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Delivery of *Ppd1* tools & novel allelic effects useful to UK/EU wheat improvement

by

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ABBREVIATIONS

CIMMYT	International Maize and Wheat Improvement Centre, Mexico
JIC	John Innes Centre, Norwich
Ppd	Photoperiod genes
Eps	Earliness *per se* genes
NIL(s)	Near iso-genic lines
Vrn	Vernalisation genes
AA	Diploid *Triticum* A-genome
BB	Diploid *Triticum* B-genome
DD	Diploid *Triticum* D-genome
AABBDD	Hexaploid wheat genome
SHW(s)	Synthetic hexaploid wheat (*T. durum* x *Aegilops tauschii*)
SHW-D	Synthetic-derived wheat
FFD	Factor Form Density
PaS	Paragon x SHW
XS	Xi-19 x SHW
QTL	Quantitative trait locus
PS	Photoperiod sensitive
PI	Photoperiod insensitive
SP / EP / NP	Short / Extended / Natural photoperiod
SNP	Single nucleotide polymorphism
SSR	Simple Sequence Repeat polymorphism (microsatellite)
DArT	Diversity Arrays Technology
GS39	Zadocks growth stage 39: flag leaf fully emerged
GS55	Zadocks growth stage 55: spike half emerged
GS61	Zadocks growth stage 61: start of flowering
BC_1, BC_2	Backcross 1 (F_1 x recurrent parent), backcross 2 (BC_1 x recurrent parent)
TGW	Thousand gain weight
WGIN	Wheat Genetic Improvement Network
NIAB	National Institute of Agricultural Botany, Cambridge
ANOVA	Analysis Of Variance
FT	Flowering time
E / L	Early / Late flowering
DH	Doubled haploid
SSD	Single Seed Descent
LoLa	BBSRC Wheat Pre-breeding Long and Large (LoLa) project
CSSL	Chromosome Segment substitution Lines

CONTENTS

1. ABSTRACT

Agro-environmental adaptation is critical to many aspects of crop performance and yield. A key component of adaptation to specific environments is developmental rate and flowering time, controlled in part by photoperiod response (*Ppd*) and earliness *per se* (*Eps*) genes. Another important factor in successful crop improvement is the availability of sufficient genetic variation from which to select plant types with optimal performance for target environments. The objectives of this study were threefold: (1) development of near iso-genic lines (NILs) carrying individual *Ppd* and *Eps* genes, (2) characterisation of *Ppd* and *Eps* NILs for flowering time and yield and (3) introduction of novel genetic variation from synthetic hexaploid wheat lines (SHW) developed at CIMMYT.

NILs were developed carrying *Ppd-1* gene variants on chromosomes 2A, 2B and 2D, some of which were previously uncharacterised. The relative potency of the reduction in flowering time for the photoperiod insensitive variants was determined as follows: *Ppd-D1a=Ppd-A1a>Ppd-B1a,* indicating that *Ppd-A1a* (previously uncharacterised in bread wheat) is a potent novel source of photoperiod insensitivity for wheat breeding. Contrary to the prevailing consensus from previous studies in north-western Europe, photoperiod insensitive early flowering NILs out-yielded the corresponding later flowering lines in 2011-12, although this may be an artefact of the unusual season.

Eps QTL in the current study were identified using data from previous studies carried out by project partners at the John Innes Centre (JIC) in Norwich. Although the accepted definition suggests that *Eps* loci reduce the time to flowering regardless of prevailing conditions, it is clear from previous studies at the JIC that environment had a significant influence on the expression of *Eps* genes over experimental years. In contrast to these previous results, *Eps* effects on flowering time in the current study were found to be relatively reproducible over experiments and years with a consensus emerging as follows (derivative population Spark x Rialto (SR) and Charger x Badger (CB), followed by chromosomal location of the gene): SR-1D>SR-3A>SR-7A>SR-3B>SR-6B>[CB-3A, CB-3B, CB-6A, CB-6B & CB-7A].

The current project represents the first systematic introduction of novel genetic variation into UK wheat germplasm from the D-genome ancestor of wheat (*Aegilops tauschii*) via synthetic hexaploids since early studies at the Plant Breeding Institute in the 1940s and 50s. The SHWs used here were developed at CIMMYT by crossing elite tetraploid durum wheat with *A. tauschii.* Markers were used to identify a representative subset of CIMMYT SHWs which were backcrossed into two UK wheat varieties, Paragon and Xi-19, generating over 5600 recombinant lines in total.

Field selection took a pre-breeding approach, with a focus on yield components including increased biomass, and the first yield trials indicated several lines which out-performed Xi-19. The best germplasm is being integrated into commercial breeding programmes.

2. SUMMARY

2.1. Background and aims

Genes controlling flowering time and the selection of genetic variation contributing to environmental adaptation are important considerations for plant breeders seeking to increase yields. However, only a few genes controlling flowering time have so far been studied in wheat, and there is still much to be understood regarding their effect on agronomic performance. Despite these constraints, genetic improvement by UK wheat breeders has continued to contribute to significant year-on-year yield gains in trials. However, yields on-farm have stagnated, giving rise to a growing gap between trial yield and farm yield. There is some evidence that better water-use efficiency and drought resistance can contribute to stability of yield over years and environments. In addition, improved efficiency in the use of other agricultural inputs, including nitrogen fertilizer, and pesticides, is another key factor in addressing wider environmental concerns.

Photoperiod response (*Ppd*) genes and floral transition in wheat

The timing of floral transition, the switch from vegetative to floral growth, is a major component of agro-environmental adaptation. In cold climates, inappropriately early transition exposes delicate floral primordia to the risk of frost. Conversely, in hot environments, late flowering can reduce reproductive success as seasonal drought and heat stress can affect microspore survival and seed set. The timing of this transition is, therefore, a key environmental adaptation that was selected unconsciously by early farmers for thousands of years, and more recently, with greater precision, by plant breeders. Research undertaken in both model plant species and crops in the past 20 years has revealed that floral transition is controlled by complex overlapping gene pathways.

Wheat is a long-day species in which floral initiation is accelerated by exposure to lengthening days. Key determinants in the pathway controlling floral transition in wheat are the photoperiod response (*Ppd*) genes on the Group 2 chromosomes. *Ppd-D1* and *Ppd-B1* in bread wheat and *Ppd-A1* in durum wheat have been cloned by colleagues at the John Innes Centre in Norwich. At the *Ppd-D1* and *Ppd-A1* loci, a large deletion is responsible for day-length neutrality (photoperiod insensitivity, denoted by an *a* suffix) and early flowering. However, early flowering is caused by copy number variation at *Ppd-B1* (more gene copies = earlier flowering). In bread wheat, numerous previous studies report that *Ppd-D1a* and, to a lesser extent, *Ppd-B1a* have a relatively large effect, reducing flowering time by between 5-10 days depending on environment.

Ppd and yield

In a seminal study over 14 years, near iso-genic lines (NILs) for *Ppd-D1* carrying early flowering (mutant; *Ppd-D1a*) and wild-type (non-mutant; *Ppd-D1b*) alleles were trialled in contrasting agro-

environments in north-western, central and southern Europe. *Ppd-D1a* was estimated to reduce flowering time by between 6-14 days, depending on season. In addition, numerous interaction effects were reported, including reduced height and spikelet number. Importantly, compared to wild-type NILs, lines carrying the early flowering allele produced significantly higher yields in southern Europe. Under these conditions, the shorter life cycle of *Ppd-D1a* NILs provided a yield advantage by reducing exposure to late season high temperatures and drought. In contrast, under generally cooler UK conditions, wild-type *Ppd-D1b* NILs produced the higher yields because they were able to more fully exploit the longer growing season, particularly the crucial period of grain fill. In a more recent two year study focussing on the UK, yield and flowering effects of *Ppd-D1a* and *Ppd-D1b* were assessed in NILs developed in two winter wheat cultivars. Under the temperate UK conditions, the effect of *Ppd-D1* on drought-resistance traits, such as water-use efficiency and maximum rooting depth, appeared to be neutral.

Although there have been numerous studies on the influence of *Ppd-D1* on yield, far less has been published on the yield effects of *Ppd-B1*. In a comparative study, *Ppd-B1* NILs were insensitive to photoperiod during the pre-anthesis late reproductive phase, but as sensitive to photoperiod as wild-type controls during the early reproductive phase. In contrast*, Ppd-D1* NILs were insensitive to photoperiod during both the early and late phases. Although it is the late, rather than early, reproductive phase that has the most important influence on fertile floret number and hence, potential yield, it is important to note that the photoperiod sensitivity of individual developmental phases is at least partially independent, and that there is, therefore, potential for genetic manipulation. However, Canadian field trials investigating the yield and agronomic performance of NILs carrying *Ppd-D1a* and *Ppd-B1a* were unable to determine which allele conferred the lower yield penalty.

Flowering time and earliness per se* (Eps) *genes

Apart from *Ppd*, other genes influence flowering time in wheat. For example, the vernalisation (*Vrn*) genes play an important role: winter varieties require vernalisation (prolonged exposure to cool temperatures as seedlings) before the floral transition is triggered, but spring varieties do not. Whereas, the effect of *Ppd* and *Vrn* genes on flowering is relatively well understood, much less is known about the influence of genetic factors that modify flowering-time once any *Ppd* and *Vrn* requirements have been satisfied. These residual effects are commonly known as earliness *per se* (*Eps*), since they appear to influence developmental rate regardless of environmental cues. Although they have a relatively small influence compared to *Ppd* and *Vrn*, they are potentially important to plant breeders seeking to optimise flowering-time for specific environments.

Most *Eps* studies have focused on their influence on flowering time, however, there is evidence to suggest that certain loci may affect yield and yield components by modifying the duration of specific developmental phases. For example, comparison of the duration of vegetative and floral phases at the shoot apex indicated that NILs carrying the *Eps-A1*^{m} locus from a wild relative of wheat, *Triticum monococcum*, initiated flowering up to 35 days earlier than lines carrying the wild-

type allele. It is worth noting that in the same study, a locus controlling spikelet number per spike was found to be closely linked to, but not an interaction effect of, *Eps-A1^m*. Similarly, authors of a field study detected QTL for plant height, thousand kernel weight and kernel number per spike that co-located with an *Eps* locus on 3AS in bread wheat. The question arising from these studies is, do any *Eps* loci have an independent effect on yield and its components?

Novel genetic diversity from synthetic hexaploid wheat (SHW)

Common bread wheat is clearly highly adaptable since it is grown all over the world. This adaptability is undoubtedly due to its complex genetic origins, combining within a single hexaploid genome (BBAADD) those of its progenitors: *Triticum urartu* (AA), a species related to *Aegilops speltoides* (BB) and *Aegilops tauschii* (DD).

There is good evidence to suggest that an important component of yield instability (yield variation from site to site and year to year) is soil water availability, even in temperate environments like the UK. For this reason, tolerance to environmental stresses is considered to be a key component to future-proofing wheat cultivars against climate change. Globally, drought causes greater yield losses than any other single factor. It is estimated that as much as 50% of the wheat production area is regularly affected by drought. For example, the UK is one of the world's most efficient wheat producers, yet approximately 30% of the current wheat area is grown on drought-prone land where yield losses average 1-2 t ha^{-1}, costing growers >£60m per year. This means that even in years with 'normal' rainfall, potential yield and grain quality are affected by insufficient water at some time during crop development. Furthermore, climate change models predict that extreme weather patterns such as prolonged droughts will worsen, which will intensify the competition between agriculture, urban needs and environmentally-sensitive areas for limited water resources.

A reduction in the use of other agricultural inputs (fertilizer, plant growth regulators and pesticides) is key to addressing wider environmental concerns. Attaining the right balance – growing more food on less land, with fewer inputs and in a more challenging environment – was described as a "perfect storm" by the UK government's Chief Scientific Advisor, and is certainly an enormous challenge to plant breeders, farmers and agronomists alike. The consensus is that food security cannot be taken for granted if things simply continue the way they are, and that different approaches in breeding and agronomy need to be taken. With respect to breeding, this includes mining genetic resources in the search for novel variation. A major constraint on progress in the selection for wider adaptation is the relative paucity of variation that results from the genetic bottleneck associated with plant domestication and subsequent selection. This lack of diversity has made modern crop plants more vulnerable to environmental stresses. A major objective of modern breeding is to screen wild ancestors of crop plants, identify valuable "left behind" alleles and introduce them into elite breeding material.

The wild goat-grass, *Aegilops tauschii,* is the diploid D-genome donor of cultivated wheat, and freely recombines with the D-genome of bread wheat. Natural hybridization occurred 10,000 years ago between tetraploid wild emmer (*Triticum dicoccoides*, BBAA) and *Ae. tauschii* (DD), which gave rise to hexaploid bread wheat, *T. aestivum* (BBAADD). *Triticeae* species (such as *T. dicoccoides and Ae. tauschii)* represent a rich source of additional genetic variation for crop improvement.

Several groups have alleviated this genetic bottleneck by creating synthetic hexaploid wheats (SHWs) through the artificial hybridization of tetraploid wheat species with *Ae. tauschii*. SHWs are potentially a rich source of novel traits that are readily crossed into elite varieties. Their promise for wheat improvement is illustrated by the fact that 25% of breeding lines in CIMMYT's international nurseries in 2003 were derived from SHWs. Whilst SHWs possess favorable disease and insect resistance traits, they also contribute to improved yield potential in well-watered, semi-arid and hot environments. Studies of CIMMYT germplasm indicate that characteristics inherited from SHW contribute to improved performance under water-limited conditions. For example, several authors have demonstrated that improved water extraction of synthetic derived wheat (SHW-D) relative to respective recurrent parents was due to a greater distribution of root biomass deeper in the soil profile (90-120 cm). SHW-D lines were also shown to have a significantly better water use efficiency than recurrent parents. In addition, it has been reported that SHW-D germplasm is a valuable source of variation for genes conditioning improved adaptation to low input farming which encompasses tolerance to drought and reduced agricultural inputs.

A breeding programme was run from 2007-10 to introduce novel variation from CIMMYT SHW into French germplasm, primarily to integrate novel sources of host resistance to several pathogens including septoria tritici blotch and fusarium head blight. However, no systematic evaluation of yield stability or breeding value was carried out. In China, breeders began to cross CIMMYT SHWs with their local varieties in the mid-1990's, and released their first SHW-derived variety in 2003, which yielded over 20% more than checks in provincial trials. SHW derivatives are now reported to be grown on over five million hectares in China, some 25% of the wheat acreage.

Two strategies to maintain and accelerate genetic improvement in UK wheat breeding were addressed in the current project: (1) characterisation of the flowering time and yield effects of genes controlling developmental rate (*Eps*) and floral initiation (*Ppd*) and (2) introduction of novel genetic variation from wheat progenitor species (*Aegilops tauschii*) via synthetic hexaploids. To facilitate the dissection of flowering time and its interaction with yield, two sets of BC_2-derived NILs were developed: a series of ten *Ppd* gene variants in the winter wheat cultivars Alchemy and Robigus, and ten *Eps* NILs developed from two doubled haploid mapping populations, Charger x

Badger and Spark x Rialto. Genetic diversity from 50 CIMMYT synthetic hexaploids was backcrossed into Paragon and Xi-19 to produce over 5600 BC_1-derived lines for field selection.

2.2. Materials and methods

2.2.1. Plant material

To study photoperiod response genes effecting floral transition (*Ppd*), ten sets of near-isogenic lines (NILs) were developed by repeatedly crossing lines carrying *Ppd* variants (the donors) with two UK winter wheat cultivars, Alchemy and Robigus (the recurrent parents). The genes of interest were selected at each backcross generation using molecular markers. A similar approach was used to develop NILs carrying ten different developmental rate (*Eps*) QTL, derived by backcrossing early-flowering lines from the Spark x Rialto (SR) and Charger x Badger (CB) mapping populations with their respective late-flowering parents for each QTL.

Synthetic hexaploid wheat (SHW) lines (durum x *Aegilops tauschii*) from the International Wheat and Maize Research Centre (CIMMYT) in Mexico were used as sources of general novel genetic diversity. SHW lines were backcrossed to the UK wheat cultivars Paragon and Xi-19 and then inbred prior to field testing.

2.2.2. Glasshouse & growth room experiments with *Eps* & *Ppd* NILs

Glasshouse and growth room experiments were used to investigate how the lines carrying different *Ppd* genes and *Eps* QTLs responded to short (8 hours) and long (16 hours) photoperiods. The potency of flowering effects were characterised for *Ppd* and *Eps* variants by assessing the amount by which flowering was accelerated under long days, or slowed under short days.

2.2.3. Field experiments with *Eps* & *Ppd* NILs

Field experiments were carried out under natural day-length at NIAB in Cambridge. Extended vs. natural day-length experiments were also carried out in the field at KWS near Thriplow, Cambridgeshire. For the extended day-length treatment, 16 hour days were provided from the early seedling stage through to the initiation of flowering, by extending the natural day using 30W electric lights suspended above the plots.

2.2.4. Field selection of synthetic wheat backcross lines

A representative subset of CIMMYT synthetic hexaploid wheat (SHW) lines was backcrossed with Paragon and Xi-19 to create lines for field selection. Two streams of lines were created: (1) F_2- and BC_1F_2-derived lines selected in the field each season; and (2) material inbred to BC_1F_5

without selection. Pre-breeding selections within this material were made primarily for yield components, including high biomass.

2.3. Results

2.3.1. Field experiments with *Ppd* & *Eps* NILs

In 2011, flowering time (FT) for both *Ppd* and *Eps* NILs was recorded on small replicated plots. For each gene, up to eight NILs from each donor/recipient combination were tested (up to 16 NILs in total for each donor). This meant that variation caused by residual genetic background variation could be taken into account statistically. FT was evaluated again in 2012, including on some plots that were harvested for yield analysis.

2.3.1.1. Field experiments with Ppd *NILs*

Flowering time assessment (days from sowing to GS-55) was made on field plots in 2011 and 2012, making it possible to compare effects within and between years.

In 2011, five early flowering (photoperiod-insensitive, PI) *Ppd* variants were evaluated in the Alchemy and Robigus backgrounds, including two sources each of D-genome and B-genome variants, plus a single A-genome variant. Several photoperiod-sensitive (PS) variants were also tested, but showed no significant difference in flowering time in either background. In the Alchemy background, D-genome PI NILs had the most potent effect, reducing FT by an average of eight to nine days relative to the corresponding non-mutant NILs. The A-genome variant and one B-genome variant both reduced FT by an average of eight days. The second B-genome variant had the least potent effect, reducing FT by four days. In the Robigus background, a D-genome variant had the most potent FT-reducing effect (-10 days), followed by an A-genome variant (-5 days). Both B-genome variants reduced FT by two days.

In 2012, six PI variants were assessed in Alchemy and five in Robigus. In Alchemy, D-genome variants reduced FT by five to eight days and B-genome NILs reduced FT by three to five days. The A-genome variant reduced FT by an average of two days. In Robigus, one D-genome variant produced the most potent FT-reducing effect (-6 days) whilst the second was non-significant. The B-genome variants both reduced FT by an average of two days. The effect of the A-genome variant was comparable to that of the most potent D-genome variant (-6 days). Again, no significant effects were found with PS variants in either background.

Plot yield was recorded on *Ppd* NILs in both the Alchemy and Robigus genetic backgrounds in 2012. In the Alchemy background, early-flowering NILs significantly out-yielded the corresponding

wild-type NILs, except for one D-genome variant. Yield differences for PS variants were non-significant. In the Robigus background, the only significant yield difference across all the NILs was for one of the Ppd-*B1a* early flowering variants, which yielded less than the wild-type NILs.

ANOVA indicated that genotype x year interaction was non-significant (P>0.05) and coefficients of correlation for donor (0.91) and recipient (0.81) variants for each NIL set were highly significant (P<0.001).

2.3.1.2. Field experiments with Eps NILs

Under field conditions in 2011, significant reductions in flowering time (days from sowing to GS55) were observed for three QTL-NILs, with NILs carrying "early" alleles flowering 2-3 days earlier than those carrying "late" alleles. Five other sets of NILs were of borderline significance, with early alleles reducing flowering time by an average of 1 day. One QTL had a non-significant effect on FT.

In 2012, three assessments were made on all eight QTL-NILs: time to GS39 (flag leaf fully emerged), GS55 (50% ears fully emerged) and GS61 (flowering started). For most QTLs, the most significant early allele FT reduction was observed at GS55. The maximum FT reducing effect of early alleles measured was 4.2 days earlier, with three other QTLs showing significant effects. Two QTLs showed borderline effects and four showed no significant effect.

2.3.2. Field selection of synthetic wheat backcross lines

A panel of 448 synthetic hexaploid wheat (SHW) lines from CIMMYT was genotyped with 12 genome-wide SSR markers. These data, combined with pedigree information, were used to identify a sub-set of 50 SHWs which represented the range of genetic diversity present in the full set. These 50 were backcrossed to the varieties Paragon and Xi-19 and then inbred. In total, over 5,600 BC_1-derived lines underwent field assessment and selection. The strategy for development of the SHW-derived lines was twofold, involving (1) selection from F_2 or BC_1F_2 populations and line advancement through field selection and (2) no selection applied, with lines selfed through successive generations of single seed descent to BC_1F_5. In a large co-ordinated yield trial of 1000 Xi-19 / SHW BC_1F_6 lines in 2011-12, around a third of lines tested yielded at least as much as Xi-19. The best of this material is being integrated into commercial breeding programmes.

Other projects have also used this novel germplasm. For example, drought tolerance trials within the DEFRA WGIN2 programme involved Xi-19 / SHW material. Above ground biomass, harvest index and grain yield were recorded on BC_1F_3 and BC_1F_4 lines in 2010 and 2011, respectively, grown on very light, drought-prone land. Three lines out-yielded Xi-19 in individual years, and partitioning work suggests that this was largely down to increased biomass.

Some Paragon / SHW lines were grown under high (180-200 kg/ha) and low (0-40 kg/ha) nitrogen by the University of Nottingham and Rothamsted Research as part of the BBSRC public sector pre-breeding LoLa project. Yields between sites and treatments correlated well with several lines achieving consistent yield levels under both treatment regimes and across sites. In particular, some SHW-derivatives appeared to maintain much of their yield under reduced nitrogen levels.

2.4. Discussion/Conclusions and implications

The development of a series of BC_2-derived *Ppd* NILs has provided the opportunity to comprehensively characterise the flowering time effects of a range of photoperiod insensitive gene variants in two genetic backgrounds. In the current study, BC_2-derived lines carrying various *Ppd-1* variants on chromosomes 2A, 2B and 2D, some of which were previously uncharacterised, were developed. The relative potency of their flowering time effect was: *Ppd-D1a=Ppd-A1a>Ppd-B1a,* indicating that *Ppd-A1a* is a potent novel source of photoperiod insensitivity for wheat breeding. Contrary to the prevailing consensus from previous studies in north-western Europe, photoperiod insensitive early flowering NILs out-yielded the corresponding later flowering lines in the 2012 trial, although the relatively good performance of early-flowering types was a feature of many variety trials during 2011-12. Continued study of the NILs developed in this project under a range of contrasting environments across Europe will add to our understanding of the interaction between yield and photoperiod sensitivity.

