RS29
27	RS30
28	RS31
29	RS32
30	RS33
31	RS34
32	RS35
33	RS36
34	RS37
35	RS38

Variety no	Variety name
49	RS52
50	RS53
51	RS54
52	RS55
53	RS56
54	RS57
55	RS58
56	RS59
57	RS60
58	RS61
59	RS62
60	RS63
61	RS64
62	RS65
63	RS66
64	RS67
65	RS68
66	RS69
67	RS70
68	RS71
69	RS72
70	RS73
71	RS74
72	RS76
73	RS77
74	RS78
75	RS79
76	RS81
77	RS82
78	RS83
79	RS84
80	RS85
81	RS86
82	RS87
83	RS88

Variety no	Variety name
97	RS104
98	RS105
99	RS106
100	RS107
101	RS108
102	RS109
103	RS110
104	RS111
105	RS112
106	RS113
107	RS114
108	RS115
109	RS116
110	RS117
111	RS118
112	RS119
113	RS120
114	RS121
115	RS122
116	RS123
117	RS124
118	RS125
119	RS126
120	RS127
121	RS128
122	RS129
123	RS130
124	RS131
125	RS132
126	RS133
127	RS135
128	RS136
V.1	Alchemy
V.2	Edmunds
V.3	Gravitas

36	RS39
37	RS40
38	RS41
39	RS42
40	RS43
41	RS44
42	RS45
43	RS46
44	RS47
45	RS48
46	RS49
47	RS50
48	RS51

84	RS89
85	RS90
86	RS91
87	RS92
88	RS93
89	RS95
90	RS96
91	RS97
92	RS98
93	RS99
94	RS100
95	RS102
96	RS103

V.4	Invicta
V.5	Lear
V.6	Oakley
V.7	RS Rialto
V.8	RS Savanna
V.9	Stigg
V.10	WW25
V.11	WW29
V.12	WW31
V.13	WW32
V.14	WW33
V.15	WW35
V.16	WW36

Appendix 2. Rialto x Savannah DH lines & elite varieties used in HM11

Variety no.	Variety name
1	RS1
2	RS2
3	RS3
4	RS5
5	RS6
6	RS7
7	RS8
8	RS9
9	RS10
10	RS11
11	RS12
12	RS13
13	RS14
14	RS16
15	RS17
16	RS18
17	RS19
18	RS20
19	RS21
20	RS23
21	RS24
22	RS25
23	RS26
24	RS27
25	RS28
26	RS29
27	RS30
28	RS31
29	RS32
30	RS33
31	RS34
32	RS35
33	RS36
34	RS37

Variety no	Variety name
49	RS52
50	RS53
51	RS54
52	RS55
53	RS56
54	RS57
55	RS58
56	RS59
57	RS60
58	RS61
59	RS62
60	RS63
61	RS64
62	RS65
63	RS66
64	RS67
65	RS68
66	RS69
67	RS70
68	RS71
69	RS72
70	RS73
71	RS74
72	RS76
73	RS77
74	RS78
75	RS79
76	RS81
77	RS82
78	RS83
79	RS84
80	RS85
81	RS86
82	RS87

Variety no	Variety name
97	RS104
98	RS105
99	RS106
100	RS107
101	RS108
102	RS109
103	RS110
104	RS111
105	RS112
106	RS113
107	RS114
108	RS115
109	RS116
110	RS117
111	RS118
112	RS119
113	RS120
114	RS121
115	RS122
116	RS123
117	RS124
118	RS125
119	RS126
120	RS127
121	RS128
122	RS129
123	RS130
124	RS131
125	RS132
126	RS133
127	RS135
128	RS136
V.1	Alchemy
V.2	Edmunds

35	RS38
36	RS39
37	RS40
38	RS41
39	RS42
40	RS43
41	RS44
42	RS45
43	RS46
44	RS47
45	RS48
46	RS49
47	RS50
48	RS51

83	RS88
84	RS89
85	RS90
86	RS91
87	RS92
88	RS93
89	RS95
90	RS96
91	RS97
92	RS98
93	RS99
94	RS100
95	RS102
96	RS103

V.3	Gravitas
V.4	Invicta
V.5	Lear
V.6	Oakley
V.7	RS Rialto
V.8	RS Savannah
V.9	Stigg
V.10	WW25
V.11	WW32
V.12	WW37
V.13	WW39
V.14	WW41
V.15	WW42
V.16	WW44

Appendix 3. Subset of Rialto x Savannah DH lines used for detailed growth analysis at HM10 and HM11. PGR untreated plots.