Eps QTL in the current study were identified using data from previous studies carried out by project partners at the John Innes Centre (JIC) in Norwich. Although the accepted definition suggests that *Eps* loci reduce the time to flowering regardless of prevailing conditions, it is clear from previous studies carried out at the JIC that environment had a significant influence on the expression of *Eps* genes over experimental years. Such fluctuations in relative potency have led some authors to question whether the influence of *Eps* on developmental rate is truly independent of environmental influence. In contrast, *Eps* effects on flowering time in the current study were found to be relatively reproducible over experiments and years with a consensus emerging as follows (derivative doubled haploid population followed by chromosomal location of the gene): SR-1D>SR-3A>SR-7A>SR-3B>SR-6B>[CB-3A, CB-3B, CB-6A, CB-6B & CB-7A]. Although yield analysis of *Eps* NILs in the current study carries a caveat since unreplicated small $1m^2$ plots were used, our initial analysis suggests that early flowering variants of the *Eps* genes tested do not have a negative effect on yield under UK conditions. Taken together, *Eps* genes may provide plant breeders with an alternative route to incrementally reduce flowering time for their target environments.

The current project represents the first systematic introduction of novel genetic variation into UK varieties from the D-genome ancestor of wheat (*Aegilops tauschii*) via synthetic hexaploids since early studies at the Plant Breeding Institute in Cambridge. Pre-breeding mainly focussed on the

development of backcross-derived germplasm for transfer to commercial UK breeders, and a wealth of phenotypic and methodological information has also been generated that will inform the future development and utilization of germplasm from wide-cross programmes.

Several key findings/observations have been made in the synthetics programme which can inform future breeding and selection work with SHWs, such as the recently funded BBSRC public sector wheat pre-breeding project:

- Many crosses between SHWs and elite UK varieties result in F_1s with severe hybrid necrosis, rendering them unsuitable for further breeding. The selection of elite parents for crossing with SHWs and derivatives, therefore, needs careful consideration and ideally a pre-screening step involving test-crosses.
- UK elite x SHW crosses are much wider in terms of genetic diversity than commercial breeders are used to, necessitating a different selection approach with increased emphasis on plant-by-plant evaluation even in later selfing generations.
- A conservative pre-breeding approach of “deselect the worst” should be employed in order to capture maximum diversity, rather than the aggressive “select the best” approach typical of commercial breeding.
- A sub-set of SHW donors were found to have particularly high breeding potential suggesting that some lines can be more easily integrated into conventional breeding programmes.
- Yield and yield component analysis indicates that many SHWs appear to harbour potentially novel yield-promoting genetic loci.

3. TECHNICAL DETAIL

3.1. Introduction

The manipulation of specific genes (when available) and exploitation of genome-wide variation contributing to agro-environmental adaptation are important considerations for plant breeders seeking to increase yields. However, due to its large genome size, only a few genes influencing environmental adaptation have so far been cloned in wheat. For those that have, there is still much to be understood regarding their pleiotropic interactions and their contribution to agronomic performance. Despite these constraints, genetic improvement by UK wheat breeders has continued to contribute to significant year-on-year yield gains in field trials (Mackay *et al*., 2010). However, farm yields have stagnated and there is a growing gap between yields in trial and on farm, even in high-yielding environments like the UK (Fischer & Edmeades, 2010). The development of novel cultivars with more efficient water-use and greater drought resistance capacity may contribute to yield stability and improved farm yield. In addition, improved efficiency in the use of other agricultural inputs, including fertilizer and pesticides, is a key factor in addressing wider environmental concerns (Reynolds *et al*., 2009).

Photoperiod response (*Ppd*) genes and floral transition in wheat

The timing of floral transition (the switch from vegetative to floral growth) is of major importance to agro-environmental adaptation. In cold climates, inappropriate early transition exposes delicate floral primordia to the risk of frost (Worland, 1996). Conversely, in hot environments, late flowering can reduce reproductive success, as seasonal drought and heat stress can adversely affect microspore survival and grain fill (Dolferus *et al*., 2011). Timing of the transition to flowering is, therefore, a key environmental adaptation that was selected unconsciously by early farmers for thousands of years, and more recently, with a greater precision, by plant breeders.

Research undertaken in both model and crop plants in the past 20 years has revealed that floral transition is controlled by complex overlapping gene pathways (reviewed by Cockram *et al*., 2007 and Colasanti & Coneva, 2009). Wheat is a long-day species in which floral initiation is accelerated by exposure to lengthening days. Key determinants in the pathway controlling floral transition in wheat are the photoperiod response (*Ppd*) genes on the Group 2 chromosomes. *Ppd-D1* and *Ppd-B1* in bread wheat and *Ppd-A1* in durum wheat have been cloned (Beales *et al*., 2007; Wilhelm *et al*., 2009). At the *Ppd-D1* and *Ppd-A1* loci, large deletions in the upstream promoter region are responsible for day-length neutrality and early flowering; however, at *Ppd-B1*, early flowering is caused by copy number variation (Diaz *et al*., 2012). In bread wheat, numerous previous studies report that *Ppd-D1* and *Ppd-B1* have relatively large effects, reducing flowering time by between 5-10 days depending on environment (summarised in Gonzalez *et al*., 2005).

Ppd *and yield*

An important contribution to understanding the pleiotropic effect of the *Ppd-D1* locus on yield has been the work carried out by Worland and colleagues in the 1980's and 90's (reviewed by Snape *et al*., 2001). In a seminal study over 14 years, near iso-genic lines (NILs) carrying contrasting early flowering (*Ppd-D1a)* and wild-type (*Ppd-D1b*) alleles were grown in different agro-environments across Europe. *Ppd-D1a* was estimated to reduce flowering time by between 6-14 days depending on season, with numerous pleiotropic effects including reduced height and spikelet number. Importantly, compared to wild-type NILs, lines carrying the early flowering allele produced significantly higher yields in southern Europe. Under these conditions, the shorter life cycle of *Ppd-D1a* NILs provided a yield advantage over wild-type NILs by reducing exposure to late season high temperatures and drought.

In contrast, under generally cooler UK conditions, early-flowering NILs produced lower yields than their later-flowering counterparts: their shorter life cycle left them unable to exploit the longer growing season, particularly the crucial period of grain fill (Worland & Sayers, 1995). In a more recent two-year study focussing on the UK, yield and flowering effects of *Ppd-D1a* and *Ppd-D1b* were assessed in NILs developed in winter wheat cultivars Mercia and Cappelle-Desprez. Under temperate UK conditions, the effect of *Ppd-D1* on drought-resistance traits such as water-use efficiency and maximum rooting depth appeared to be neutral. It was concluded that the effects of the *Ppd-D1a* allele appeared to be largely neutral on yield potential and late-season drought resistance under UK conditions (Foulkes *et al*., 2004).

Although there have been numerous studies of the influence on yield of *Ppd-D1*, far less has been published on the yield effects of *Ppd-B1*. In a comparative study of NILs carrying the D and B-genome alleles, Gonzalez *et al*. (2005) reported that *Ppd-B1* NILs were insensitive to photoperiod during the pre-anthesis late reproductive phase, but as sensitive to photoperiod as wild-type controls during the early reproductive phase. In contrast, *Ppd-D1* was insensitive to photoperiod during both the early and late phases. The late reproductive phase has the most important influence on fertile floret number and hence, potential yield. However, if the photoperiod sensitivity of individual developmental phases is at least partially independent, this suggests that there is potential for genetic manipulation (Gonzalez *et al*. 2005). In the field, authors of a study of yield and agronomic performance of NILs carrying *Ppd-D1* and *Ppd-B1* in Canada were unable to determine whether the B-allele conferred a lower yield penalty than the D-allele (Dyck *et al*., 2004).

Flowering time and earliness per se (Eps) *genes*

Whereas the effect of *Ppd* genes on flowering is well documented and understood, the influence of genetic factors that modify flowering-time once the requirements of the *Ppd* and vernalisation (*Vrn*) genes have been satisfied is much less well understood. These residual effects are commonly known as earliness *per se (Eps)* since they appear to influence developmental rate regardless of environmental cues. Although they have a relatively small influence compared to *Ppd* and *Vrn*, they are potentially important to plant breeders seeking to optimise flowering-time for specific environments (Snape *et al*., 2001).

Many *Eps* loci have been mapped in both wheat and barley (reviewed by Cockram *et al*., 2007); however, it is still uncertain whether the action of *Eps* is truly unaffected by the environment (Colasanti & Coneva, 2009). It is clear that the number of *Eps* genes in wheat is potentially very large. In a comprehensive genetic analysis of four doubled haploid mapping populations, Griffiths *et al*., (2009) detected *Eps* meta-QTL on all seven chromosome groups. Their large number and dispersed genomic location suggests that *Eps* genes are likely to be highly heterogeneous with respect to their mode of action and effect on flowering time. Most *Eps* studies have focussed on assessing their influence on flowering time, however, there is evidence to suggest that certain loci may affect yield and yield components by modifying the duration of specific developmental phases. For example, comparison of the duration of vegetative and floral phases at the shoot apex indicated that NILs carrying the *Eps-A1*^{m} locus from *Triticum monococcum* initiated floral apices up to 35 days earlier than lines carrying the wild-type allele (Lewis *et al*., 2008). It is worth noting that in the same study, a locus controlling spikelet number per spike was found to be closely linked to, but not a pleiotropic effect of, *Eps-A1*^{m}. Similarly, authors of a field study detected QTL for plant height, thousand kernel weight and kernel number per spike that co-located with an *Eps* locus on 3AS (Shah *et al*., 1999).

Novel genetic diversity from synthetic hexaploid wheat (SHW)

Common bread wheat is clearly highly adaptable since it is so widely grown. This adaptability is undoubtedly due to its allohexaploid origins, combining within a single genome (BBAADD) those of *Trititcum urartu*, (AA), a species related to *Aegilops speltoides* (BB), and *Aegilops tauschii* (DD) (Dvorak *et al*., 1992; Feldman *et al*., 2005). Further increases in yield potential and stability, coupled with improvements in disease resistance and adaptation to abiotic stress, will be needed to ensure that yield gains can be maintained in the face of climate change (Reynolds *et al*., 2009). There is good evidence to suggest that an important component of yield instability (yield variation from site to site and year to year) is due to soil water availability even in temperate environments like the UK (Foulkes *et al*., 2002). Tolerance to environmental stresses will clearly be important in future-proofing wheat cultivars against climate change (Reynolds *et al*., 2009; Warburton *et al*., 2006).

Globally, drought causes greater yield losses than any other single pest or environmental factor (Boyer, 1982) and it is estimated that as much as 50% of the wheat production area is regularly affected by drought (Pfeiffer *et al*., 2005). The UK is one of the world's most efficient producers of arable crops, yet approximately 30% of the current UK wheat area is grown on drought-prone land and drought losses are on average 1-2 t ha^{-1}, which costs >£60M per year (Foulkes *et al*., 2007). This means that even in the temperate UK climate, and in years with 'normal' rainfall, potential yield and grain quality are affected by insufficient water at some time during crop development.

Furthermore, climate change models predict that extreme weather patterns such as prolonged droughts will worsen (Jones *et al*., 2003; Richter and Semenov, 2005), which will intensify the competition between agriculture, urban needs and environmentally-sensitive areas for limited water resources. A reduction in other agricultural inputs, including fertilizer, plant growth regulators and pesticides, is key to addressing wider environmental concerns (Reynolds *et al*., 2009).

Balancing these competing concerns – growing more food on less land, with fewer inputs and in a more challenging environment – was described as a “perfect storm” by the UK government’s Chief Scientific Advisor, and is certainly an enormous challenge to plant breeders, farmers and agronomists alike (Beddington, 2009). The consensus is that food security cannot simply be taken for granted, and that different approaches in breeding and agronomy must be taken in order to secure food production in the future. With respect to breeding, this means mining genetic resources in the search for novel variation.

A major constraint on progress in the selection for wider adaptation is the relative paucity of variation that results from the genetic ‘bottleneck’ associated with plant domestication and subsequent selection by early farmers and, latterly, breeders. This lack of diversity has left our crop plants vulnerable to environmental stresses. A major long-term objective of modern breeding is to screen wild ancestors of crop plants, identify valuable "left behind" alleles and introduce them into elite breeding material (Tanksley and McCouch 1997; Gur and Zamir 2004).

The wild goat-grass, *Aegilops tauschii,* is the D-genome donor of cultivated wheat, and freely recombines with the D-genome of bread wheat. Hexaploid bread wheat (*Triticum aestivum;* genome BBAADD) arose following the inter-specific hybridization 10,000 years ago of the tetraploid, wild emmer (*Triticum dicoccoides,* BBAA), with the diploid *Ae. tauschii* (DD) (Feldman, 2001). The more general genetic bottleneck of crop domestication is exaggerated for wheat because the interspecific hybridization that formed hexaploids probably occurred only a few times. *Triticeae* species (such as *T. dicoccoides and Ae. tauschii),* therefore, represent a rich source of additional genetic variation for crop improvement.

This bottleneck can be alleviated by creating synthetic hexaploid wheats (SHWs) through the artificial hybridization of tetraploid wheats species with *Ae. tauschii* (Mujeeb-Kazi *et al*., 1996; Lage *et al*., 2001). SHWs are potentially a rich source of novel traits that can be readily crossed into elite varieties. Their promise for wheat improvement is illustrated by the fact that 25% of CIMMYT cultivars transferred to international nurseries in 2003 were derived from SHWs (Zhang *et al*., 2005). Whilst SHWs possess favorable disease and insect resistance traits (Lage *et al*., 2002; Mujeeb-Kazi *et al*., 2001), critically they also contribute to improved yield potential in well-watered, semi-arid and hot environments (Gororo *et al*., 2002; Reynolds *et al*., 2007). Studies of synthetic

derived (SHW-D) germplasm at CIMMYT indicate that characteristics inherited from SHW contribute to improved performance under water-limited conditions. For example, it has been demonstrated that improved water extraction of SHW-D relative to respective bread wheat parents was due to a greater distribution of root biomass deeper in the soil profile (Reynolds *et al*., 2007). SHW-D lines also displayed significantly better water use efficiency than their bread wheat parents. In addition, it has been reported that SHW-D germplasm is a valuable source of variation for improved performance under low input farming, which encompasses tolerance to drought and reduced agricultural inputs (Valkoun, 2001).

A breeding programme was run from 2007-10 to introduce novel variation from CIMMYT SHW into French germplasm, primarily to integrate novel sources of host resistance to several pathogens including septoria tritici blotch and fusarium head blight. However, no systematic evaluation of yield stability or breeding value was carried out. In China, breeders began to cross CIMMYT SHWs with their local varieties in the mid-1990's, and released their first SHW-derived variety in 2003, which yielded over 20% more than checks in provincial trials (Yang *et al*., 2009). SHW derivatives are now reported to be grown on over five million hectares in China, 25% of the wheat acreage.

Two strategies to maintain and accelerate genetic improvement in UK wheat breeding were addressed in the current project: (1) characterisation of the flowering time (FT) and yield effects of genes controlling developmental rate (*Eps*) and floral initiation (*Ppd*) and (2) introduction of novel genetic variation from wheat progenitor species (*Aegilops tauschii*) via synthetic hexaploids. To facilitate the dissection of their FT and yield effects, two sets of BC_2-derived near iso-genic lines (NILs) were developed: an allelic series of ten *Ppd* gene variants in the winter wheat cultivars Alchemy and Robigus, and ten *Eps* QTL NILs developed from two doubled haploid mapping populations, Charger x Badger and Spark x Rialto. Genetic diversity from 50 CIMMYT synthetic hexaploids was backcrossed into Paragon and Xi-19 to produce over 5,600 BC_1-derived lines for field selection.

3.2. Materials and methods

3.2.1. *Ppd* NIL experiments

3.2.1.1. Plant material

The elite UK-adapted winter wheat cultivars 'Robigus' and 'Alchemy' (neither of which has a *Ppd-1a* allele, and are both, therefore, PS) were selected as recurrent parents (Table 1). Development of BC_2F_4 lines in two recurrent backgrounds facilitated the evaluation of genetic background effects. *Ppd* allele-donors included five cultivars, three synthetic hexaploid wheat (SHW) lines and two chromosome substitution lines (Table 1). With the exception of SHW lines SHW_131 (Cid: 154094) and SHW_173 (Cid: 160224) provided by CIMMYT, seeds of recurrent parents and

donors were sourced from the John Innes Centre (JIC) Germplasm Resources Unit. To ensure no interaction with photoperiod response in controlled-environment experiments, the vernalisation requirement of winter-adapted germplasm was satisfied with eight weeks cold treatment at 6ºC with 8 hr photoperiod applied to two-week old seedlings.

Table 1. Source and description/predicted functionality of introgressed photoperiod insensitive (PI) and photoperiod sensitive (PS) *Ppd* alleles

Source	Type[a]	Allele	Chr	Associated polymorphism[(Reference)]	Predicted allele functionality
CIMMYT SHW_131 (Cid:154094)	D	*Ppd-A1a*	2A	1117bp upstream deletion[1]	PI early
CIMMYT SHW_173 (Cid:160224)	D	*Ppd-A1a*	2A	1117bp upstream deletion[1]	PI early
Cappelle-Desprez'	D	*Ppd-A1b*	2A	Exon 5/6 deletion[2]	Null
Soissons'	D	*Ppd-A1b*	2A	Exon 5/6 deletion[2]	Null
Chinese Spring'	D	*Ppd-B1a*	2B	4 x copy number variant[3] [A/G SNP in exon 3][2]	PI early
Chinese Spring (Timstein 2B)'	D	*Ppd-B1a*	2B	3 x copy number variant[3]	PI early
Mercia (Ciano67 2D)'	D	*Ppd-D1a*	2D	2kb deletion in 5' upstream sequence[2]	PI early
Soissons'	D	*Ppd-D1a*	2D	2kb deletion in 5' upstream sequence[2]	PI early
JIC SHW_GBR011 (Acc: 9553)	D	*Ppd-D1b*	2D	16bp deletion in exon 8[2]	PS wild type
Mercia'	D	*Ppd-D1b*	2D	Mariner transposon in intron 1[2]	Null or weakly PS
Norstar'	D	*Ppd-D1b*	2D	5bp deletion in exon 7[2]	Null or weakly PS
Robigus'	RP	*Ppd-D1b*	2D	Mariner transposon in intron 1[2]	Null or weakly PS
Alchemy'	RP	*Ppd-D1b*	2D	5bp deletion in exon 7[2]	Null or weakly PS

[a] D = *Ppd* allele donor; RP = recurrent parent

[1] Wilhelm *et al.* (2009)

[2] Beales *et al.* (2007)

3 Diaz *et al.* (2012)

3.2.1.2. BC_2F_4 line development

Primary crosses were made between recurrent parents and allele donors (Table 1). F_1 progeny from primary crosses were backcrossed twice, using the recurrent parent as the pollen recipient, to derive BC_2 plants. Allele specific markers were used to select *Ppd* heterozygous progeny from each backcross generation, with information on the BC_1/BC_2 stream derivation recorded to allow analysis via family structure to ameliorate latent background heterozygosity. Homozygous progeny lines with (+) or without (-) the donor *Ppd-1* allele were marker-selected from the BC_2F_2 generation and selfed to produce BC_2F_4 lines for phenotyping. Marker-assisted backcrossing has continued to BC_4 to provide fixed near-isogenic lines for future analysis.

3.2.1.3. DNA extraction and **Ppd** *allele detection*

Genomic DNA was extracted from two-week old seedlings using a modified Tanksley extraction method (Fulton *et al*., 1995). The 'GS-105' *Ppd-A1a* allele was detected using previously described primers (Wilhelm *et al*., 2009) in a modified single reaction as described by Bentley *et al.,* (2011). The 'Chinese Spring' *Ppd-B1a*, 'Ciano 67' *Ppd-D1a*, 'Soissons' *Ppd-D1a* and 'JIC SHW_GBR011' *Ppd-D1b* alleles were detected using previously described primers and amplification conditions (Beales *et al*., 2007). The 'Timstein' *Ppd-B1a* copy number variant was detected using a *Ppd-B1* specific quantitative TaqMan® assay at IDna Genetics Ltd (Norwich, UK) (Diaz *et al*., 2012).
A nested PCR reaction was used to detect the 'Cappelle-Desprez' *Ppd-A1b* allele using primers AgF3 (agtcagagatatgcagcaac), HvR6-1 (tcttcccgaagttcctctc) and 219-R2 (tgccgttgattggcgagac). The primary amplification reaction in 10µl consisted of 20ng of total genomic DNA, 1µl 10 x buffer (NEB; 10mM Tris-HCl, 50mM KCl, 1.5mM $MgCl_2$ pH8.3), 0.2mM dNTPs, 1µM of primer AgF3 and HvR6-1 and 0.5U *Taq* polymerase (NEB). The secondary reaction used a 1:10 dilution in PCR-grade H_2O of the primary product as template, 1µl 10 x buffer (NEB), 0.2mM dNTPs, 1µM of primer AgF3 and 219-R2 and 0.5U *Taq* polymerase (NEB). For both steps the amplification conditions were: initial denaturation 94ºC for 5 min followed by 30 cycles of 94ºC for 30 sec, 55ºC for 30 sec, 72ºC for 90 sec, with a final extension of 72ºC for 5 min. Secondary PCR products were electrophoresed through a 1.5% agarose gel stained with ethidium bromide and visualised under UV light. Amplification of an intact sequence gave an 850bp product whilst a 550bp product was amplified in the presence of a deletion.

The 'Mercia' *Ppd-D1b* allele was detected with the primers 414F-F1 (atgatggtattcttgggtgc), Mer-10KbIns-R5 (gcaagtcttctccatggcg) and DgR4 (ggcaccatttgacaggcag). PCR amplification was performed in a 10µl volume with 20ng genomic DNA, 1µl 10 x buffer (Roche), 0.2mM dNTPs, 1µM of each primer and 0.5U *Taq* polymerase (Roche). For both steps 40 cycles of 30 sec annealing at 55ºC were used and visualised as above. The presence of a mariner transposon gave a 727bp product whilst a 1232bp product was amplified in the absence of the transposon.
To detect the 'Norstar' *Ppd-D1b* allele, nested PCR amplification in 10µl volumes with primers HvF5-1 (ttgagctgagcctgaagag), Dg-R2 (gtctaaatagtaggtactagg), HvF3 (aggaggaacagaggaac) and TaPpdD1_NorDel_2 (ggcggtgcagggttggagc) was performed on 20ng of total genomic DNA, 1µl 10 x buffer (NEB), 0.2mM dNTPs, 1µM of primer HvF5-1 and Dg-R2 and 0.5U Taq polymerase (NEB). The secondary reaction used a 1:10 dilution of the primary product as above, 1µl 10 x buffer (NEB), 0.2mM dNTPs, 1µM of primer HvF3 and TaPpdD1_NorDel_2 and 0.5U *Taq* polymerase (NEB). For both steps 30 cycles of 30 sec annealing at 55ºC (PCR1)/60ºC (PCR2) were used and visualised as above. Amplification of an intact sequence gave a 900bp product whilst a 750bp product was amplified when the 'Norstar' deletion occurred.

Syntenic resources in cereals, and markers from previous studies on the location of *Ppd* in wheat (Hanocq *et al*., 2004) were used in an attempt to identify polymorphic markers flanking *Ppd* on 2A, 2B and 2D in order to minimise linkage drag and pleiotropic interactions (data not shown).

3.2.1.4. Assessment of BC_2F_4 lines in a short photoperiod glasshouse

BC_2F_4 lines were grown under a fixed short photoperiod (SP) to assess flowering time at JIC, Norwich, UK. A SP was provided by a photoperiod glasshouse with moving benches that transferred plants to a dark chamber after 8 h natural day light from 15 July, 2010. Seven 'Alchemy' and four 'Robigus' allele variant combinations were included in the experiment, along with the recurrent parents 'Alchemy' and 'Robigus' (Table 2). *Ppd* lines were arranged adjacent to one another on the bench as full sib pairs (+/- donor allele, from a single segregating BC_1 family) with BC_2F_4 pairs randomised across two benches (blocks). The number of days to Zadoks GS55 (50% ear emerged from the flag leaf) (Zadoks *et al*., 1974) was recorded on the primary stem of each plant. The experiment was unequally replicated due to limited seed, but a minimum of six replicate plants were assessed for each BC_2F_4 line (Table 2). After 123 days exposure to SP the experiment was terminated.

Table 2. Predicted mean days to GS55 and average predicted reduction in flowering time relative to the recurrent parent controls for homozygote +/- donor allele BC_2F_4 lines in a short (8 hr) photoperiod

	# lines		+ donor allele lines	
BC_2F_4 line	**+/- donor allele**	**+ donor observations**	**Mean days to GS55[1]**	**Mean allele effect (d)[2]**
Robigus	5	16	116[e]	
Robigus (Cappelle-Desprez *Ppd-A1b*)	10/16	2	87[c]	-29
Robigus (Timstein *Ppd-B1a*)	11/5	22	92[c]	-24
Robigus (Ciano67 *Ppd-D1a*)	4/6	43	92[c]	-24
Robigus (Soissons *Ppd-D1a*)	14/13	69	74[b]	-42
Alchemy	8	23	110[e]	
Alchemy (CIMMYT SHW_131 *Ppd-A1a*)	2/1	10	72[b]	-38
Alchemy (Chinese Spring *Ppd-B1a*)	6/2	17	75[b]	-35
Alchemy (Timstein *Ppd-B1a*)	½	20	96[c]	-14
Alchemy (Ciano67 *Ppd-D1a*)	8/8	47	77[b]	-33
Alchemy (Soissons *Ppd-D1a*)	14/13	69	68[a]	-42
Alchemy (JIC SHW_GBR011 *Ppd-D1b*)	4/0	4	104[d]	-6
Alchemy (Mercia *Ppd-D1b*)	3/19	4	100[cd]	-10

[1] Total number of individual plant observations made within the 123d timeframe of the experiment

[2] Predicted means from REML accounting for BC_1/BC_2 family structure (letters assigned to values based on significant differences at a 95% confidence level)
[3] Average reductions in predicted mean relative to the recurrent parent

Analysis of the data for lines flowering within the 123 d timeframe of the experiment was performed using the REML (residual maximum likelihood) function in Genstat v12. Background and allele donor terms were fixed in the model, with the BC_2 stream nested in the BC_1 stream and both assigned as random terms, accounting for non-independence, as above.

3.2.1.5. Field assessment of BC_2F_4 lines in a natural and extended photoperiod

Two field trials were conducted in 2010/11, one at NIAB, Cambridge (52°13´N, 04°59´E) and one at KWS near Thriplow (52°6´N, 0°6´E), both in Cambridgeshire, UK. Flowering time was recorded in the field under natural photoperiod (NP) in both trials. In addition, at KWS, flowering time was recorded under an extended long day (16 hr) photoperiod (EP).

At NIAB, 'Alchemy' (nine allele variant combinations) and 'Robigus' (eight allele variant combinations) BC_2F_4 lines (Table 3) were sown on the 25 October 2010. Each line was planted as an individual Hege 90 plot (six rows) in a randomised complete block design. The number of days from sowing to GS55 (Zadoks *et al*., 1974) was recorded for each line. Data recorded on individual lines was analysed using REML with fitted and random terms assigned as in the SP experiment.

At KWS, comparison of NP versus EP was recorded. Seeds of selected lines (Table 4) in both genetic backgrounds were sown on 22 October 2010. Each accession was sown as a single row with a Hege 95B precision drill. Pairs of contrasting homozygote BC_2F_4 lines were planted as adjacent rows in both experiments. EP was achieved with supplementary field lighting (25W clear glass tungsten bulbs suspended 1m above the centre of plot-pairs at 37.5 cm intervals) during the pre-dawn night to extend the daylength to 16 hr. The EP regime ran from the shortest day (22 December 2010) until 23 May 2011, two weeks after GS31 (first node detectable; Zadoks *et al*., 1974). Plots under NP treatment were separated from EP plots by at least 3m. The number of days to GS39 (flag leaf emergence), GS59 (ear emergence) and GS61 (start of anthesis) (Zadoks *et al*., 1974) after 1 April 2011 were recorded on the primary ears for each row (line). Scores were adjusted to number of days to flowering from sowing and all data was analysed with REML, with photoperiod treatment added to the fitted model terms described above. The BC_1/BC_2 terms were removed from the random model as they had negative estimates.

Table 3. Days to 50% ear emergence (GS55) for BC_2F_4 lines in field conditions; NIAB, natural photoperiod (NP)

	# lines		Mean days to GS55[2]		Mean GS55 allele effect (d)[3]	
BC_2F_4 line	+/- donor allele	# plots[1] (+/-)	+ donor allele	- donor allele	RP	+/-
Robigus	8	42		234		
Robigus (CIMMYT SHW_173 *Ppd-A1a*)	4/4	4/4	224	229	-10	-5
Robigus (Cappelle-Desprez *Ppd-A1b*)	8/8	8/8	234	233	0	1
Robigus (Chinese Spring *Ppd-B1a*)	4/3	4/3	230	232	-4	-2
Robigus (Timstein *Ppd-B1a*)	8/7	8/7	228	230	-6	-2
Robigus (Ciano67 *Ppd-D1a*)	4/5	4/5	230	236	-4	-6
Robigus (Soissons *Ppd-D1a*)	8/8	8/8	224	233	-10	-9
Robigus (JIC SHW_GBR011 *Ppd-D1b*)	7/9	7/9	233	231	-1	2
Robigus (Norstar Ppd-D1b)	7/7	7/7	233	232	-1	1
Alchemy	10	46		233		
Alchemy (CIMMYT SHW_131 *Ppd-A1a*)	4/2	4/2	223	230	-10	-7
Alchemy (CIMMYT SHW_173 *Ppd-A1a*)	5/4	5/4	224	229	-9	-5
Alchemy (Cappelle-Desprez *Ppd-A1b*)	8/8	8/8	234	234	1	0
Alchemy (Chinese Spring *Ppd-B1a*)	4/7	4/7	229	231	-4	-2
Alchemy (Timstein *Ppd-B1a*)	8/6	8/6	225	230	-8	-5
Alchemy (Ciano67 *Ppd-D1a*)	7/7	7/7	224	233	-9	-9
Alchemy (Soissons *Ppd-D1a*)	8/8	8/8	225	234	-8	-9
Alchemy (JIC SHW_GBR011 *Ppd-D1b*)	8/8	8/8	230	231	-3	-1
Alchemy (Mercia *Ppd-D1b*)	8/5	8/5	233	233	0	0

[1] Number of individual field plots assessed per BC_2F_4 line

[2] Predicted means from REML accounting for BC_1/BC_2 family structure (letters assigned to values based on differences at a 95% confidence level)

[3] Average reductions in predicted mean relative to the recurrent parent

[4] Average reductions in predicted mean relative to the corresponding - donor allele homozygote

Table 4. Days to flag leaf emergence (GS39) and ear emergence (GS59) of BC_2F_4 lines in field conditions; experiment 2, KWS, natural (NP) and extended (EP) photoperiod

BC_2F_4 line[1]	# lines +/- donor allele	# rows[2] +/-	Mean d to GS39[3] +		Mean d to GS39[3] -		GS39 +/- allele effect (d)[4]		Mean d to GS59[5] +		Mean d to GS59[5] -		GS59 +/- allele effect (d)	
			NP	EP	NP	EP	NP	EP	NP	EP	NP	EP	NP	EP
Robigus	8	57			217^{c}	211^{d}					232b^{c}	231^{d}		
Alchemy	11	55			219^{d}	221^{e}					238^{e}	243^{e}		
CIMMYT SHW_131 *Ppd-A1a*	2/1	2/1	207^{a}	204^{a}	217^{cd}	206^{ab}	10	-2	226^{a}	220^{a}	234^{d}	222^{ab}	-8	-2
Cappelle-Desprez *Ppd-A1b*	8/8	8/8	217^{c}	207^{b}	216^{c}	208^{c}	1	-1	235^{d}	227^{c}	234^{d}	227^{c}	1	0
Chinese Spring *Ppd-B1a*	6/2	6/2	220^{d}	208^{c}	218^{c}	207^{b}	2	1	235de	220^{a}	235^{d}	224^{b}	0	-4
Timstein *Ppd-B1a*	3/6	3/6	212^{b}	205^{a}	215^{c}	205^{a}	-3	0	230^{b}	220^{a}	231^{b}	219^{a}	-1	1
Ciano67 *Ppd-D1a*	4/5	4/5	217^{c}	206^{ab}	222^{d}	207^{b}	-5	-1	234^{d}	223^{b}	238^{e}	224^{b}	-4	-1
Soissons *Ppd-D1a*	15/12	15/12	212^{b}	205^{a}	218^{c}	208^{b}	-6	-3	228^{a}	220^{a}	236^{d}	225^{b}	-8	-5
JIC SHW_GBR011 *Ppd-D1b*	5/0	5/0	219^{d}	208^{b}					231^{b}	224^{b}				
Mercia *Ppd-D1b*	1/3	1/3	220^{d}	209^{c}	220^{d}	207^{b}	0	2	236d^{e}	228^{c}	238^{e}	228^{c}	-2	0
Norstar *Ppd-D1b*	7/4	7/4	220^{d}	206^{ab}	219^{d}	206^{ab}	1	0	237^{e}	223^{b}	238^{e}	225^{b}	-1	-2

[1] No significant recipient background effects were detected so results for each donor allele across the two backgrounds are given in the table

[2] Number of individual rows assessed per BC_2F_4 line

[3] Predicted means to GS39 from REML accounting for BC_1/BC_2 family structure (letters assigned to values based on differences at a 95% confidence level separately for each photoperiod treatment)

[4] Average reductions in predicted mean relative to the corresponding - donor allele homozygote

[5] Predicted means to GS59 from REML accounting for BC_1/BC_2 family structure (letters assigned to values based on differences at a 95% confidence level separately for each photoperiod treatment)

3.2.2. Eps NIL experiments

3.2.2.1. BC2F4 QTL-NIL development

Map and phenotype data generated on the Charger x Badger and Spark x Rialto doubled haploid (DH) mapping populations (Griffiths *et al*., 2009; Pánková *et al*., 2008) was used to identify ten *Eps* QTL with a 95% confidence interval corresponding to a map distance of 20-30 cM. Average R^2 values for each QTL and the number of field trials on which the data are based are presented in Table 5. Where possible, using both published (Griffiths *et al*., 2009) and unpublished mapping data, flanking and centre markers were selected to delineate the 95% confidence interval for each QTL (Table 6). Based on the above QTL mapping data, four DH progeny lines carrying the early allele at each of ten QTL, five from Spark x Rialto and five from Charger x Badger were selected for backcrossing to the late allele parent within each population. Marker data indicated that these DH progeny lines had the minimum contribution of alleles from the early allele parent across the whole genome (data not shown). Selected DH lines were backcrossed twice to the late allele parent for each QTL. At BC_1, SSR markers subtending the QTL interval were used to select heterozygotes. At BC2, early and late allele homozygotes (eight lines of each allele) were selected for rapid selfing through single seed descent to F4. Backcrossing and marker selection was continued to produce BC4 iso-genic lines for future analyses.

Table 5. QTL chromosomal location, doubled haploid (DH) mapping population and donor allele source for *Eps* QTL.

QTL	DH mapping population	Late allele recipient
1D	Spark/Rialto	Rialto
3A	Spark/Rialto	Spark
3B	Spark/Rialto	Spark
6B	Spark/Rialto	Rialto
7A	Spark/Rialto	Spark
3A	Charger/Badger	Badger
3B	Charger/Badger	Badger
6A	Charger/Badger	Badger
6B	Charger/Badger	Badger
7A	Charger/Badger	Badger

Table 6. Flanking and centre SSR markers used to track *Eps* QTL through the backcross generations.

Recipient	QTL	Markers
Rialto	1D	Gdm111; Barc062
Spark	3A	Xgwm155; Xpsp3047; Wmc264
Spark	3B	Barc229; Xgwm376; Wmc291Spark
Rialto	6B	Xgwm193; Xgwm361; Xgwm219Rialto
Spark	7A	Xgwm130; Xpsp3001; Barc195
Badger	3A	Xgwm369; Wmc050; Wmc264
Badger	3B	Xgwm389; Xgwm299; Xgwm566
Badger	6A	Gdm36; Xgwm518; Wmc179
Badger	6B	Wmc106; Xgwm193; Xgwm219
Badger	7A	Barc127; Barc108; Xgwm63

3.2.2.2. DNA extraction and Eps *QTL marker screening*

Genomic DNA was extracted from two-week old seedlings using a modified Tanksley extraction method (Fulton *et al*., 1995). Primer sequences for SSRs with the GWM prefix were obtained from data published in Roder *et al*. (1998), Pestova *et al*. (2000) for markers with GDM, Song *et al*. (2005) for markers with BARC, Bryan *et al*.(1997) and Stephenson *et al*. (1998) for markers with PSP and Gupta *et al*. (2003) for markers with WMC. At BC_1 and BC_2, markers were used to identify a minimum of 16 progeny lines that were heterozygous at flanking and centre makers. Backcrossing was terminated at BC_2 and heterozygous progeny were allowed to self-fertilize. The resulting BC_2F_2 progeny were screened with markers to identify a minimum of eight homozygous lines for each of the early and late allele classes.

3.2.2.3. Controlled environment assessments of BC_2F_4 lines under extended photoperiod

Glasshouse

After eight weeks of vernalisation (5°C at 8hr photoperiod) twelve seedlings of each of the ten QTL NIL pairs (Table 5) were transplanted individually into 1 litre pots and arranged in a randomised in-complete block design on the floor of a heated glasshouse in November 2009. An extended photoperiod, natural daylight extended to 16hrs from astronomical dusk, was provided by 60W light bulbs suspended overhead. Day and night temperature was maintained at a constant 20 °C. For each plant, flowering date was recorded on the primary tiller at GS55 (half of ear emerged).

Growth room

Vernalised seedlings of selected QTL NILs: SR-1D (eight NILs: four early; four late), SR-3A (four NILs: two early; two late), SR-3B (six NILs: 3 early; 3 late) and SR-7A (eight NILs: four early; four late) were transplanted individually into 1 litre pots. These were arranged in a controlled environment cabinet (PGR-15, Conviron Ltd), running at a 16-hour photoperiod with a constant temperature of 20 °C. Three replicate seedlings per NIL were grown in a randomised complete block design. Development was monitored on the primary tiller of each plant by recording the dates of GS39 (flag leaf fully emerged), GS55 (half of ear emerged) and GS61 (anthesis).

3.2.2.4. Field assessments of* Eps *NILs under natural and extended photoperiods

Field trials were conducted in 2011 and 2012 at NIAB, Cambridge (52°13´N, 04°59´E), and at KWS near Thriplow (52°6´N, 0°6´E, 2012 only). Flowering time was recorded in the field under natural photoperiod (NP) in all three trials. At NIAB, plot weight was recorded on small $1m^2$ observation plots in 2012. In 2012, at KWS, flowering time was also recorded under an extended long day (16 hr) photoperiod (EP).

At NIAB, Spark (three QTL), Rialto (two QTL) and Badger (five QTL) BC_2F_5 lines were sown in two separate field trials in October 2010 and 2011. Each line was planted as two replicate Hege 90 plots (six rows) in a randomised complete block design. The number of days from sowing to GS55 (Zadoks *et al*., 1974) was recorded. For the trial planted in October 2011, the dates of GS39 (flag leaf emergence) and GS61 (start of flowering) were also recorded. Data recorded on individual plots (lines) was analysed using REML with fitted and random terms assigned as previously.

At KWS, comparison of NP versus EP was recorded. Seeds of selected lines were sown on 24 October 2011. Each accession was sown as a single row of a Hege 90 drill plot. Pairs of contrasting homozygote BC_2F_5 lines were planted as adjacent rows in both experiments. EP was achieved with supplementary field lighting (30W clear glass tungsten bulbs suspended 1m above the centre of plot-pairs at 37.5 cm intervals) during the pre-dawn night to extend the daylength to 16 hr. The duration of supplementary lighting was adjusted each week to accommodate for increasing NP as the experiment progressed. The EP regime ran from the shortest day (22 December 2011) until two weeks after GS31 (first node detectable, Zadoks *et al*., 1974). Plots under NP treatment were separated from EP plots by at least 3m. The number of days to GS39 (flag leaf emergence), GS59 (ear emergence) and GS61 (start of anthesis) (Zadoks *et al*., 1974) after 1 April 2012 were recorded on the primary ears for each row (line). Scores were adjusted to number of days from sowing to flowering and all data was analysed with REML, with photoperiod treatment added to the fitted model terms described above.

3.2.3. Synthetic hexaploid wheat (SHW) backcross programme

3.2.3.1. Plant material

An international nursery of 448 SHW lines was supplied by CIMMYT in spring 2006 (Appendix 1). Seed increase was carried out in an unheated glasshouse by planting four seeds of each line in separate 4 litre pots. Flowering date (days to GS55 from 1 February) and straw height were recorded and used as additional factors for selection of lines for backcrossing. Two elite UK genotypes, the spring cultivar Paragon and the facultative cultivar Xi-19, were selected as recurrent parents for the backcrossing programme.

3.2.3.2. Selection of lines for backcrossing

As the inclusion of all SHWs in the backcrossing programme was considered to be impractical, an 81 line subset (Appendix 1) was identified. In order that as much of the D-genome (e.g. *Ae. tauschii*) diversity from the whole nursery as possible was represented within the subset, phylogenetic analysis (Rogers' distance) was carried out on all 448 lines using 12 genome-wide EST-derived microsatellite markers (Eujayl *et al*., 2002). Lines were additionally genotyped with diagnostic markers for the *Ppd-D1* (Beales *et al*., 2007) and *Ppd-A1* (Wilhelm *et al*., 2009) loci. The selected subset was genotyped with DArT markers. DNAs of 81 SHW were submitted to Diversity Arrays Technology PTY Ltd, Yarralumla, ACT 2600, Australia and hybridized to the high density wheat array (Wheat *Pst*1 (*Taq*1) v2.5).

3.2.3.3. Evaluation of primary SHWs

The full set of 448 SHWs was sown as a gridded array of tussocks (10-15 seeds per tussock) at NIAB in spring 2009. The tussocks were assessed for flowering time, mildew resistance and plant height. A bulk sample of grain was harvested from each tussock. The subset of 50 SHWs used as donors for backcrossing was also tested in a number of different experiments.

Grain samples were tested using SDS-polyacrylamide gel electrophoresis, in order to determine which high molecular weight glutenin sub-units were carried by each SHW line. Testing was carried out in the laboratory of Dr J Seekings, RAGT Seeds, Ickleton, Cambs, in accordance with their standard protocol.

These SHW donors were also grown in standard pathology tests at NIAB in spring 2010 to determine seedling resistance to the YRW 08/21 ('Solstice / Oakley') race of yellow rust (*Puccinia striiformis)*. Seedlings were grown in spore-proof growth rooms under metal halide lights, with a 16-hour daylength, at 18°C day / 11°C night. Inoculation occurred 8 days after sowing, and disease symptoms were assessed a further 14-17 days later.

The same lines, plus spreaders and suitable controls, were sown as two replicates within a gridded array of tussocks as a standard yellow rust nursery at NIAB in autumn 2010. Seedlings of spreader varieties were inoculated with three races of yellow rust in growth rooms and once symptoms were apparent these were transplanted into spreader rows within the tussock trial. Assessments of yellow rust were made at three dates in May and June 2011 in order to investigate adult plant resistance. The races used for inoculation were 08/21 ('Solstice / Oakley'), 03/07 ('Brock') and 08/501 ('Timber').

The SHW donors were also sent for assessment in fusarium head blight experiments run by Dr Paul Nicholson, John Innes Centre in 2010 as part of the INSPYR LINK project.

3.2.3.4. Backcrossing and line development

The strategy for development of synthetic wheat-derived backcross lines was twofold involving (1) selection from F_2 or BC_1F_2 populations and line advancement through conventional field selection and (2) lines selfed through successive generations of single seed descent (SSD) without selection.

An initial round of crossing was carried out between Paragon (female parent) and 50 SHWs (male parents) in spring 2007. The same 50 SHWs were crossed with Xi-19 (female parent) in summer 2007. Paragon / SHW (PaS) F_1 plants (male parent) were crossed again onto Paragon during winter 2007 to produce BC_1 populations, and selfed to produce F_2 seed. Similarly, Xi-19 / SHW (XS) F_1 plants were backcrossed and selfed during spring 2008. No inbreeding was directly carried out from XS F_1 material as F_2 seed production was very poor for most of these crosses. BC_1 plants were grown in the greenhouse (PaS spring/summer 2008; XS autumn/winter 2008) and allowed to self-fertilize. Open-pollinated BC_1F_2 seed was harvested from each BC_1 plant.

BC_1F_2 seed was sown into 96-cell modular seed trays and grown in the glasshouse to maturity, where a single ear was harvested from each plant, with no selection practised. For PaS material, 64 BC_1F_2 seeds were typically sown per SHW donor, sampling all the BC_1 progenitors, although up to 400 seeds were sown for five populations. For XS material, 8-48 BC_1F_2 seeds were sown from each BC_1 progenitor. During single-seed descent (SSD), one seed from each harvested ear was planted for the next generation, maintaining the original population structure during inbreeding. This procedure was repeated for $BC_1F_2 \rightarrow BC_1F_3 \rightarrow BC_1F_4$, although BC_1F_4 seedlings were transplanted into 1 litre pots in order to maximise seed multiplication prior to field testing of BC_1F_5 plant progenies. BC_1F_5 material was available for drilling in spring 2010 and spring 2011 for PaS and XS, respectively.

Hybrid necrosis

Following the observation of contrasting levels of hybrid necrosis in F_1s between CIMMYT SHWs and Paragon or Xi-19 (for the 50 SHW donor parents) and Robigus or Alchemy (for crosses with SHW *Ppd-A1* donors), a small panel of UK wheat varieties was crossed with SHWs to produce F_1s. These were then grown in the glasshouse and hybrid necrosis symptoms were visually assessed.

3.2.3.5. Inbreeding and field selection strategy

Just as for a commercial breeding programme, promising lines were identified at each stage of field testing and carried forwards for further testing through iterative cycles of evaluation. Selection was broadly based upon the same characters as commercial breeding programmes, i.e. yield potential, appropriate adaptation and, to a lesser extent, adequate disease resistance. In addition, the commercial partners encouraged the retention of "exotic" traits such as delayed senescence, elevated above-ground biomass, and floral characters such as anther extrusion and high pollen productivity. For the 2011 XS BC_1F_5 material in particular, the breeders were keen to explore the maximum level of diversity from the SHW donors. Rather than making positive selections in this nursery, it was instead decided to make negative de-selections, i.e. material was discarded if it was too tall, too weak, too short, showed too much chlorosis or necrosis, or appeared aneuploid.

Field nurseries were treated with a robust early fungicide but then left untreated until harvest to encourage disease symptoms. Fertilizer levels were lower than for the surrounding farm crop, in accordance with usual breeding practice for small nursery plots. Except for the F_2, all nursery plots were drilled as standard breeding nursery plots with a Hege 90 drill, and comprised of six rows each approx 1.2m long. For the F_2 nursery, up to 800 progeny per cross were planted at 12 cm intervals using a Hege 95b pneumatic drill. Ears or plants of selected lines were taken by hand, and bulks were taken either using a hand-held Minibatt harvester (Agricultural Supply Services, Dursley, Gloucs) or using a sickle. Later generation plot bulks were harvested with a Haldrup plot combine. Hand-harvested samples were threshed out using either a Hege 16 ear thresher for ears and plants or Wintersteiger LD350 thresher for sickled bulks (Trials Equipment UK Ltd., Braintree, Essex). Plots containing reference samples of the parents (Paragon, Xi-19 and the SHWs) were nested within the nurseries, demarcating material derived from different SHWs. For benchmarking purposes, varieties from HGCA winter- and spring-wheat Recommended List Trials (RLT) were also sown as short rows within the nurseries.