		Height reducing alleles present?		
Variety no.	Variety name	7D	3A	6A
8	**RS9**	N	Y	N
9	**RS10**	N	Y	N
27	**RS30**	N	Y	Y
28	**RS31**	N	Y	Y
33	**RS36**	N	Y	Y
34	**RS37**	N	Y	N
50	**RS53**	N	Y	N
55	**RS58**	N	Y	N
65	**RS68**	N	Y?	Y
69	**RS72**	N	Y	N
73	**RS77**	N	Y	Y
82	**RS87**	N	Y	Y
84	**RS89**	N	Y	Y
89	**RS95**	N	Y?	Y
90	**RS96**	N	Y	N
1	**RS1**	Y	Y	Y
6	**RS7**	Y	N	Y
12	**RS13**	Y	N	Y
13	**RS14**	Y	Y	Y
17	**RS19**	Y	N	Y
46	**RS49**	Y	Y	Y
47	**RS50**	Y	Y	N
49	**RS52**	Y	Y	Y
56	**RS59**	Y	N	Y
57	**RS60**	Y	Y	Y
64	**RS67**	Y	Y	N
67	**RS70**	Y	Y	Y
68	**RS71**	Y	N	Y
71	**RS74**	Y	Y	Y
76	**RS81**	Y	N	Y

Appendix 4. Subset of Avalon Cadenza 3A NILs and Spark Rialto 6A NILs used in the HM12 experiment.

NIL no	3A NIL name
1	Avalon
2	Cadenza
3	AC113-113-10 A
4	AC113-113-10 C
5	AC144-32-1 A
6	AC144-32-1 C
7	AC179-27-2 A
8	AC179-27-2 C
9	AC179-27-8-A
10	AC179-27-8-C

NIL no	6A NIL name
1	Spark
2	Rialto
3	6A-SPXRI-3-125 S
4	6A-SPXRI-2-126 R
5	6A-SPXRI-2-127 S
6	6A-SPXRI-2-128 R
7	6A-SPXRI-1-129 S
8	6A-SPXRI-2-130 R
9	6A- SPXRI-1-131 S
10	6A-SPXRI-2-132 4

Appendix 5. Subset of Avalon Cadenza 3A NILs, Spark Rialto 6A NILs and Savannah Rialto 7D NILs used in the GT1, HM 14 and LM14 experiments.

NIL no	3A NIL name
1	Avalon
2	Cadenza
3	AC113-113-10 A
4	AC113-113-10 C
5	AC144-32-1 A
6	AC144-32-1 C
7	AC179-27-2 A
8	AC179-27-2 C
9	AC179-27-8 A
10	AC179-27-8 C

NIL no	6A NIL name
1	Spark
2	Rialto
3	6A-SPXRI-3-125 S
4	6A-SPXRI-2-126 R
5	6A-SPXRI-2-127 S
6	6A-SPXRI-2-128 R
7	6A-SPXRI-1-129 S
8	6A-SPXRI-2-130 R
9	6A- SPXRI-1-131 S
10	6A-SPXRI-2-132 4

NIL no	7D NIL name
1	Savannah
2	Rialto
3	328-114A
4	328-114B
5	352-043A
6	352-043B
7	115-040A
8	115-040B

Appendix 6. Subset of Avalon Cadenza 3A NILs Avalon Cadenza 6A NILs and Savannah Rialto 7D NILs used in the TT14 and LIM14 experiment.

NIL no	3A NIL name
1	Avalon
2	Cadenza
3	AC113-113-10 A
4	AC113-113-10 C
5	AC144-32-1 A
6	AC144-32-1 C
7	AC179-27-2 A
8	AC179-27-2 C
9	AC179-27-8 A
10	AC179-27-8 C

NIL no	6A NIL name
1	Avalon
2	Cadenza
3	6A-height-AC89-5-1 A
4	6A-height-AC89-5-1 C
5	6A-height-AC43-E55-6 A
6	6A-height-AC43-E55-6 C
7	6A-height-AC104-6-9 A
8	6A-height-AC104-6-9 C
9	6A-height-AC43-E55-4 A
10	6A-height-AC43-E55-4 C

NIL no	7D NIL name
1	Savannah
2	Rialto
3	328-114A
4	328-114B
5	352-043A
6	352-043B
7	115-040A
8	115-040B