All field selections were taken forwards one generation per calendar year, except for plants selected from the 2008 F_2 nursery which were sown as F_3 families in modular trays and rapidly advanced to F_4 through the glasshouse during autumn/winter 2008. Material progressed through nurseries and trials at NIAB and elsewhere as follows:

Harvest	Field selections			Material ex. SSD	
2008	PaS F_2				
2009	PaS F_4	PaS BC_1F_2	XS BC_1F_2		
2010	[a,b]PaS F_5	[b]PaS BC_1F_3	[b,c]XS BC_1F_3	PaS BC_1F_5	
2011	[d]PaS F_6	PaS BC_1F_4	[c]XS BC_1F_4	[d]PaS BC_1F_6	XS BC_1F_5
2012	[d]PaS F_7	PaS BC_1F_5	XS BC_1F_5	[d]PaS BC_1F_7	[c,e]XS BC_1F_6

[a] Also grown in 2010 yield trials (Limagrain)

[b] Also grown in 2010 observation nurseries (KWS and Limagrain)

[c] Subset also grown in drought tolerance trials (University of Nottingham on behalf of WGIN)

[d] Subset also grown in LoLa phenotyping trials (University of Nottingham and Rothamsted)

[e] 1000 lines also grown in yield trials (NIAB, KWS, Limagrain and RAGT)

The 2010 nurseries were irrigated shortly after sowing to promote germination and establishment, but then left, in order to increase drought stress. This facilitated selection for drought tolerance characters, especially across the previously unselected PaS BC_1F_5 material. There was a similar prolonged drought during spring 2011, but this time the nurseries were irrigated several times in order to ensure that yield potential and seed production were maximised, especially for the XS BC_1F_5 material.

For 2011-12, 1000 XS BC_1F_6 lines were entered into a co-ordinated yield trial, with 200 lines sown at each of five locations (two NIAB locations and one each from the three breeding companies) in October 2011 as single-replicate 6m plots. Material was divided equally between the five trials, i.e. there was equal representation of lines from each SHW donor in each trial. A high proportion of Xi-19 control plots were included in each trial to allow for some comparison across trials. Trials were grown for maximum yield and were harvested in late August and early September 2012. Yields were calculated relative to the mean of the Xi-19 plots within each trial, using row/column spatial analysis on Genstat.

A small, partially-replicated yield trial was sown at NIAB in spring 2012 for a number of selections taken from the 2011 nursery. This was grown as a treated trial for maximum yield, in small plots ($8m^2$ harvested area).

Grain size assessments

Once the 2010 NIAB BC_1F_3 nurseries had been prepared, residue BC_1F_3 seed was sent to RAGT Seeds (Ickleton, Cambs) where it was passed over a non-destructive MARVIN grain analyzer (GTA; Sensorik) and weighed. This generated data on the average grain length (L), width (W) and area (A) for each sample, plus the thousand grain weight (TGW). These data were also used to

calculate two additional parameters, the L/W ratio and the factor form density (FFD), which have previously been used to study the variation of grain size in wheat populations (Gegas *et al*., 2010). This seed was then split into two samples which were distributed to KWS and Limagrain.

Development of near-isogenic lines for specific traits

On several occasions it was noted that specific traits of interest (e.g. gross characters like presence or absence of awns, hairy glumes versus smooth glumes, or canopy / ear glaucosity) were segregating within ear-rows or plant progeny plots. For example, near-isogenic lines for glaucosity have been developed by selecting multiple non-glaucous plants from segregating rows (based on the assumption that the non-glaucous character is dominant) during inbreeding, and finally taking pure-breeding glaucous and non-glaucous types for subsequent testing.

WGIN and LoLa trials

WGIN drought-tolerance trials were planted on a sandy loam site in Nottinghamshire (run by Dr J Foulkes, University of Nottingham, Sutton Bonington) during the late autumn of 2009, 2010 and 2011, and grown as yield trials without irrigation. At maturity, the crop was harvested at ground level and total biomass was weighed, before partitioning into ears (which were then further partitioned into grains and chaff) and straw (further partitioned into lamina and stem + sheath) in order to calculate the harvest index. Tiller number (fertile shoots/m^2) and plant height were also recorded.

LoLa phenotyping trials were planted at two locations in Hertfordshire (run by Dr M Hawkesford, Rothamsted Research, Harpenden) and Nottinghamshire (run by Dr J Foulkes, University of Nottingham, Sutton Bonington) in the late autumn of 2010, 2011 and 2012 as replicated small plots grown under conventional or reduced/zero levels of N fertilizer.

3.2.4. Variation from CIMMYT synthetic-derived breeding lines (SHW-D)

Compared to the primary synthetics backcross programme described above, a more targeted approach was employed to exploit potentially valuable novel genetic variation from synthetic - derived wheat (SHW-D) germplasm supplied by CIMMYT. Such an approach capitalises on breeding and selection work already carried out by CIMMYT over many years. The strategy was to identify genomic blocks inherited from the synthetic component of the derived lines, identify markers delineating SHW-D genomic blocks, introgress individual blocks into two elite UK varieties (Paragon and Xi-19) and associate trait value with them using yield as a proxy for general adaptation and potential breeding value.

3.2.4.1. Synthetic-derived line selection

The rationale behind the identification of CIMMYT SHW-D germplasm for inclusion in the current study was based on previous CIMMYT work (Zhang *et al*., 2005). The underpinning hypothesis assumes that CIMMYT breeders selecting for specific traits of interest, e.g. yield and its components, agronomic characters and pathogen resistance, will accumulate breeding lines carrying positive alleles for these traits. It could be expected that markers linked to such alleles will be inherited in distorted, non-Mendelian proportions due to positive selection (Diaby and Casler, 2003). Zhang and colleagues deployed an informative set of 90 microsatellite (SSR) markers to genotype the genetic diversity present in 11 SHWs, their backcross derived families, and their durum and bread wheat parents to test for the selective advantage of SHW alleles in SHW-D families after several generations of selection. They reported that certain D-genome *(Ae. tauschii*) and AABB- genome (*T. turgidum*) SHW components were inherited in non-Mendelian proportions in corresponding SHW-D genotypes, suggesting that these conferred a selective breeding advantage. Using lines from this study, CIMMYT SHW-D were selected for backcrossing to Paragon and Xi-19. In order to track specific marker haplotypes during backcrossing, pedigree components (SHW, bread wheat, durum and *Ae. tauschii)* for nine SHW-D genotypes were kindly supplied by CIMMYT.

3.2.4.2. Marker selection of synthetic-derived genomic blocks and BC_2 line development

Germplasm supplied by CIMMYT was genotyped with markers identified by Zhang *et al*. (2005) as being inherited in non-Mendelian proportions. Additional SSR markers were identified from consensus wheat maps and, to further delineate the selected genomic regions and identify break-points, the same germplasm was submitted to Diversity Arrays Technology PTY Ltd, Yarralumla, ACT 2600, Australia and hybridized to the high density wheat array of DArT markers (Wheat *Pst*1 (*Taq*1) v2.5). Unfortunately at the time of backcrossing, many of the selected DArTs had no sequence data available to allow primer design for conversion to the PCR platform. This problem was circumvented by identifying closely linked SSR markers from a consensus map developed at NIAB (J. White, unpublished).

For each SHW-derived genomic block, flanking and centre markers were used to track the introgressions through the backcross generations to BC_2. Homozygous progeny were extracted from the BC_2F_2 generation. To facilitate paired analysis of the potential agronomic and yield advantage of each introgression, up to eight paired full sib homozygotes were identified (16 lines per introgression) so that each pair consisted of the SHW-derived and recurrent parent in an identical genetic background.

3.2.4.3. Field trial of BC_2F_5 lines

At NIAB in spring 2012, plots of full sib pairs of BC_2F_5 introgression NILs were drilled in both a single replicate observation nursery ($1m^2$) to provide pure seed stocks and in a fully randomised three replicate yield trial ($8m^2$ plots). Plot yield was recorded at harvest.

3.3. Results

3.3.1. Experiments with the *Ppd* allelic series

A total of 341 BC_2F_4 lines ('Alchemy': 84 + donor allele / 83 – donor allele; 'Robigus': 70 + donor allele / 104 – donor allele) were developed in the current study. Potential flanking markers were identified, but were not used in selecting the BC_2F_4 lines as they were not polymorphic on the parents (data not shown).

Statistically significant flowering time effects were recorded for all photoperiod treatments. In the SP and NP (NIAB) experiments, a significant interaction was detected between the recipient genetic background and allele donor ($P<0.001$ and $P=0.041$, respectively), with the flowering time (days to GS55) effect conferred by an allele dependent on the genetic background. In the KWS NP and EP experiments, no significant interaction with background was detected at any growth stage, although individual allele effects were significant ($P<0.001$). In the SP experiment not all lines flowered within the 123 day timeframe, so comparisons were made to the relevant recurrent parent. In the NP and EP experiments, all lines flowered, and comparisons were made between BC_2F_4 lines with (+) and without (-) the donor allele.

3.3.1.1. D-genome effects

The *Ppd-D1a* allele gave the earliest flowering phenotype in the SP (Table 2) and NP (NIAB) (Table 3) experiments, although background and allele donor source effects were observed. In the SP experiment, the 'Soissons' *Ppd-D1a* allele reduced the time to GS55 by 42 d in both 'Alchemy' and 'Robigus' backgrounds, and by 9 d in the NP (NIAB) experiment. The 'Ciano67' *Ppd-D1a* allele gave the same 9 d reduction in the 'Alchemy' background in NP (NIAB), but reduced flowering to a lesser extent than the 'Soissons' *Ppd-D1a* allele in the SP experiment (33 d 'Alchemy' background; 24 d 'Robigus' background) and by only 6 d in a 'Robigus' background in the NP (NIAB) experiment.

A similar effect was also detected for the 'Ciano67' *Ppd-D1a* allele in the NP and EP (KWS) experiments. In NP (KWS) the 'Ciano67' *Ppd-D1a* allele reduced days to GS39 by 5 d and to GS59 by 4 d, in contrast to the same allele from 'Soissons' which reduced days to GS39 by 6 d and to GS59 by 8 d. The EP treatment compressed the early flowering time effect, reducing time to GS39 by 3 d and GS59 by 5d in 'Soissons' lines and more severely in the 'Ciano67' lines (1 d reduction to both GS39 and GS59).

There was little evidence that the PS *Ppd-D1b* (JIC SHW_GBR011; 'Mercia'; 'Norstar') alleles either promoted or delayed flowering in any of the photoperiod treatments (Tables 2, 3 and 4).

3.3.1.2. *A-genome effects*

Major reductions in flowering time were also observed for the PI *Ppd-A1a* allele. In the SP experiment (Table 2) the allele reduced days to GS55 by 38 d in an 'Alchemy' background, a stronger effect than observed for the 'Ciano67' *Ppd-D1a* allele. In the NP (NIAB) experiment the allele gave either a 7 or 5 d reduction, dependent on allele source (from SHW_131 and SHW_173, respectively), with this being slightly less than the reduction conferred by the *Ppd-D1a* allele in the same experiment. The SHW_131 *Ppd-A1a* allele gave the strongest recorded reduction in days to GS39 in the NP (KWS) experiment (10 d) (compared to an 8 d reduction from the 'Soissons' *Ppd-D1a* allele). This strong early flowering was not seen in the EP treatment, with the *Ppd-A1a* allele giving a 2 d reduction to both GS39 and GS59.

There was little evidence that the PS *Ppd-A1b* ('Cappelle-Desprez') allele either promoted or delayed flowering in any of the photoperiod treatments (Tables 2, 3 and 4).

3.3.1.3. B-genome effects

The *Ppd-B1a* allele gave moderate flowering time reductions across experiments. In the SP experiment the 'Chinese Spring' *Ppd-B1a* allele gave a large (35 d) reduction in 'Alchemy', with the 'Timstein' *Ppd-B1a* allele giving a more moderate 24 d ('Robigus') and 14 d ('Alchemy') reduction. Under NP (NIAB) the 'Timstein' allele gave a 5 d reduction in 'Alchemy', compared to a 2 d reduction in 'Robigus', with the 'Chinese Spring' allele also giving a 2 d reduction ('Robigus' only).

In the NP (KWS) experiment no early flowering effect was recorded for the 'Chinese Spring' allele, with the 'Timstein' allele again giving a moderate 3 d (GS39) and 1 d (GS59) reduction. In the EP (KWS) treatment no effect was detected for the 'Timstein' allele, and the 'Chinese Spring' allele gave no reduction to GS39, but relatively strong reduction to GS59 (4 d).

3.3.1.4. Repeat flowering-time analysis across 2011-12

In 2012, flowering assessments were made on yield trials plots making it possible to compare effects over years (Figures 1 and 2). ANOVA indicated that genotype x year interaction was non-significant ($P>0.05$) and coefficients of correlation for donor (0.91) and recipient (0.81) alleles for each NIL set was highly significant ($P<0.001$).

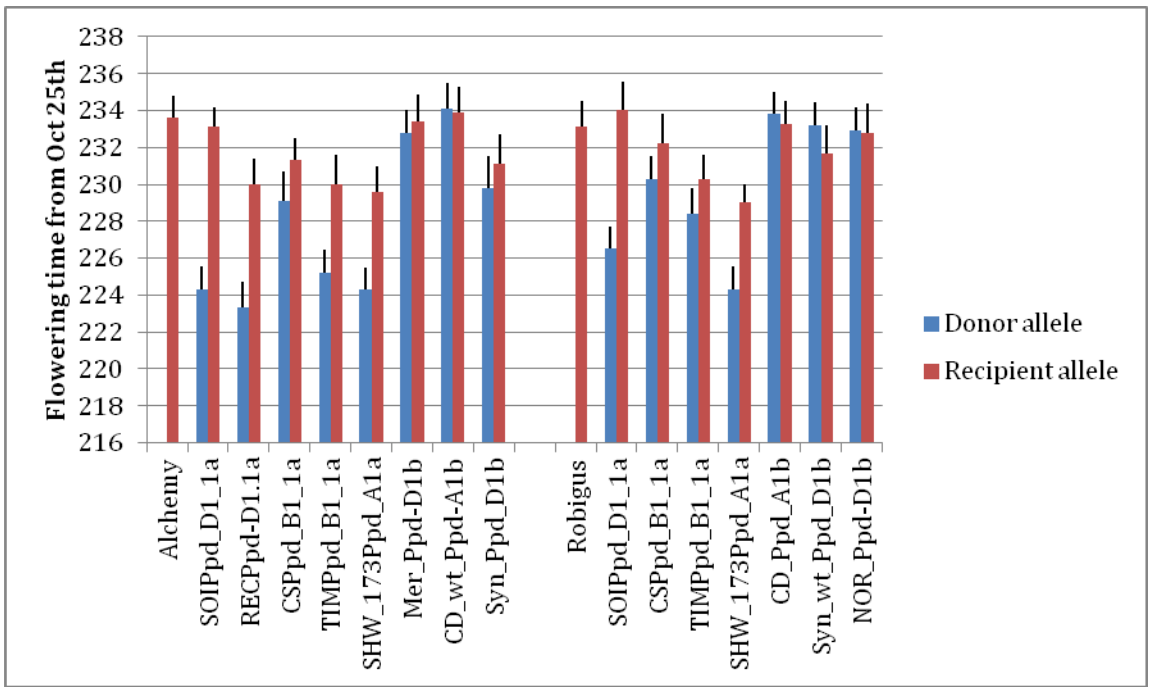


Figure 1. Average flowering time from sowing for the NIAB 2011 field trial of the *Ppd* allelic series in the Alchemy (left panel) and Robigus (right panel) genetic backgrounds. For each NIL set, the average flowering time of lines carrying the donor and corresponding wild-type alleles were compared.

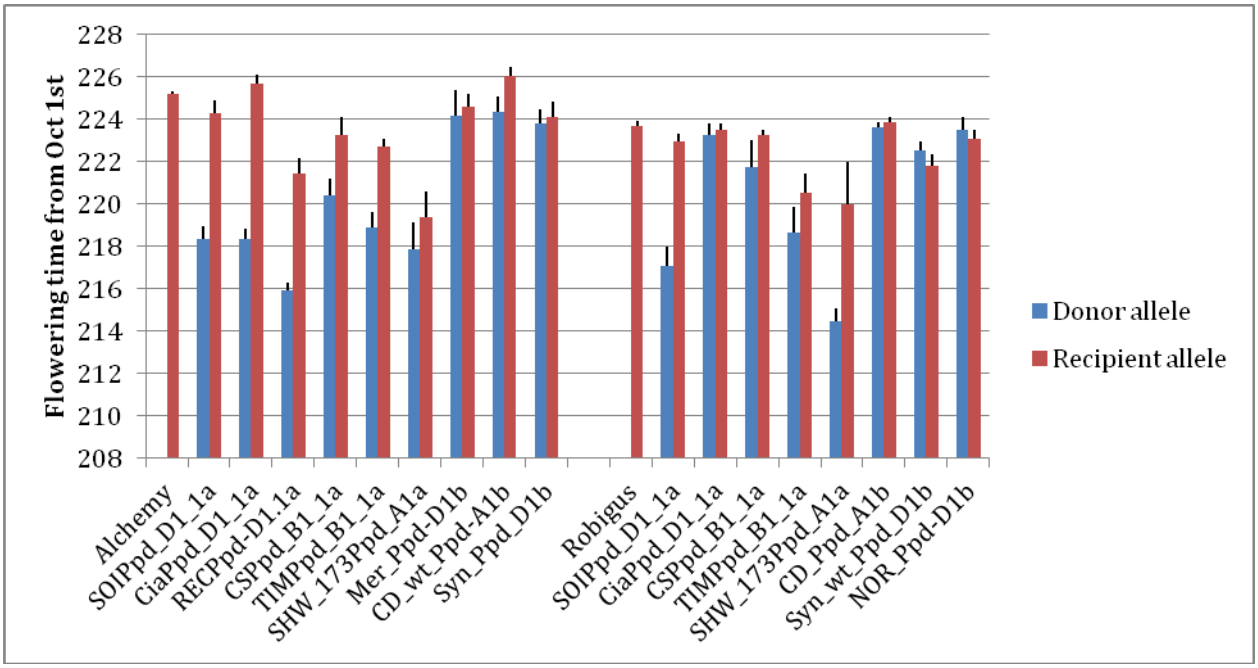


Figure 2. Average flowering time from sowing for the NIAB 2012 field trial of the *Ppd* allelic series in the Alchemy (left panel) and Robigus (right panel) genetic backgrounds. For each NIL set, the average flowering time of lines carrying the donor and corresponding wild-type alleles were compared.

3.3.1.5. Yield trials

In 2012, plot yield was recorded on *Ppd* NILs in both the Alchemy and Robigus genetic backgrounds (Figures 3 and 4). In the Alchemy background (Figure 3), analysis of lines carrying mutant (+) early-flowering alleles on the A, B, and D-genomes indicated that NILs carrying the *Ppd-D1a* allele introgressed from Soissons and Ciano-67, the *Ppd-B1a* allele from Chinese Spring and Timstein and the *Ppd-A1a* allele from SHW-173 significantly (P<0.05) out-yielded corresponding NILs carrying the Alchemy allele. However, there was a non-significant yield difference observed for lines derived from Recital (*Ppd-D1a* + *Ppd-B1a*). For putative wild-type variants *Ppd-D1b* and *Ppd-A1b* introgressed from Mercia and Cappelle Desprez respectively, yield differences were non-significant (data not shown).

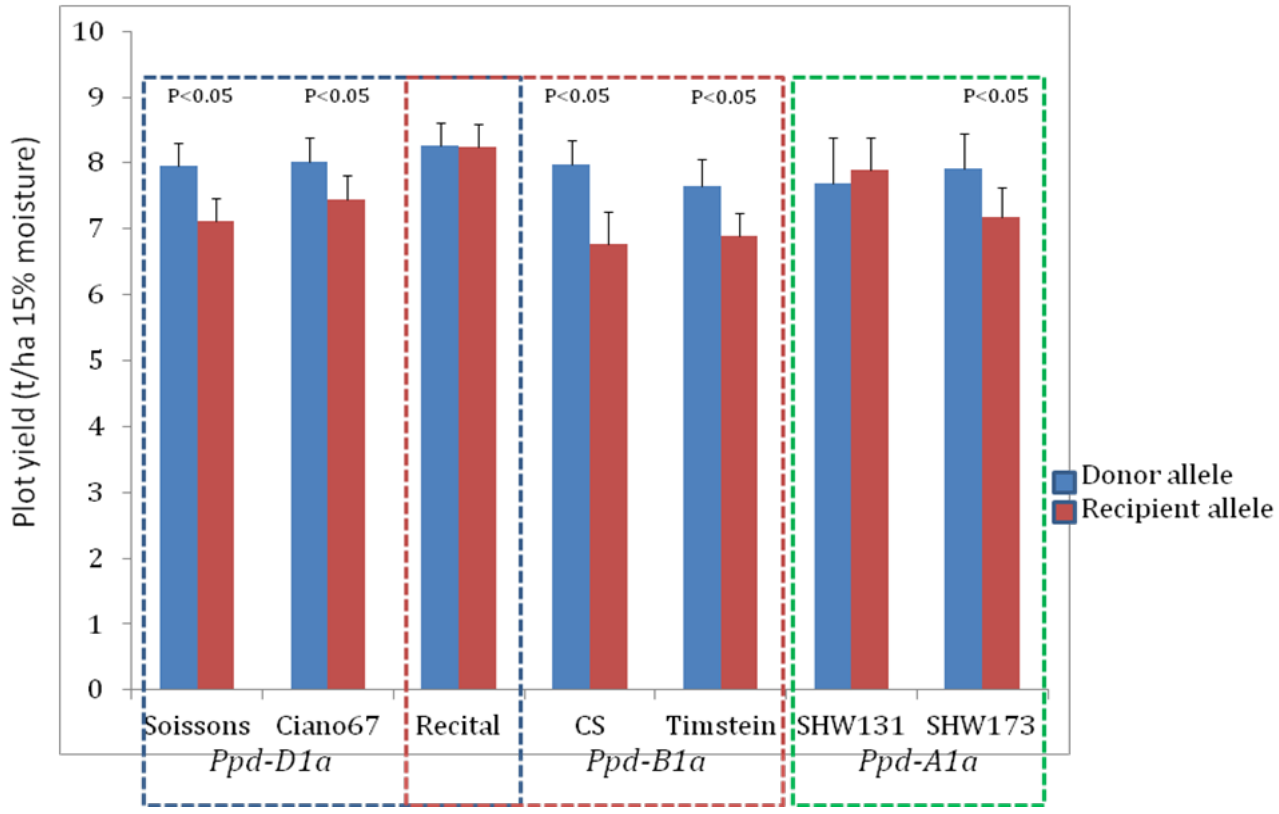


Figure 3. Average plot yield (tonnes per hectare) from the NIAB 2012 field trial of the *Ppd* allelic series of NILs in the Alchemy genetic background. For each NIL set, the average plot yield of lines carrying the donor and corresponding wild-type alleles are compared.

In the Robigus genetic background, mutant (+) early-flowering alleles did not show significant yield differences, except for those with the *Ppd-B1a* allele from Timstein, in which the recipient allele out-yielded the corresponding allele from Timstein (Figure 4). Similarly, for lines carrying putative wild-type variants *Ppd-D1b* and *Ppd-A1b* introgressed from the synthetic hexaploid, GBR-011 and Cappelle Desprez respectively, yield differences were also non-significant (data not shown).

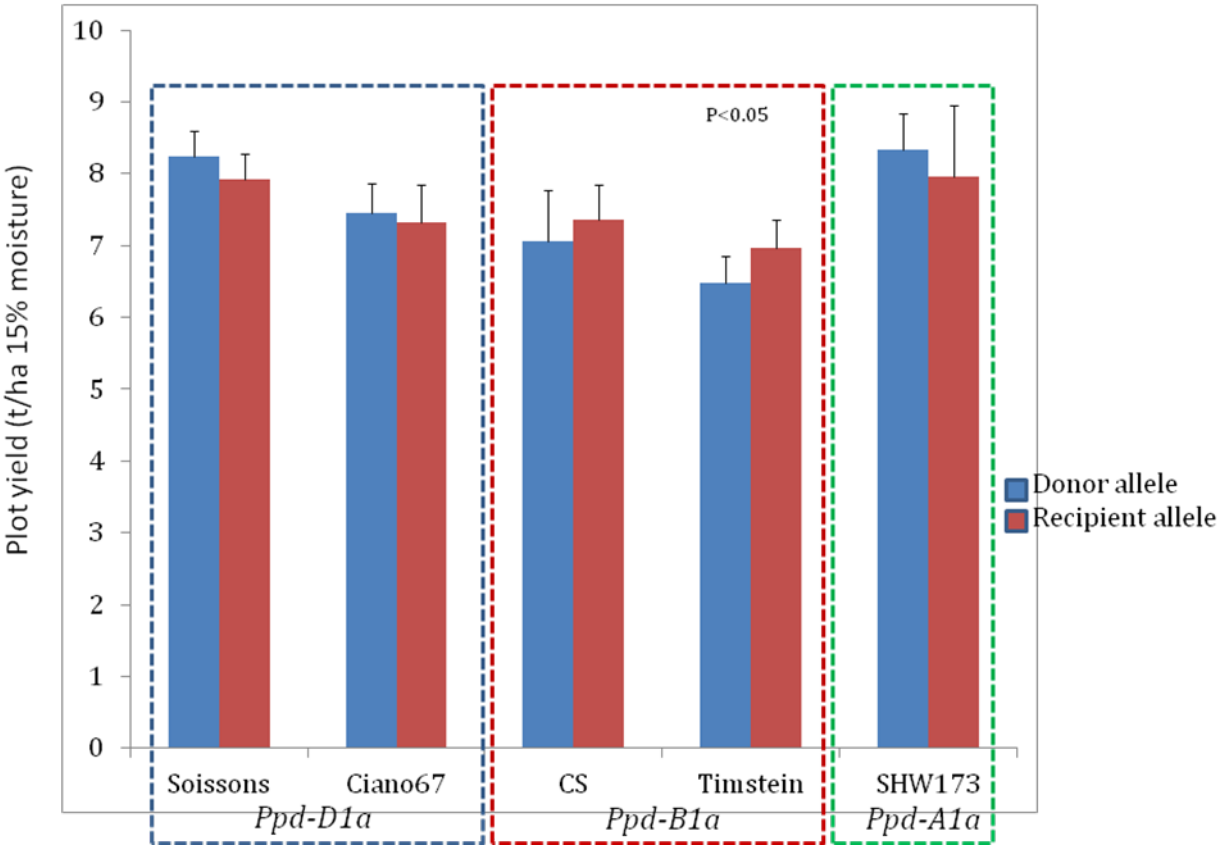


Figure 4. Average plot yield (tonnes per hectare) from the NIAB 2012 field trial of the *Ppd* allelic series of NILs in the Robigus genetic background. For each NIL set, the average plot yield of lines carrying the donor and corresponding wild-type alleles are compared.

3.3.2. Experiments with Eps *QTL-NILs*

3.3.2.1. Controlled environment experiments

Ten *Eps* BC_2F_4 QTL-NILs derived from the Spark x Rialto (SR-1D, SR-3A, SR-3B, SR-6B, SR-7A) and Charger x Badger (CB-3A,CB-3B, CB-6A, CB-6B, CB-7A) were tested under extended photoperiod (16hr) in a heated and lit glasshouse at NIAB during October 2010 to February 2011. A second confirmatory extended photoperiod experiment in a growth chamber was performed on QTL-NILs that had the most potent effect on flowering time (FT) in the previous glasshouse experiment and under natural photoperiod in the field (see below).

3.3.2.1.1. Glasshouse extended photoperiod, 2010-11

Primary spikes (spikes produced on the first stem to flower) were individually visually assessed for flowering time (time in days from potting up from vernalisation to GS55) on each of 12 replicate plants per QTL-NIL. Unbalanced analysis of variance (ANOVA) of pooled data indicated that block, replicate and genotype x block effects were non-significant ($P > 0.05$). Predicted values were for individual FT scores using Genstat regression (general linear model – GLM) indicated that, relative

to their corresponding late allele full-sib lines, the early flowering allele significantly reduced FT in SR-1D (P < 0.001; early allele FT reduction: 10 days), SR-3A (P < 0.001; early allele FT reduction: 7 days), SR-7A (P<0.01; early allele FT reduction: 6 days) and SR-6B (P=0.05; early allele FT reduction: 2.5 days). For SR-3B, the effect of the early allele was non-significant (P>0.05) (Figure 5). An identical analysis carried out on Charger x Badger derived NILs did not identify any significant FT effect for the early allele (data not shown).

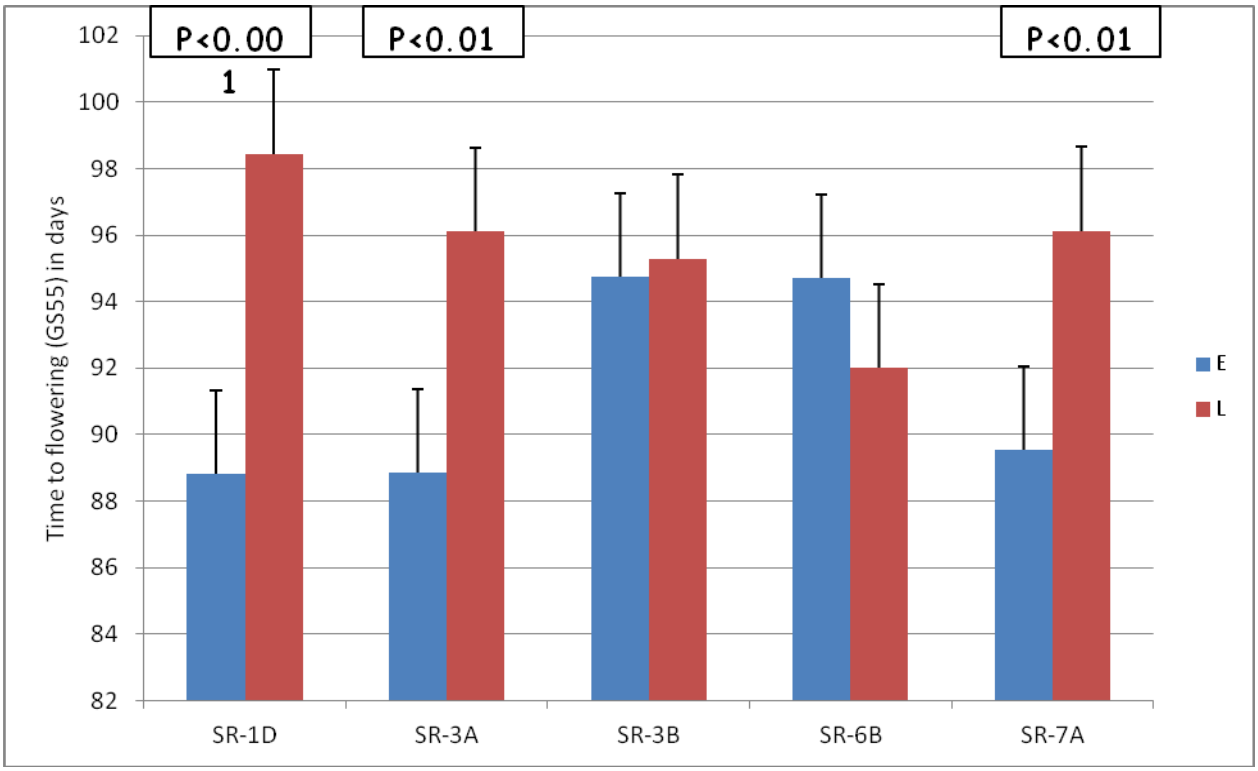


Figure 5. Average time from the end of vernalisation to flowering (GS55) of Spark x Rialto *Eps* BC_2F_4 NILs under extended photoperiod (16hrs) in the glasshouse. For each QTL NIL set, the average flowering time of early (E) and late (L) allele lines derived from the Spark x Rialto (SR) doubled haploid population are compared.

3.3.2.1.2. Growth room extended photoperiod

QTL-NILs SR-1D, SR-3A, SR-3B and SR-7A were evaluated in a growth chamber. FT from the end of vernalisation to GS39, GS55 and GS61 was recorded on primary spikes from 3 replicate plants per NIL. REML of the fully randomised complete block design indicated that, relative to corresponding late allele full sib lines, the early allele at SR-1D significantly (P < 0.001) reduced FT at all growth stages by a similar number of days (average reduction 6.5 days) (Table 7). The most significant (P<0.01) effect for SR-3A was observed at GS55 (reduction of 3.9 days), values for GS39 and GS61 were non-significant (P>0.05). At SR-7A, FT reduction was significant at GS55 (P<0.01) and GS61 (P<0.05), but non-significant (P>0.05) at GS39. SR-3B was non-significant at all three growth stages.

Table 7. Average time from the end of vernalisation to flowering (GS39, GS55 & GS61) of Spark x Rialto *Eps* BC_2F_4 NILs under extended photoperiod (16hrs) in a growth chamber. For each QTL NIL set, the average reduction in flowering time of early compared to late allele lines derived from the Spark x Rialto DH population are presented.

	Reduction in days to		
QTL	GS39	GS55	GS61
1D	-5.7*	-7.4*	-6.6*
3A	-0.6	-3.9*	-1.8
3B	-1.3	-0.9	-1.5
7A	-0.4	-4.0*	-2.7*

3.3.2.2. Field assessments of BC_2F_4 NILs in a natural and extended photoperiod

FT assessment at NIAB was carried out on all ten NILs derived from both SR and CB populations in 2011 and 2012. Harvested plot weight was additionally recorded for the 2012 trial. At KWS in Thriplow, near Cambridge, all NILs were evaluated for FT under both natural (NP) extended photoperiod (EP).

3.3.2.2.1. NIAB field trials under natural photoperiod in 2011 and 2012

Under field conditions in 2011, early alleles of QTL with the most potent FT (time in days to GS55) reducing effect relative to corresponding late allele full sib lines were SR-1D ($P<0.001$; early allele FT reduction: 3 days) SR-3B ($P<0.01$; early allele FT reduction: 2 days) and CB-3A ($P<0.01$; early allele FT reduction: 2 days) (Figure 6). SR-6B, SR-7A, CB-3B, CB-6B and CB-7A were all of borderline significance ($P=0.05$), reducing FT by an average of 1 day. CB-6A had a non-significant effect on FT.

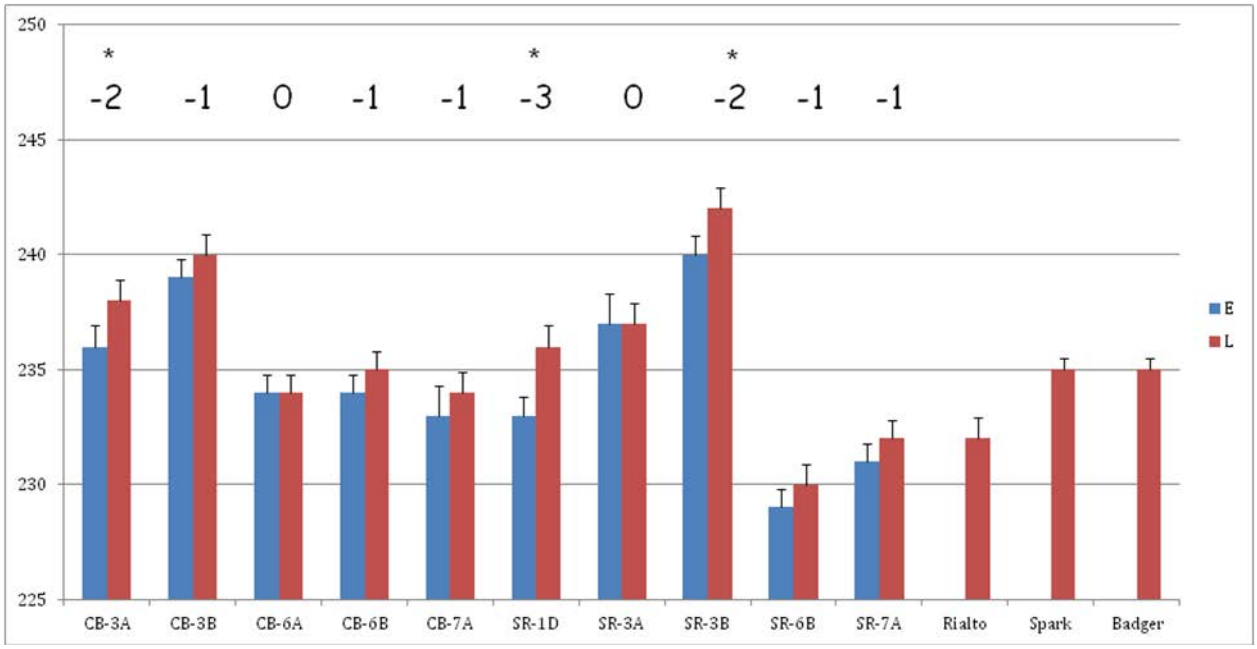


Figure 6. NIAB 2011 *Eps* field trial. Time from 1 October 2010 to flowering (GS55) of Spark x Rialto (SR) and Charger x Badger (CB) BC_2F_4 NILs. For each QTL NIL set, the average flowering time of early (E) and late (L) allele lines are compared.

In 2012, FT to GS39, GS55 and GS61 were evaluated on all ten QTL-NILs. With the exception of CB-3A, CB-3B and CB-6A, the most significant early allele FT reduction was observed for GS55 (Tables 8 and 9). The early allele of SR-3A had the most significant FT reducing effect (P<0.001; FT reduction: 4.2 days) closely followed by SR-1D (P<0.01; FT reduction: 2.5 days). Effects of lesser significance (P<0.05) were observed for SR-3B and SR-7A (FT reduction: 1 and 1.6 days respectively). Borderline significance (P=0.05) was observed for SR-6B and CB-3A; however, CB-3B, CB-6A, CB-6B and CB-7A all had a non-significant effect on FT.

In 2012, plot weight was recorded for all QTL NILs on the same small ($1m^2$) plots used to assess flowering. Analysis of the mean yield of early and late allele full sib NIL pairs revealed a small reduction of borderline significance (P≥0.05) associated with the late allele (Figure 7). There were, however, significant differences in yield within and between QTL NIL sets and *Eps* parental cultivars. For example, Rialto was the highest yielding followed by Badger and Spark. Compared to Soissons (carrying the *Ppd-D1b* early-flowering allele), Spark had a similar yield, however, both Soissons and Spark were significantly (P<0.01) out-yielded by Rialto and Badger. Within Charger x Badger derived NILs, yields were non-significantly different and similar to Badger. However, of NILs derived from the Spark x Rialto population, only SR-1D lines had an average yield that was similar to Rialto, the average yields of other NIL groups were all significantly lower (P<0.01). For Spark x Rialto NILs, an interesting correlation was observed between the donor of the early allele *Eps* QTL and yield relative to the parental cultivars. Despite the fact that Rialto significantly (P<0.01) out-yielded Spark, NILs carrying contrasting alleles donated by Rialto (SR-3A, SR-3B,

SR-7A, were significantly (P<0.01) lower yielding than SR-1D and SR-6B that carry contrasting *Eps* QTL alleles from Spark.

Table 8. NIAB 2012 *Eps* field trial. Flowering time (GS39, GS55 & GS61) of Spark x Rialto BC_2F_4 NILs. For each QTL NIL set, the average reduction in flowering time of early compared to late allele lines derived from the Spark x Rialto doubled haploid population are presented.

	Reduction in days to		
QTL	GS39	GS55	GS61
1D	-1.3*	-2.5*	-0.5*
3A	-0.3	-4.2*	-2.5*
3B	-0.6*	-1.0*	-0.9*
6B	-0.6*	-0.7*	0
7A	-0.5	-1.6*	-0.6*

Table 9. NIAB 2012 *Eps* field trial. Flowering time (GS39, GS55 & GS61) of Charger x Badger BC_2F_4 NILs. For each QTL NIL set, the average reduction in flowering time of early compared to late allele lines derived from the Charger x Badger doubled haploid population are presented.

	Reduction in days to		
QTL	GS39	GS55	GS61
3A	0.1	-0.8*	-1.6*
3B	-0.8	0	-0.3
6A	-0.6*	-0.2	-0.1
6B	-0.1	-0.6	-1.0*
7A	-0.7*	0.9	0.9

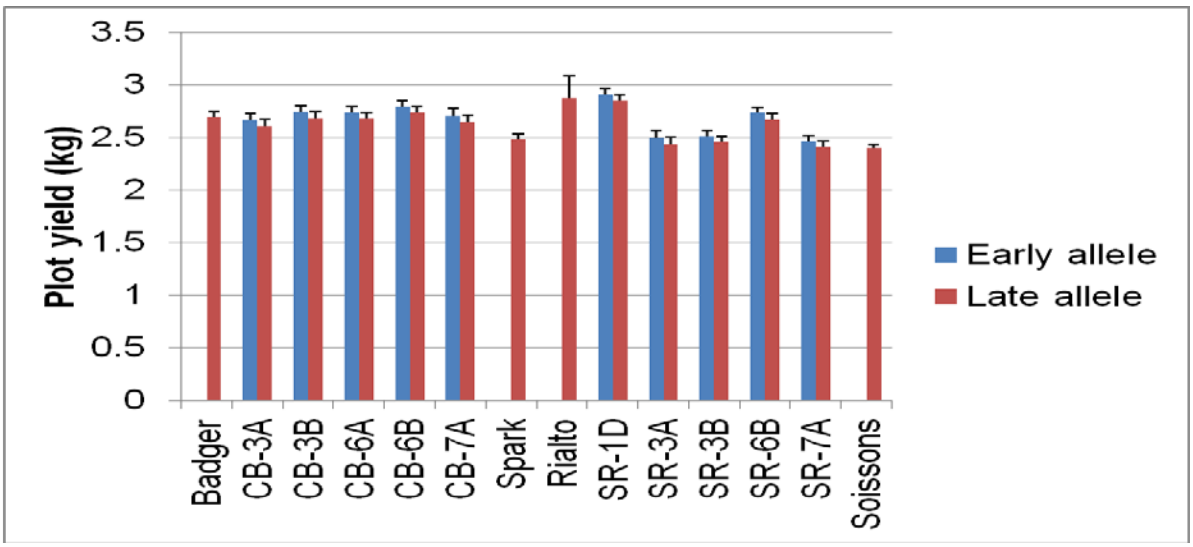


Figure 7. NIAB 2012 *Eps* field trial. Average plot yield (kg) of Spark x Rialto (SR) and Charger x Badger (CB) BC_2F_4 NILs. For each QTL NIL set, the average plot yield of early (E) and late (L) allele lines are compared.

3.3.2.2.2. KWS field experiment under natural and extended photoperiod in 2012

All eight QTL-NILs derived from Spark x Rialto and Charger x Badger were assessed for FT under both natural and extended photoperiod. No significant effect on FT was observed (Tables 10 & 11).

Table 10. KWS 2012 *Eps* field trial under natural (NP) and extended (EP) photoperiod. Flowering time (GS39, GS55 & GS61) of Spark x Rialto BC_2F_4 NILs. For each QTL NIL set, the average reduction in flowering time of early compared to late allele lines derived from the Spark x Rialto doubled haploid population are presented.

	NP		EP	
QTL	GS39	GS55	GS39	GS55
1D	0.9	1.1	2.8	0.8
3A	4.5	0.3	1.3	3.8
3B	0.7	0.2	0.7	2.2
6B	-1.2	-0.7	-1.9	-1.7
7A	1.4	0.0	0.4	-0.4

Table 11. KWS 2012 *Eps* field trial under natural (NP) and extended (EP) photoperiod Flowering time (GS39 & GS55) of Charger x Badger BC_2F_4 NILs. For each QTL NIL set, the average reduction in flowering time of early compared to late allele lines derived from the Charger x Badger doubled haploid population are presented.

	NP		EP	
QTL	GS39	GS55	GS39	GS55
CB 3A	2.1	-1.1	0.6	2.1
CB 3B	0.0	-0.1	-1.9	-1.9
CB 6A	1.1	2.1	0.6	0.4
CB 6B	0.9	0.4	0.3	0.1
CB 7A	-0.1	-0.2	0.5	2.0

3.3.3. Synthetic hexaploid wheat (SHW) backcross programme

3.3.3.1. Selection of primary SHW lines for backcrossing

All 448 primary SHW lines provided by CIMMYT were genotyped with 12 genome-wide SSR markers. Rogers distance calculated with PowerMarker v3.25 based on these 12 markers suggested that the collection comprised up to 30 diversity groups at the 95% level of similarity (Figure 8). Using both pedigree data supplied by CIMMYT and the above phylogenetic analysis to identify a numerically balanced selection from within each diversity group, a subset of 81 lines was identified for further analysis with DArT markers (Appendix 1). Considering allele call quality (Q) and polymorphism information content (PIC) values, 2,700 polymorphic DArT markers, from a total of 5,000 provided by Diversity Arrays Technology PTY Ltd, were used to perform a second diversity analysis in PowerMarker. Where chromosomal location was known (288 DArT loci) it was possible to produce genome-specific dendrograms (Figures 9-10). Analysis of the AABB genome (147 markers) suggested that the 50 elite Mexican durum cultivars used to develop the SHW provided by CIMMYT were drawn from three distinctive germplasm streams (Figure 9). A small number of SHWs clustered together with the bread wheats based on these AABB genome markers.

Phylogenetic analysis (using 141 markers) of the D-genome donors (*Aegilops tauschii*) indicated that their genetic diversity was significantly greater than the AABB component (average gene

diversity 0.79 for *Ae. tauschii* vs 0.55 for durum parents). Although levels of diversity were generally high on the D-genome, a distinctive closely related sub-group of lines was identified (Figure 10). In addition, comparison with the D-genome of the bread wheat recurrent parents (Paragon and Xi-19) used in the backcross program indicated that several synthetic lines were relatively closely related to bread wheat (Figure 10). In particular, SHW-216, -217 and -219 appear closely related to bread wheat on both the AB genome (Figure 9) and D genome (Figure 10) dendrograms.

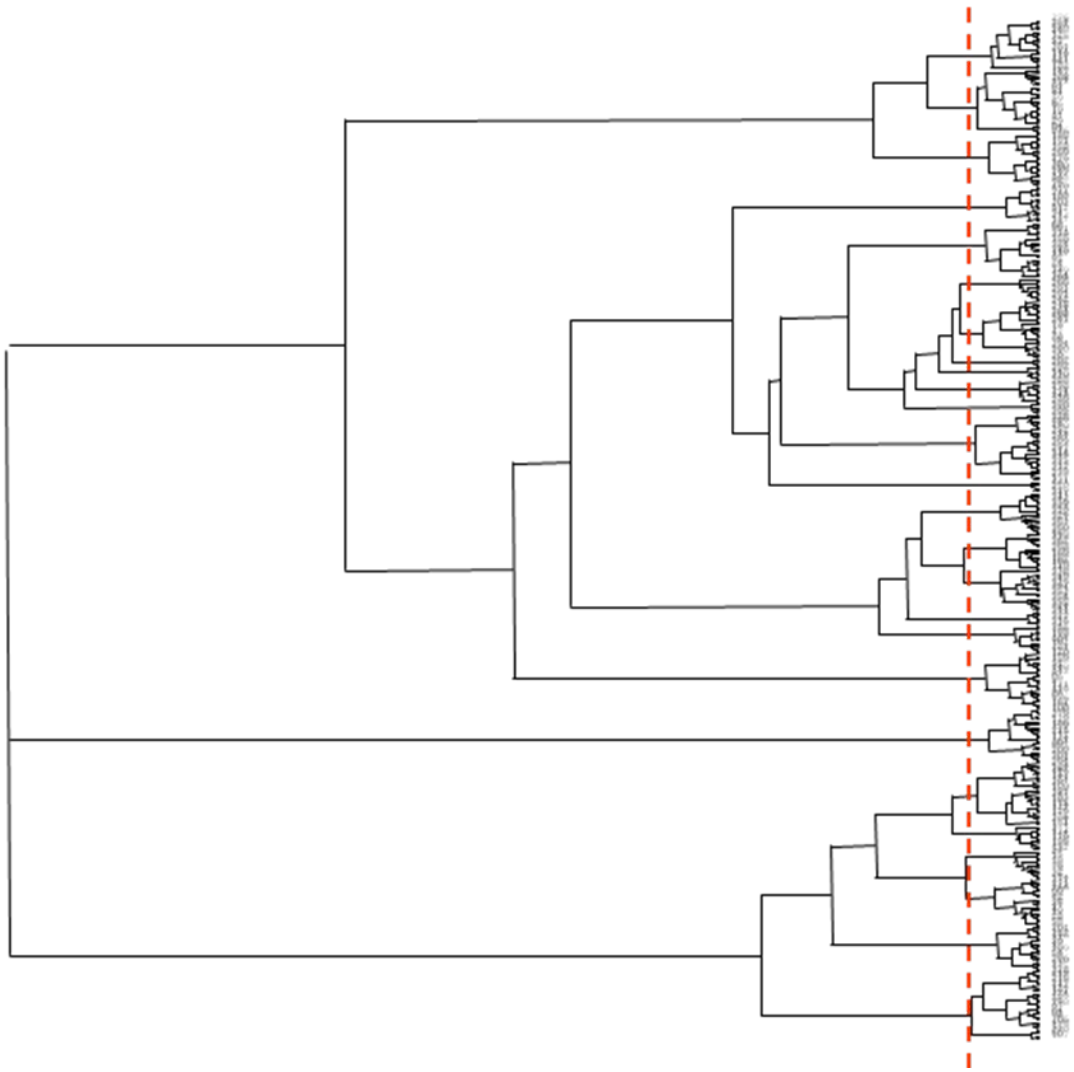


Figure 8. Dendrogram of 448 CIMMYT synthetic hexaploid wheat lines calculated using PowerMarker based on 12 genome-wide SSR markers. Red dotted line indicates the 95% level of similarity used to identify diversity groups for line selection.

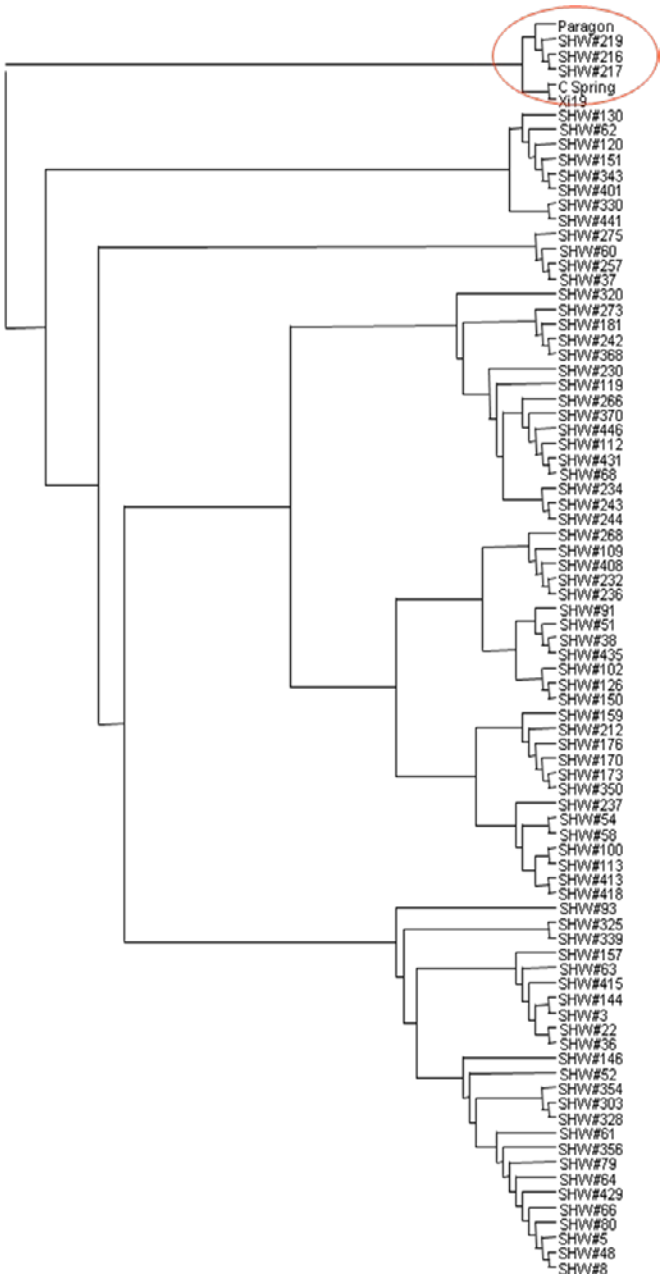


Figure 9. Dendrogram of 81 CIMMYT synthetic hexaploid wheat lines, including recurrent parents, Paragon and Xi-19 (red circle) calculated using PowerMarker based on 147 AB-genome (tetraploid parents) specific DArT markers

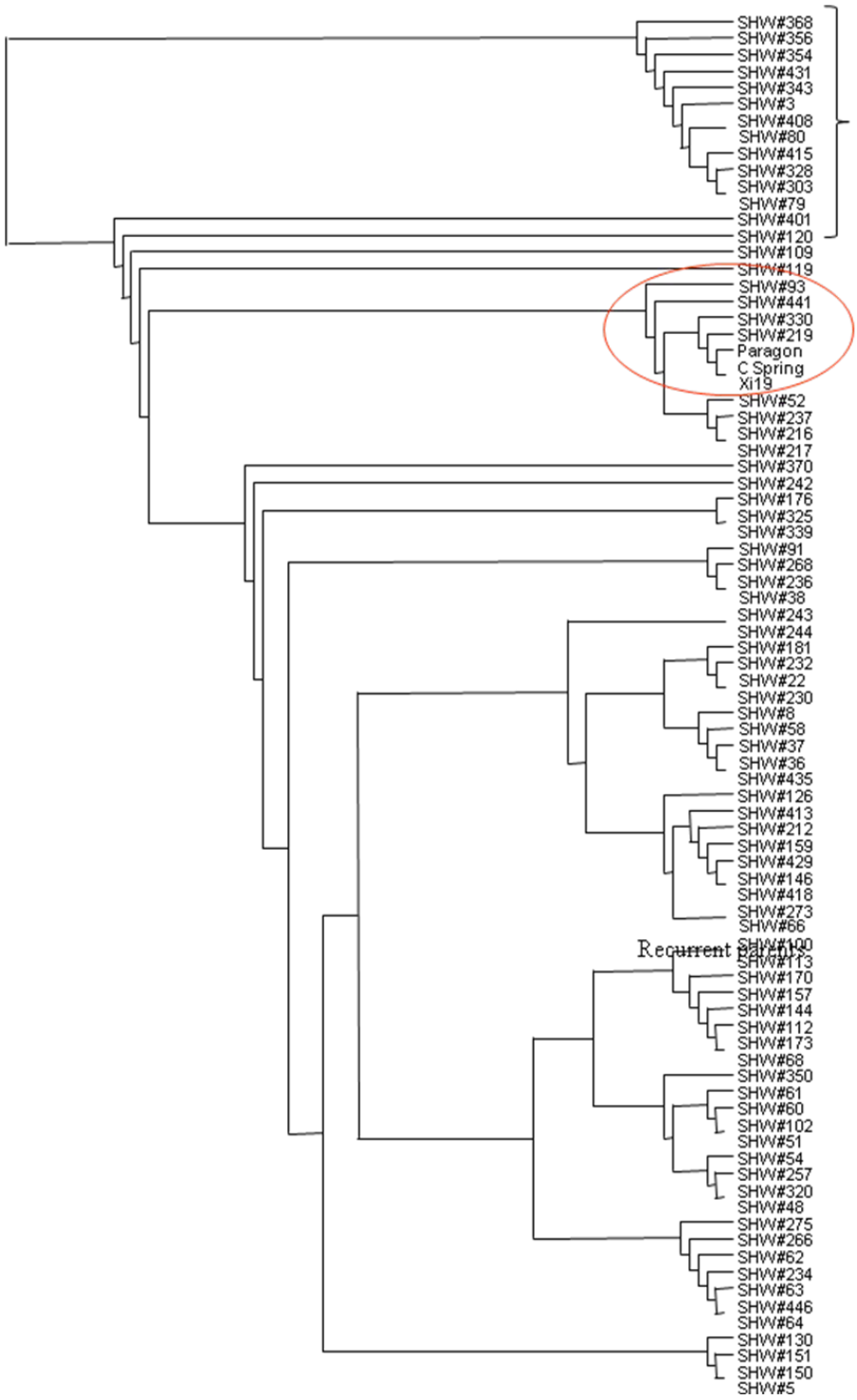


Figure 10. Dendrogram of 81 CIMMYT synthetic hexaploid wheat lines including recurrent parents, Paragon and Xi-19 (red circle) calculated using PowerMarker based on 141 D-genome (*Aegilops tauschii* parents) specific DArT markers. Outlying group of lines bracketed.

3.3.3.2. Phenotypic evaluation of primary SHW lines

3.3.3.2.1 Tussock trial 2009

The majority of the CIMMYT SHWs were considerably earlier flowering than typical UK spring varieties (Figures 11 and 12). This corresponded well with their haplotypes at *Ppd-A1* and *Ppd-D1* (Appendix 2). Variation for height and mildew resistance was also far greater than for the RLT varieties included within the nursery (data not shown).

Figure 11. 2009 SHW tussock trial. UK spring wheat controls (right hand column) were generally shorter, later flowering and more glaucous than the CIMMYT SHWs.

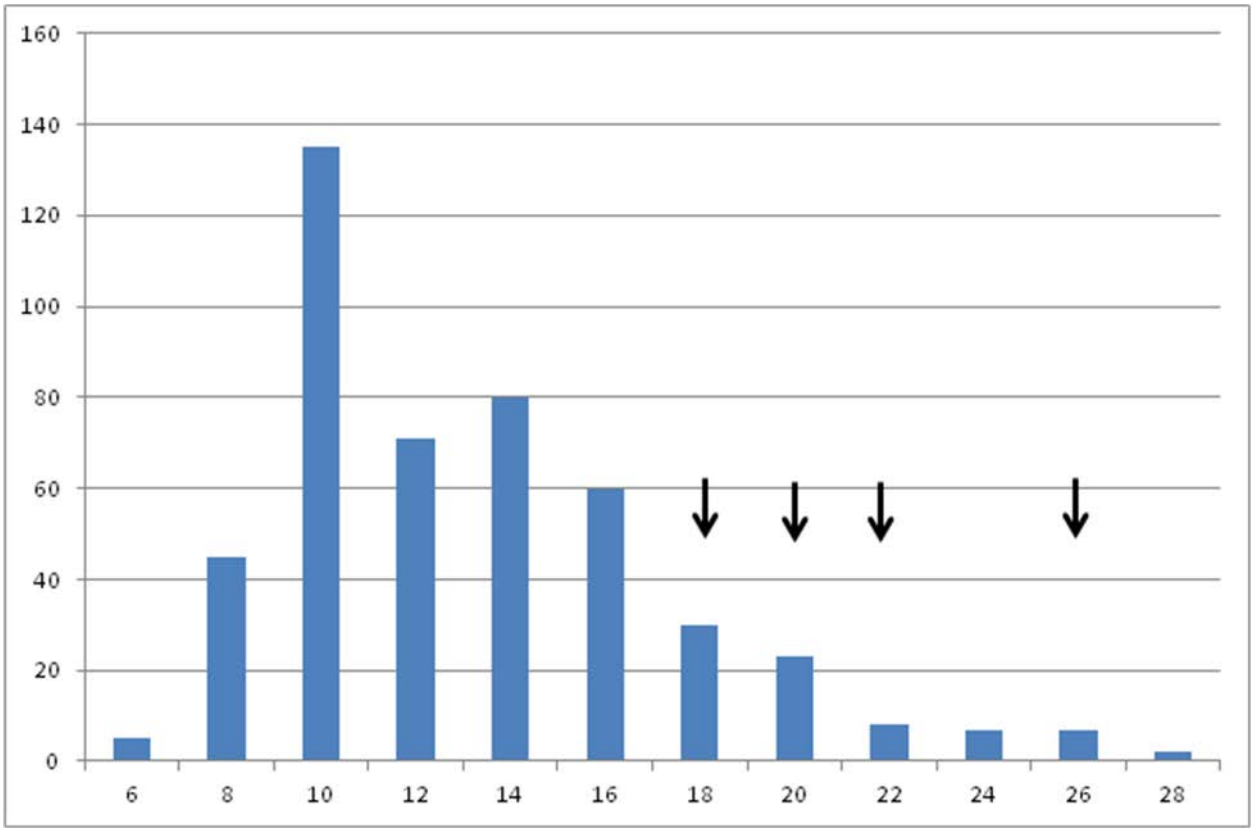


Figure 12. Frequency distribution for flowering time scores in the SHW tussock trial, 2009. For each SHW, the aggregate of three flowering time scores is shown, with low numbers denoting very early flowering and high numbers denoting late flowering. Arrows denote the relative positions of UK spring wheat varieties to the SHW lines.

3.3.3.2.2 HMW glutenin subunits

The sub-units carried at the Glu-1A, Glu-1B and Glu-1D loci are shown for the SHW used as donors during backcrossing are shown in Appendix 3.

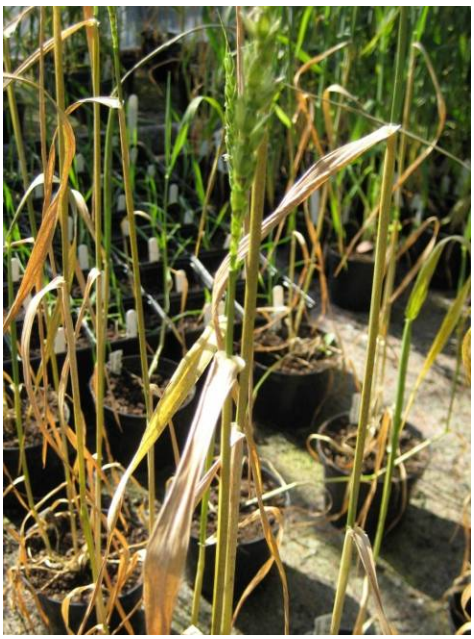
3.3.3.2.3. Pathology tests

The results for the fusarium head blight inoculation experiments are shown in Appendix 4. Disease scores for seedling and adult plant resistance to the 'Solstice / Oakley' race of yellow rust are shown in Appendix 5.

3.3.3.3. Backcrossing and line development

The breakdown by SHW donor of successful F_1 and BC_1 crosses with Xi-19 and Paragon is summarised in Table 12. Paragon / SHW (PaS) F_1s were notably chlorotic, but seed number during backcrossing and selfing did not appear to be unduly affected. Severe hybrid necrosis was noted in 44 of the Xi-19 / SHW (XS) F_1 combinations (Figure 13) to the extent that many F_1 plants did not survive to anthesis, so neither crossing to BC_1 or selfing to F_2 were possible. Rather than aiming for a consistent number of Xi-19 crosses per SHW donor, it was decided to keep on crossing with available material until pollen production ceased.

Figure 13. Hybrid necrosis symptoms in XS F_1 plants.



The number of BC_1F_5 lines produced per SHW donor after SSD inbreeding is also shown in Table 12.

Table 12. Number of lines developed during backcrossing and SSD inbreeding.

SHW donor		PaS			XS		
Code	CIMMYT ID	F_1	BC_1	BC_1F_5 SSD	F_1	BC_1	BC_1F_5 SSD
SHW-003	159512	21	15	0	10	0	0
SHW-008	159516	6	8	35	4	0	0
SHW-022	62052	22	16	24	0	0	0
SHW-036	159530	2	3	5	7	9	54
SHW-038	62061	6	13	47	3	0	0
SHW-048	62048	10	16	22	4	0	0
SHW-051	62062	12	16	40	10	24	95
SHW-052	88720	10	16	44	5	11	17
SHW-054	154089	3	5	23	8	0	0
SHW-058	154091	10	12	70	2	57	0[a]
SHW-060	62059	4	0	0	2	17	0[a]
SHW-061	62059	11	8	23	11	17	105
SHW-062	62056	19	16	62	9	2	32
SHW-063	62056	16	12	37	13	6	84
SHW-065	62049	5	7	0	9	0	0
SHW-066	159544	3	15	23	12	3	0
SHW-079	159556	7	8	49	1	16	0
SHW-080	159557	12	7	25	15	0	0
SHW-091	159565	5	7	58	1	0	0
SHW-093	159566	20	8	47	25	0	0
SHW-100	159572	16	8	48	23	41	204

SHW-109	159576	4	7	37	7	10	120
SHW-120[b]	154092			15			
SHW-126	159681	1	0		5	0	0
SHW-143	160197	8	16	44	24	6	52
SHW-144	160198	9	43	298	10	62	259
SHW-159	160213	8	8	55	7	14	66
SHW-170	160221	9	11	54	31	7	106
SHW-173[b]	160224			49			
SHW-176	160227	6	6	78	0	0	0
SHW-181	160232	20	38	252	1	0	0
SHW-216	161079	10	16	51	16	4	81
SHW-217	161079	22	11	40	20	41	182
SHW-218	161079	20	39	326	11	22	169
SHW-219	161079	10	28	35	4	4	67
SHW-232	161191	38	26	29	4	0	0
SHW-236	154095	4	2	6	5	5	73
SHW-237	161193	0	0	0	18	5	37
SHW-264	161596	1	0	0	17	31	117
SHW-330	161658	6	30	317	11	27	176
SHW-339	161667	15	1	1	8	0	0
SHW-343	161671	8	5	45	2	5	45
SHW-350	161678	6	8	40	9	5	58
SHW-354	161682	21	16	34	0	0	0
SHW-356	161684	2	0	11	0	0	0
SHW-368	161696	9	8	26	1	0	0
SHW-370	161698	16	13	28	5	11	51
SHW-372	161700	6	10	52	6	13	8
SHW-405	161733	15	0	0	6	0	0
SHW-409	161737	8	24	8	21	54	497
SHW-429	161756	7	7	22	12	2	0
SHW-441	161769	15	34	182	35	22	92

[a] All BC_1F_2 progeny from Xi-19 / SHW-058 and Xi-19 / SHW-060 looked like Xi-19 selfs, so inbreeding stopped

[b] SHW-120 and -173 were not in the original set of 50 donors but were used in crosses to bring in novel *Ppd* alleles and incorporated into the pre-breeding set of germplasm

3.3.3.3.1 Hybrid necrosis

F_1 plants were grown from crosses between a small panel of SHW donors and varieties from HGCA Recommended List Trials. Up to four F_1 plants were grown and any ambiguous results were repeated, seed permitting. In all crosses, the SHW parent was the pollen donor. Plants were

scored initially as seedlings (Figure 14) but then grown on to maturity to determine the severity of symptoms and any impacts on seed set (Figure 15).

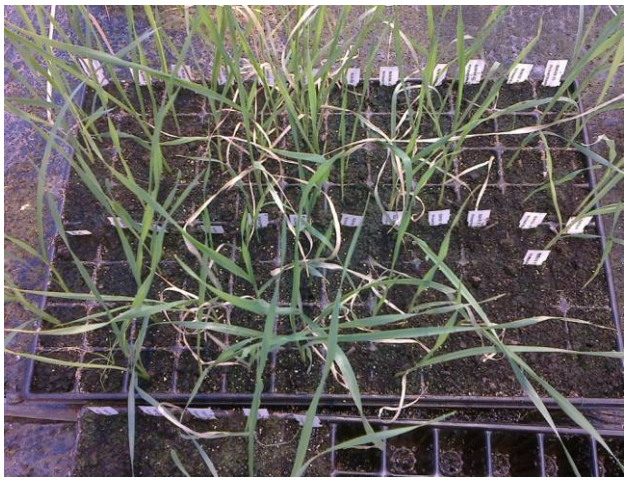


Figure 14. Elite / SHW F_1 seedlings clearly differing in their hybrid necrosis symptoms.

Figure 15. F_1 plants at anthesis. Left: Robigus / SHW-330 (no necrosis). Right: Oakley / SHW-330 (hybrid necrosis, but some pollen

The results from these screening crosses are shown below in Table 13.

Table 13. Hybrid necrosis symptoms for elite UK wheat varieties in F_1s with CIMMYT SHWs

Parent	**Notes on F_1 (x Cimmyt SHWs)**
Paragon	Chlorosis in some F_1s but seed set barely compromised
Xi-19	HN in most combinations, many not beyond stem extension
Alchemy	Similar to Xi-19
Robigus	As intervarietal crosses, no visible necrosis, full seed set
Cordiale	Similar to Xi-19, no F_2 recovered
Gallant	Drastically reduced tiller number/ear size, few F_2 recovered
Humber	Similar to Gallant
Oakley	Similar to Gallant
Q Plus	Similar to Xi-19, no F_2 recovered
Scout	Similar to Gallant
Timber	Similar to Xi-19, no F_2 recovered
Viscount	Similar to Gallant
Cortez	As intervarietal crosses, no visible necrosis, full seed set

Shamrock	Similar to Gallant
Glasgow	Similar to Gallant
Denman	Similar to Xi-19, no F_2 recovered
Conqueror	Variable from Xi-19 reaction to Robigus reaction: check
Grafton	Similar to Xi-19, no F_2 recovered
Mulika	As intervarietal crosses, no visible necrosis, full seed set
Stigg	Similar to Gallant
SY Epson	As intervarietal crosses, no visible necrosis, full seed set
Invicta	As intervarietal crosses, no visible necrosis, full seed set
KWS Target	As intervarietal crosses, no visible necrosis, full seed set
Tuxedo	As intervarietal crosses, no visible necrosis, full seed set
Warrior	As intervarietal crosses, no visible necrosis, full seed set
Beluga	No germination
Gravitas	No germination
KWS Santiago	Similar to Gallant
Tybalt	As intervarietal crosses, no visible necrosis, full seed set
Monty	As intervarietal crosses, no visible necrosis, full seed set
Zanatan	Similar to Gallant

3.3.3.3.2 Field evaluation and selection of inbreds derived from Paragon / SHW F_2

Table 14 shows the progression through inbreeding from PaS F_2 individuals**.**

Table 14. Number of field selections made from PaS material, F_2 onwards.

SHW	F_2 2008	F_4 2009	F_5 2010	F_6 2011	F_7 2012
SHW-003	0				
SHW-008	9	8	6	3	3
SHW-022	0				
SHW-036	0				
SHW-038	1	0			
SHW-048	7	4	4	0	
SHW-051	3	5	11	3	3
SHW-052	23	25	25	11	7
SHW-054	3	0			
SHW-058	4	0			
SHW-060	0				
SHW-061	3	5	0		
SHW-062	1	1	0		

SHW-063	3	2	0		
SHW-065	6	0			
SHW-066	14	8	1	1	1
SHW-079	1	0			
SHW-080	2	0			
SHW-091	0				
SHW-093	0				
SHW-100	1	1	0		
SHW-109	16	20	8	2	2
SHW-143	3	3	0		
SHW-144	12	8	10	3	3
SHW-159	0				
SHW-170	0				
SHW-176	0				
SHW-181	0				
SHW-216	1	0			
SHW-217	0				
SHW-218	2	5	3	2	2
SHW-219	4	2	3	0	
SHW-232	3	1	0		
SHW-236	6	8	8	2	2
SHW-264	0				
SHW-330	18	11	3	2	2
SHW-339	0				
SHW-343	2	0			
SHW-350	5	1	0		
SHW-354	4	0			
SHW-356	2	1	4	0	
SHW-368	2	2	0		
SHW-370	5	3	2	1	1
SHW-372	4	0			
SHW-405	4	1	0		
SHW-409	9	1	0		
SHW-429	6	1	0		
SHW-441	11	13	11	6	4

Small F_2 populations were sown from PaS crosses in 2008, in order to get a feel for the range of plant types these combinations would produce. Most crosses seemed to give a high proportion of tall, vigorous plants, with many extremely non-glaucous progenies (Figure 16).

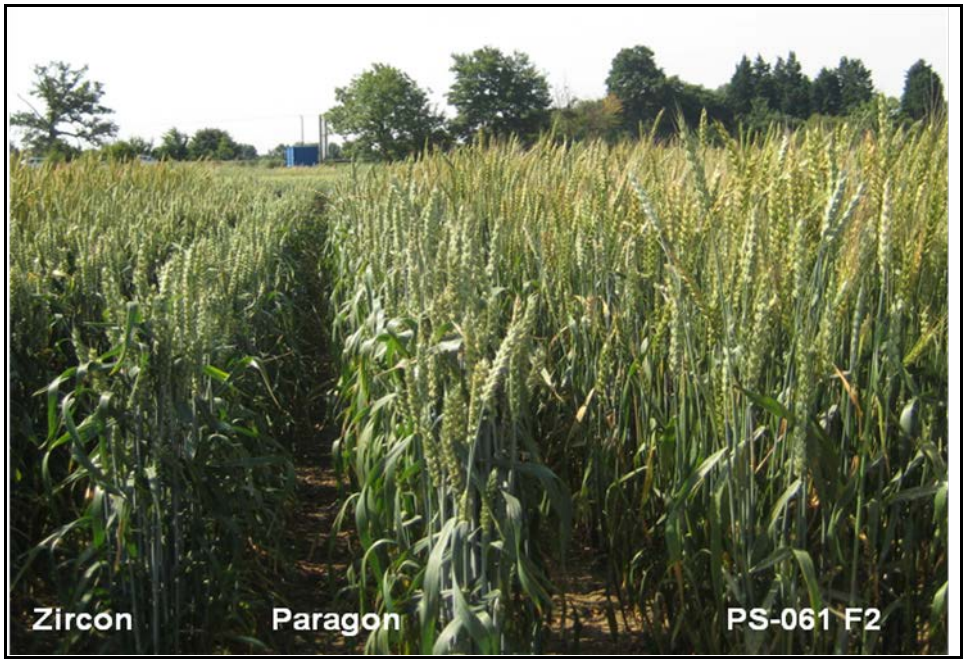


Figure 16. Variation in the PaS F_2 nursery, July 2008.

200 selections were taken from the F_2 nursery as selected single plants (Figure 17) and threshed out to provide F_3 seed which was taken through a single cycle of SSD inbreeding. For a few SHW donors which appeared to be relatively good combiners (i.e. a relatively high proportion of F_2 selections came from these crosses), increased numbers of plants were grown for backcrossing and SSD.

Figure 17. Selection of improved plants within the PaS F_2 nursery, August 2008.

One F_2 population, Paragon / SHW-144 (PS-144) was assessed in more detail as it appeared to segregate for a number of interesting characters, including glaucosity, ear morphology and yield components (Figure 18). Segregation ratios suggested that the non-glaucous / glaucous character was controlled by a single gene and that non-glaucous types were dominant (281 non-glaucous:112 glaucous, not significantly different from 3:1 ratio).

Figure 18. Comparison of single ears from a typical PS-144 F_2 plant, July 2008 (left: awned, non-glaucous, high spikelet fertility) and Paragon (right: non-awned, glaucous, moderate spikelet)

Following a single inbreeding cycle under glass, F_4 rows were sown in 2009 which traced back to 196 of the 200 F_2 plant selections. Several selections were taken forwards to F_5 on the basis of their performance in the nursery, either as six ears (93 selections) or individual plants (47 selections), which traced 86 different F_2 plants and came from 25 different SHW donor parents (Table 14). Bulk samples of F_5 grain were taken from 65 F_4 rows.

The F_5 grain bulks were grown in a single-replicate yield trial at Limagrain in 2010. Data was captured from this trial for grain yield and specific weight using on-combine sampling (Figure 19). Five lines were retained by Limagrain for further testing within their breeding programme in 2011, one of which was retested again in 2012 in their pre-National List series but did not progress further.

Most of the PaS F_2-derived materials retained beyond F_6 were only retained as near-isogenic lines (3.3.3.3.6) or because they were being used in other projects e.g. LoLa phenotyping (3.3.3.3.7).

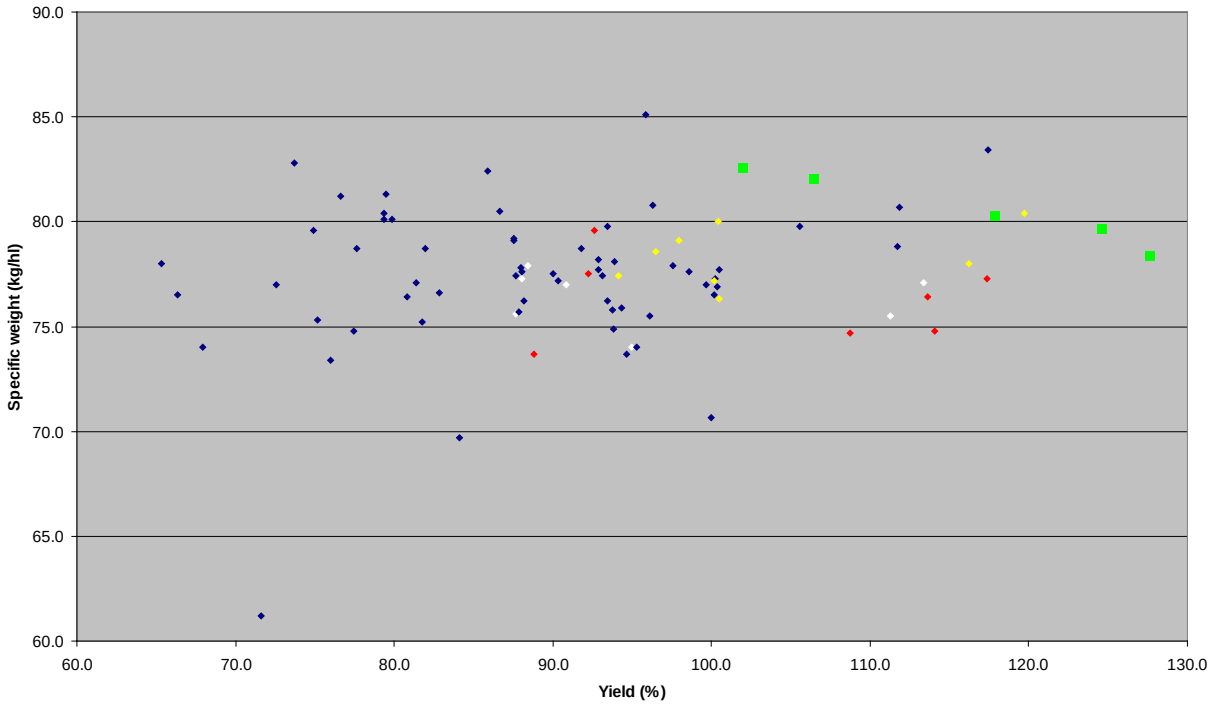


Figure 19. Yield and specific weight of PaS F_5 bulks grown in single-replicate yield trials by Limagrain, 2010. Yields are expressed as a percentage relative to the control mean (100%=mean of eight plots each of Paragon (red), Ashby (white) and Tybalt (yellow)). F_5 bulks are shown in blue except those selected for further testing, which are shown in green.

3.3.3.3.3 Field selection of inbreds derived from PaS and XS BC_1F_2

The progression of selections through inbreeding from PaS and XS BC_1F_2 is shown in Tables 15 & 16, respectively.

Table 15. Number of field selections made from PaS material, BC_1F_2 onwards.

SHW	BC_1F_2 2009		BC_1F_3 2010	BC_1F_4 2011	BC_1F_5 2012
	Sown	Selections	Selections	Selections	Selections
SHW-003	8	0			
SHW-008	5	6	1	0	
SHW-022	9	0			
SHW-036	1	0			
SHW-038	9	6	0		
SHW-048	16	8	3	0	
SHW-051	16	16	5	2	1
SHW-052	11	26	3	0	
SHW-054	4	5	0		
SHW-058	10	9	0		

SHW-061	4	7	0		
SHW-062	10	16	3	0	
SHW-063	6	12	0		
SHW-065	4	0			
SHW-066	12	15	4	0	
SHW-079	6	3	0		
SHW-091	4	9	2	0	
SHW-093	3	2	0		
SHW-100	7	4	0		
SHW-109	5	10	0		
SHW-120	3	1	0		
SHW-143	10	10	3	0	
SHW-144	25	28	10	0	
SHW-159	4	3	0		
SHW-170	6	9	0		
SHW-173	7	4	0		
SHW-176	2	0			
SHW-181	25	35	10	1	0
SHW-216	12	9	3	0	
SHW-217	6	1	1	0	
SHW-218	30	40	17	4	0
SHW-219	25	14	5	0	
SHW-232	17	18	8	1	0
SHW-264	11	3	3	0	
SHW-330	23	38	14	6	4
SHW-339	1	2	0		
SHW-343	3	4	0		
SHW-350	4	3	2	0	
SHW-354	8	7	4	1	0
SHW-368	5	10	0		
SHW-370	9	18	1	1	0
SHW-372	4	4	1	0	
SHW-409	15	0			
SHW-429	2	4	0		
SHW-441	23	31	18	6	3

Table 16. Number of field selections made from XS material, BC_1F_2 onwards.

SHW	BC_1F_2 2009		BC_1F_3 2010	BC_1F_4 2011
	Sown	Selections	Selections	Selections
SHW-036	1	6	1	0
SHW-051	10	7	2	2
SHW-052	5	12	3	2
SHW-058	41	0		
SHW-060	13	0		
SHW-061	9	28	8	0
SHW-062	2	2	0	
SHW-063	4	4	2	2
SHW-079	4	0		
SHW-100	25	36	6	0
SHW-109	6	14	5	3
SHW-143	4	1	0	
SHW-144	33	31	5	2
SHW-159	8	8	0	
SHW-170	5	8	3	1
SHW-216	2	5	2	0
SHW-217	28	31	5	1
SHW-218	22	17	8	7
SHW-219	3	0		
SHW-236	2	6	1	0
SHW-237	3	3	1	0
SHW-264	19	10	3	0
SHW-330	9	1	0	
SHW-343	3	0		
SHW-350	4	2	1	0
SHW-370	6	2	0	
SHW-372	2	0		
SHW-409	50	29	1	0
SHW-429	1	0		
SHW-441	11	8	5	2

In 2009, 430 PaS BC_1F_2 rows were sown, each tracing a different BC_1 plant. At harvest, 450 plants were selected from 197 rows: 71 rows included at least three selections and only 7 rows included six or more selections, and 38 SHW donors were represented in the selections. For the

Xi-19 background, 335 BC_1F_2 rows were sown, and 271 plants were selected which came from 98 rows: 44 rows with at least three selections and 12 rows with six or more selections, collectively representing 23 SHW donors. A number of rows appeared to be identical to their recurrent parent and were eliminated as probable parental selfs (54 Paragon and 62 Xi-19); the corresponding families were also eliminated from the SSD inbreeding process.

The “stay-green” delayed senescence trait, which seemed to be associated with some of the vivid non-glaucous types in the PaS F_2 derivatives, was again apparent in some of the lines (Figure 20).

Figure 20. Delayed senescence segregating in adjacent BC_1F_2 plants within the same row (PS-144>10), August 2009

MARVIN seed dimension and thousand grain weight data were gathered for all plant selections taken from the PaS BC_1F_2 nursery (Figure 21).

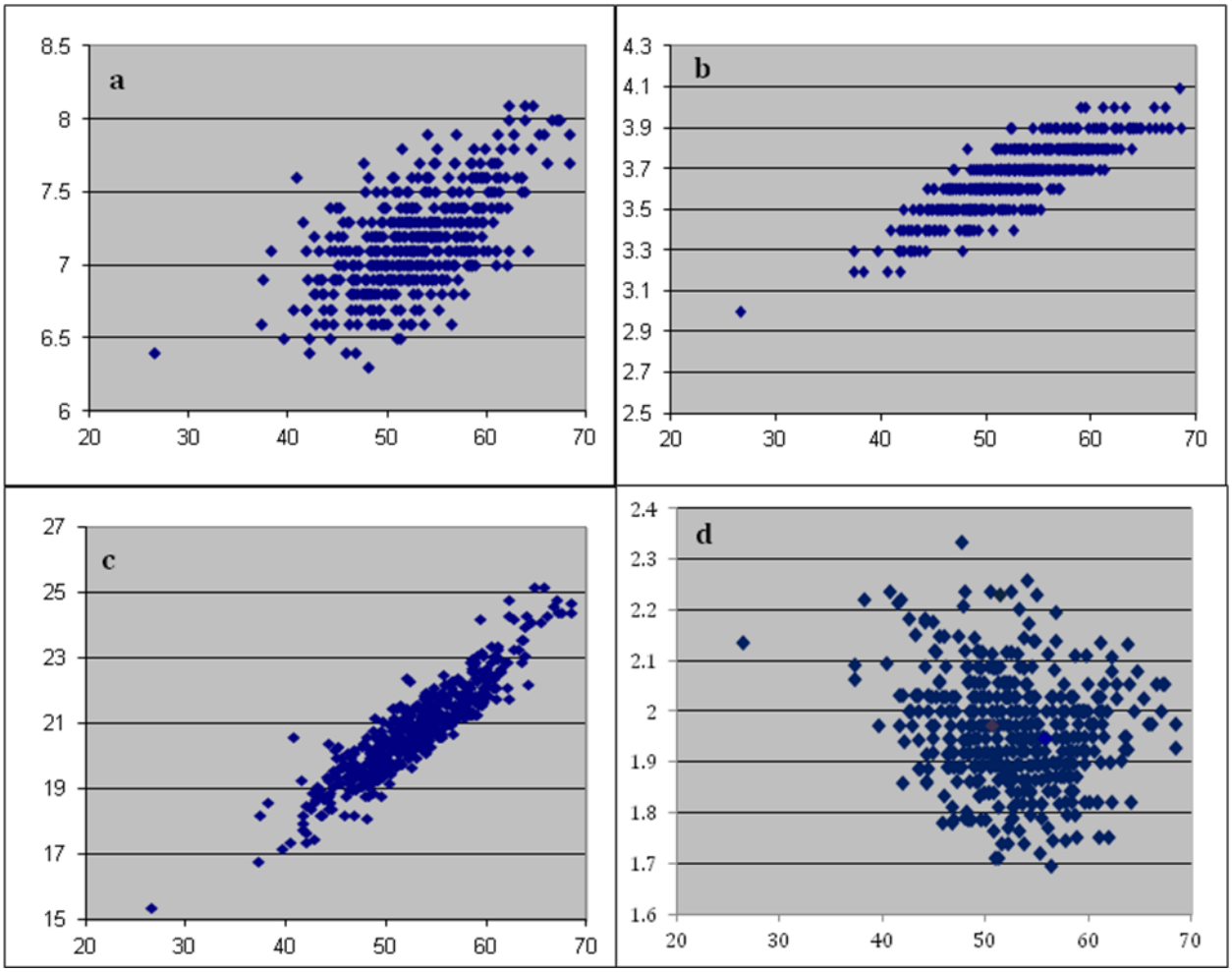


Figure 21. Distributions of physical grain size attributes (a: average grain length L (mm); b: average grain width W (mm); c: average grain area A (mm^2); d: L/W ratio), plotted against average thousand grain weight (g), for 447 BC_1F_3 PaS plant progenies harvested from the 2009 BC_1F_2 nursery.

Correlations between the different grain size characters measured are shown in Table 17. Characters are the same as shown in Figure 21, plus factor form density (FFD).

Table 17. Pearson product-moment correlation coefficients for different morphometric characters measured on the BC_1F_3 PaS plant progenies.

	TGW	Length	Width	Area	L/W
Length	0.615				
Width	0.844	0.286			
Area	0.917	0.810	0.769		
L/W	-0.164	0.622	-0.571	0.066	
FFD	0.809	0.137	0.662	0.530	-0.426

As MARVIN measures the physical dimensions of each grain within a sample, it was possible to look at the distributions for L, W and A, as well as the averages, of each sample. This was done in detail for the four lineages from which most selections had been taken: crosses between Paragon and the donors SHW-144 (PS-144), SHW-181 (PS-181), SHW-218 (PS-218) and SHW-330 (PS-330); Table 18, Figure 22.

Table 18. Average values for morphometric characters within four different PaS lineages.

		Max				Min				Mean			
Lineage	n	TGW	A	W	L	TGW	A	W	L	TGW	A	W	L
PS-144	28	68.4	24.4	3.9	7.7	45.2	20.3	3.5	7.4	52.63	21.26	3.58	7.49
PS-181	35	66.7	24.6	3.9	8	45.5	19.2	3.4	7	54.93	21.36	3.68	7.28
PS-218	40	58.4	21.3	3.8	7	37.2	16.8	3.2	6.6	49.55	19.64	3.62	6.79
PS-330	38	66.1	24.3	3.9	7.7	46.5	19.6	3.6	6.8	55.99	21.26	3.76	7.1

Thousand grain weights (TGW) and average values for grain area (A), width (W) and length (L) are shown for those individuals with the highest TGW (max) and lowest TGW (min) within each lineage, plus the mean value for the entire lineage (mean). n=number of individuals within that lineage.

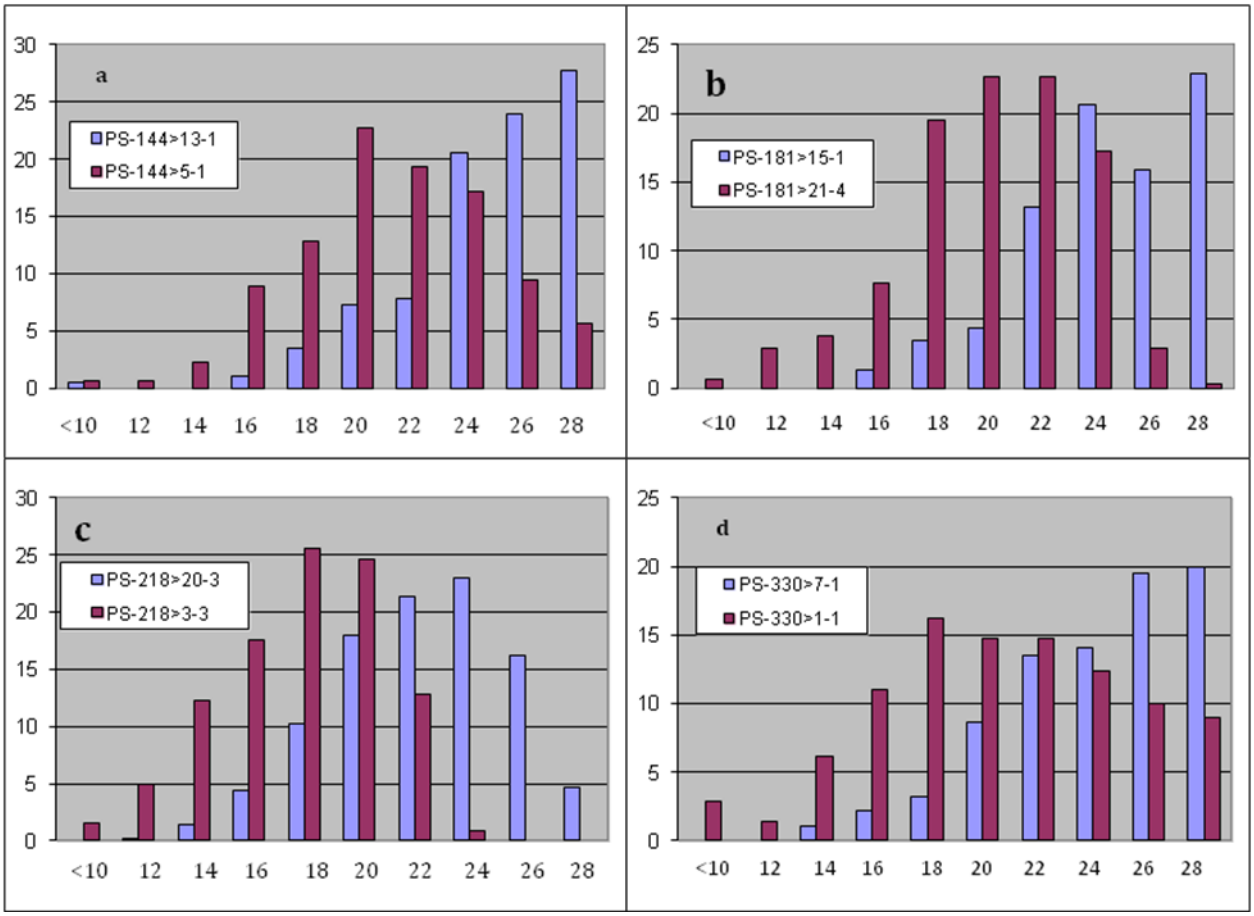


Figure 22. MARVIN frequency distributions for individual grain area within PaS BC_1F_3 grain samples. Histograms (showing % grains against size bins for grain area) are shown for two

individuals within each lineage, representing the minimum (red bars) and maximum (blue bars) TGW, respectively, for BC_1F_3 samples from lineages PS-144 (a) PS-181 (b) PS-218 (c) and PS-330 (d).

The late planting of the 2010 BC_1F_3 nurseries revealed some cryptic, partially-winter types in the Xi-19 background (Figure 23).

Figure 23. Tussock phenotype demonstrating partially vernalised plants within segregating XS BC_1F_3 row, July 2010.

For PaS material, 450 rows were sown (each tracing a single BC_1 plant): six ears were taken from each of 87 rows, and 34 single plants were harvested. These selections traced back to 65 different BC_1 plants, and came from 22 SHW donors. For XS material, 271 rows were planted: six ears were taken from 58 rows, and 4 single plants were harvested, tracing 42 different BC_1 plants and 18 SHW donors. Some recombinants were clearly superior to their parents, especially for key yield components such as ear size and spikelet fertility (Figure 24).

Figure 24. increased ear size and spikelet fertility in BC_1F_3 recombinants from Paragon / SHW-144 (left panel) and Xi-19 / SHW-441 (right panel). In each panel, the recombinant is shown in the centre, with the respective parents to the left (recurrent parent) and right (SHW donor parent).

As with PaS F_2-derived materials, at later generations most of the material retained was either as near-isogenic lines, or to maintain stock seed for other projects. For PaS, 103 BC_1F_4 plots were sown in 2011; six ears were taken from 16 selections, plus six plants, with the selections tracing just 16 BC_1 plants from 8 SHW donors. For Xi-19 material, 52 BC_1F_4 plots were sown, six ears were taken from 21 selections plus one plant; collectively these selections traced 16 BC_1 plants from 9 SHW donors. In 2012, just 4 PaS BC_1F_5 selections were retained, and no XS BC_1F_5 material was harvested.

A number of white-grained lines were noted amongst the PaS progenies of certain white-grained SHW donors, but all XS material was red-grained, regardless of SHW donor.

3.3.3.3.4 Lines derived from PaS BC_1F_2 through SSD inbreeding

PaS SSD inbreeding began with the sowing of 5168 BC_1F_2 seeds, which traced 623 BC_1 plants and originated from 47 SHW donors. Consistent plant loss occurred during SSD inbreeding, and lineages noted in field observations as being probable Paragon selfs were also removed.
The BC_1F_5 nursery of unselected progenies was drilled at NIAB on 19 April 2010, which consisted of 2817 plant progenies which traced 366 BC_1 plants, from 45 SHW donors. Progenies were

planted as half plots (3 rows per progeny, Figure 25) with Paragon, SHW donors and RL wheat varieties interspersed as references. Selection within this nursery was heavily influenced by high temperatures and low rainfall soon after sowing, with temperatures approaching 30°C well before anthesis. Although the high temperatures were not sustained, the dry conditions continued until early grain-fill. When the weather broke, pre-harvest sprouting was noted in susceptible lines, especially those which were white-grained. These conditions placed a stress on the plants which some lines appeared to tolerate more than others (Figure 25). Close examination of the plants within these plots revealed no obvious disease symptoms.

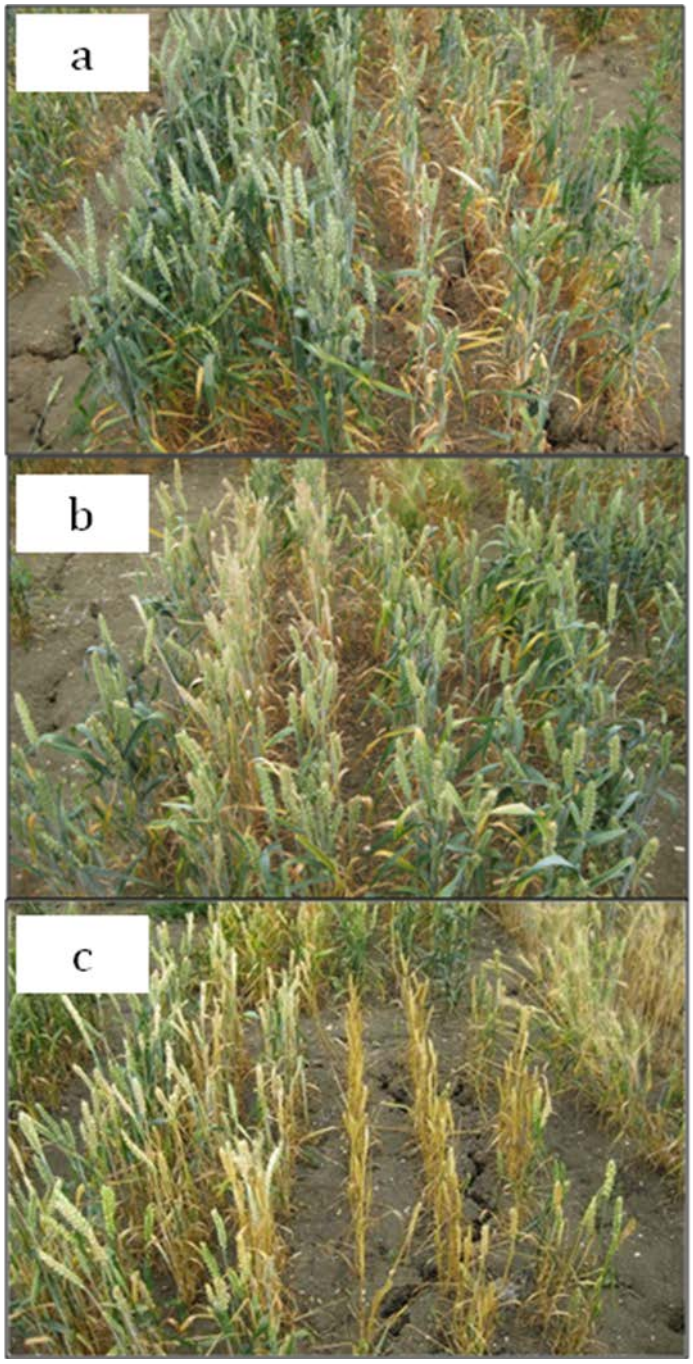


Figure 25. Drought and heat stress in PaS BC_1F_5 material. Each plot contains three rows each of two sister lines. Sister lines originate from the same BC_1 but different BC_1F_2 lineages. (a) PS-079>8, 23 July, (b) PS-144>12, 30 July and (c) PS-008>4, 3 August .

Variation was noted for yield components, in particular spikelet fertility (Figure 26), as observed elsewhere.

Figure 26. Improvements in yield components. Spikelet fertility is increased in PaS BC_1F_5 material (right), relative to the recurrent parent, Paragon (left).

At harvest, 252 lines were carried forwards as six ears, plus 83 single plants. Collectively, these traced 140 BC_1 progenitors and represented 36 SHW. One breeder selected heavily within two populations in particular, but their trials failed in the dry spring of 2011 and the material was dropped. A number of lines were also selected for inclusion in phenotyping trials as part of the BBSRC public sector pre-breeding LoLa (3.3.3.3.7).

In March 2011, 303 BC_1F_6 lines were drilled at NIAB as ear-row families or plant progeny plots within the 'XSPS' nursery which contained material selected from various sources in 2010. One breeder selected heavily within this nursery, taking many lines forward into their own programme. At harvest, 80 lines were taken forwards as six ears, plus five single plants. These traced 59 BC_1 progenitors and represented 19 SHW.

The BC_1F_7 selections were planted in March 2012 at NIAB as ear-row families or plant progeny plots within the 2012 'XSPS' nursery. 27 lines were harvested, tracing 25 BC_1 progenitors and represented 15 SHW. As for other breeding strands, most of these were near-isogenic lines or stocks of material continuing in other projects.

Progress with field selection amongst the PaS lines first tested in the BC_1F_5 nursery is shown in Table 19.

Table 19. Number of field selections made from SSD-derived PaS material, BC_1F_5 onwards.

SHW	BC_1F_5 2010		BC_1F_6 2011	BC_1F_7 2012
	Sown	Selections	Selections	Selections
SHW-008	35	7	2	1
SHW-022	24	1	0	
SHW-036	5	1	1	
SHW-038	47	10	3	1

SHW-048	22	3	0	
SHW-051	40	3	0	
SHW-052	44	7	2	2
SHW-054	23	1	0	
SHW-058	70	8	1	0
SHW-061	23	1	0	
SHW-062	62	7	0	
SHW-063	37	8	4	3
SHW-066	23	1	0	
SHW-079	49	6	2	1
SHW-080	25	6	1	1
SHW-091	58	8	1	0
SHW-093	47	3	1	0
SHW-100	48	3	0	
SHW-109	37	5	0	
SHW-120	15	0		
SHW-143	44	2	1	1
SHW-144	298	40	12	4
SHW-159	55	1	0	
SHW-170	54	9	0	
SHW-173	49	2	0	
SHW-176	78	8	1	1
SHW-181	252	23	8	2
SHW-216	51	3	1	1
SHW-217	40	7	0	
SHW-218	326	26	7	2
SHW-219	35	0		
SHW-232	29	0		
SHW-236	6	0		
SHW-330	317	60	18	2
SHW-339	1	0		
SHW-343	45	0		
SHW-350	40	0		
SHW-354	34	4	1	0
SHW-356	11	0		
SHW-368	26	6	0	
SHW-370	28	2	0	

SHW-372	52	4	3	2
SHW-409	8	0		
SHW-429	22	4	0	
SHW-441	182	39	16	3

3.3.3.3.5 Lines derived from XS BC_1F_2 through SSD inbreeding

XS SSD inbreeding began with the sowing of 6132 BC_1F_2 seeds, which traced 363 BC_1 plants and originated from 30 SHW donors. Consistent plant loss occurred during SSD inbreeding, especially as hybrid necrosis continued to affect plant survival, and lineages noted in field observations as being probable Xi-19 selfs were also removed.

The BC_1F_5 nursery of unselected progenies was drilled at NIAB in mid-March 2011, which consisted of 2847 plant progenies which traced 273 BC_1 plants, from 27 SHW donors. Following the de-selection of the worst types (too tall, too weak, double dwarf, chlorotic, necrotic, aneuploid and very tenacious glumes), 1401 lines were taken forwards as 6 ears and a bulk, which traced 252 BC_1 progenitors and 27 SHW. 1000 of these lines were put into yield trials, tracing 230 BC_1 progenitors and 26 SHW. Each trial consisted of 200 BC_1F_6 lines as single replicate plots, plus multiple replicates of Xi-19 and other varieties according to local best practice.

Yield trials of XS BC_1F_6 lines were planted in October 2011 at five locations (Table 20). The growth of these trials was heavily influenced by the 2011-12 climatic conditions. A mild, open autumn meant crops established well, and a generally mild and very dry winter / early spring kept plant populations high with virtually no losses over winter. From April through to harvest, the weather was cool and very wet, and plots grew very thick. Disease was present in most trials and there was also differential lodging and high grass weed levels (Figure 27), despite robust fungicide, PGR and herbicide programmes. There were also appreciable levels of chlorosis (Figure 28) and sterility (Figure 29) in some plots, and high levels of fusarium head blight. Whilst harvest was delayed due to poor weather, no appreciable pre-harvest sprouting was observed.

Table 20. summary of five yield trials of Xi-19/SHW BC_1F_6 lines, 2011-12

	NIAB Cam	NIAB Her	KWS	Limagrain	RAGT
Control mean[a]	9.02	8.21	7.47	11.19	8.43
Trial mean[b]	7.62	8.35	7.85	9.9	7.92
Max yield[c]	113%	133%	144%	118%	134%
≥ Xi-19[d]	11	115	130	34	72

[a] Mean yield (t/ha) of all Xi-19 plots within the trial

[b] Mean yield (t/ha) of all BC_1F_6 lines within the trial

[c] Yield (expressed as a % of control mean) of highest yielding BC_1F_6 line within each trial

[d] Number of BC_1F_6 lines within each trial which yielded at least as much as the control mean

Figure 27. Differential lodging (a) and poor grassweed control (b) in the NIAB Girton trial, June 2012.

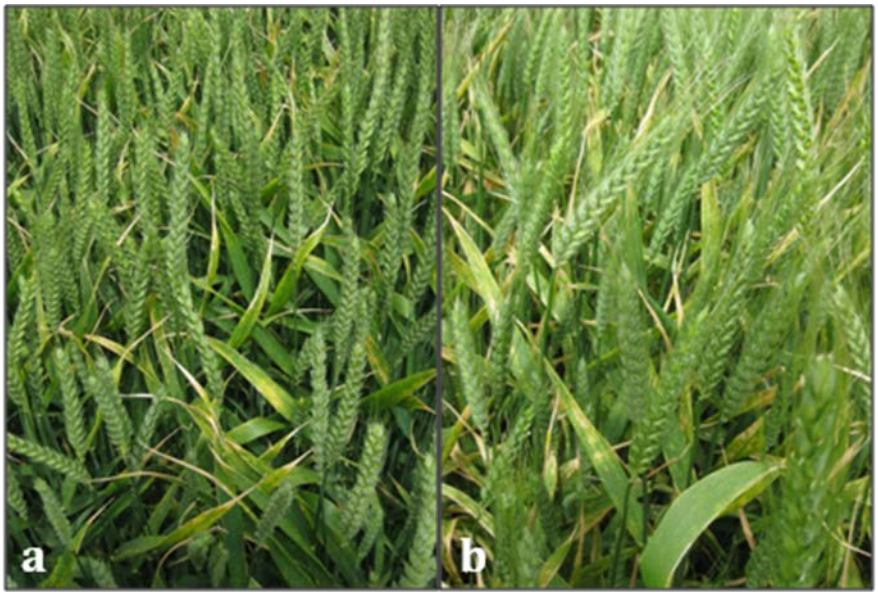


Figure 28. Chlorosis in the NIAB Girton trial, June 2012. a: XS-144>45-12; b: XS>159-6-5.

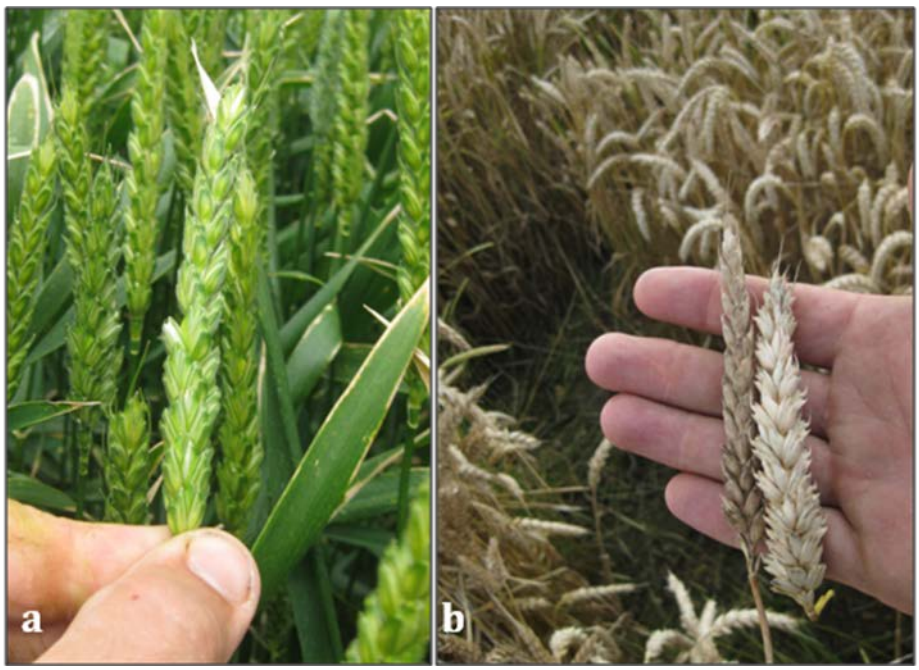


Figure 29. Sterility during early/mid (a,4 July) and late (b, 9 August) grainfill in a single plot within the NIAB Girton yield trial. An ear of Xi-19 is shown for comparison (right, panel b).

Whilst each trial featured 200 different BC_1F_6 lines, with only Xi-19 common to all trials, each trial consisted of equivalent numbers of lines from each SHW donor. For example, 28 Xi-19 / SHW-036 lines were tested in trials: six were included in each of the NIAB Cambridge, NIAB Hereford and KWS trials, and five were included in both the Limagrain and RAGT trials. The mean yield (expressed as a percentage of the control yield) was calculated for lines derived from each SHW donor, in each trial, and the trials were compared in each pairwise combination. There was an overall positive correlation between SHW parental performance in each pair of trials (Table 21).

Table 21. Pearson product-moment correlation coefficients of the mean performance of lines derived from each SHW donor across the five trial sites

	NIAB Cam	NIAB Her	KWS	Limagrain
NIAB Her	0.213			
KWS	0.300	0.145		
Limagrain	0.158	0.691	0.085	
RAGT	0.423	0.041	0.149	0.19

The nursery plots for these lines, plus the 401 lines selected in 2011 but not included in yield trials, were sown in March and were also affected by the poor weather during the spring and summer. High levels of mildew, yellow rust, brown rust and fusarium head blight were observed, despite full fungicide treatment. Seed quality was generally poor, with the thousand grain weights of lines taken forwards typically in the 35-45 g range. Differences between lines were still clear (Figure 30).

Figure 30. Variation in the XS BC_1F_6 nursery, July 2012. Clear differences in height, glaucosity, presence or absence of awns can be observed.

Based upon a combination of yield, agronomics, disease resistance and visual appearance in trials and nurseries during 2011 and 2012, 290 lines were selected to move forwards into multi-location yield, disease and agronomy testing for the 2012-13 season. A number of lines were also selected as near-isogenic lines or for other investigations. Progress with field selection amongst the XS lines first tested in the BC_1F_5 nursery is shown in Table 22.

Table 22. Number of field selections made from SSD-XS material, BC_1F_5 onwards.

SHW	BC_1F_5 2011			BC_1F_6 2012
	Sown	Harvested	Trial	Selections
SHW-036	54	33	28	3
SHW-051	95	54	49	14
SHW-052	17	17	17	5
SHW-061	105	65	58	8
SHW-062	32	24	23	3
SHW-063	84	51	42	8
SHW-100	204	112	63	27
SHW-109	120	69	59	17
SHW-143	52	22	20	3
SHW-144	259	123	64	30
SHW-159	66	40	36	7
SHW-170	106	53	48	14
SHW-216	81	60	54	12
SHW-217	182	104	62	23
SHW-218	169	93	52	17
SHW-219	67	22	20	6

SHW-236	74	42	34	7
SHW-237	37	23	19	7
SHW-264	117	50	42	11
SHW-330	176	101	65	34
SHW-343	45	12	11	1
SHW-350	58	21	20	4
SHW-370	51	26	20	6
SHW-372	8	6	5	2
SHW-409	497	139	59	21
SHW-441	92	39	30	24

3.3.3.3.6 Near-isogenic lines

Near-isogenic pairs of lines, which differ for single characters but otherwise share the same background, have been developed for several characters. The principal focus has been on generating pairs of non-glaucous / glaucous lines from segregating ear rows or plant progenies (Figure 31).

Figure 31. Segregation for the non-glaucous / glaucous character in (a) Xi-19 / SHW BC_1F_3 rows, July 2010 and (b) Paragon / SHW BC_1F_5 plant progenies, July 2010.

Closer scrutiny has identified three distinct phenotypes: non-glaucous ear and leaf; non-glaucous ear, glaucous leaf; glaucous ear and leaf (Figure 32). The fourth possible combination (glaucous ear, non-glaucous leaf) has not been observed. Both recurrent parents, Xi-19 and Paragon, have glaucous ears and canopy (as in Figure 32b).

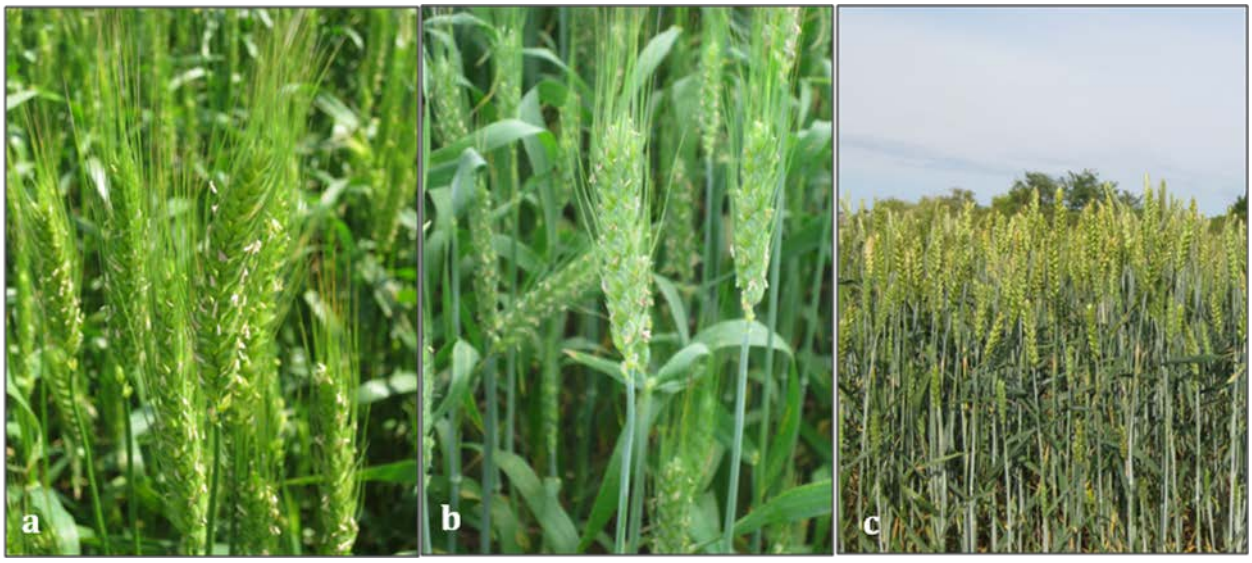


Figure 32. The three distinct glaucosity phenotypes observed in SHW derivatives: non-glaucous ear and canopy (a); glaucous ear and canopy (b); non-glaucous ear, glaucous canopy (c).

Empirically, non-glaucous types appeared to exhibit “stay-green” delayed senescence as previously reported (Figure 20) and also seemed to be more vigorous and robust under heat and drought stress than glaucous types (Figure 33).

Figure 33. Appearance of contrasting non-glaucous and glaucous PaS material grown under heat and drought stress, July 2010. a: BC_1F_3 plants; b: BC_1F_5 material.

Near-isogenic lines for the non-glaucous / glaucous character have been developed using several different SHW donors and in both Paragon and Xi-19 backgrounds. A preliminary yield trial of F_7 and BC_1F_7 PaS lines (Figure 34), drilled in spring 2012, gave very low yields but will be repeated. There were no significant differences in yield for six pairwise comparisons of non-glaucous and glaucous NILs (Table 23); similarly, the yield of all glaucous NILs taken together as a population was not significantly different from that of all non-glaucous NILs (data not shown).

Figure 34. Trial plots of F_7 near-isogenic lines, July 2012. Left: glaucous type, PS-052-3-2-1-1; right: non-glaucous type, PS-052-3-2-1-3. Both lines trace back to the same F_4 plant.

Table 23. Pairwise comparison of yields for non-glaucous/glaucous NILs

Code	Generation	Ear	Canopy	Mean yield (t/ha)	*P*[a]
PS-008-8-4-1-2	F_7	Non-glaucous	Glaucous	2.95	
PS-008-8-4-1-3	F_7	Non-glaucous	Non-glaucous	3.09	0.32
PS-052-3-2-1-1	F_7	Glaucous	Glaucous	3.96	
PS-052-3-2-1-3	F_7	Non-glaucous	Non-glaucous	3.26	0.34
PS-144-3-1-7-1	F_7	Glaucous	Glaucous	2.94	
PS-144-3-1-7-3	F_7	Non-glaucous	Non-glaucous	2.59	0.49
PS-236-4-6-7-2	F_7	Non-glaucous	Non-glaucous	2.24	
PS-236-4-6-7-4	F_7	Glaucous	Glaucous	1.62	0.11
PS-063>5-8-2	BC_1F_7	Glaucous	Glaucous	3.59	
PS-063>5-8-3	BC_1F_7	Non-glaucous	Non-glaucous	3.28	0.13
PS-144>18-1-2	BC_1F_7	Non-glaucous	Non-glaucous	3.89	
PS-144>18-1-4	BC_1F_7	Glaucous	Glaucous	3.86	0.87

[a] Probability based on yield from both replicates, using Student's two-tailed t-test, assuming unequal variance.

Pairs of lines have also been developed for the presence or absence of awns, the presence or absence of hairy glumes (Figure 35) and chlorotic versus normal leaves (Figure 36). These will be tested in future experiments.

Figure 35. Ear from SHW-derivative exhibiting the hairy glume character, June 2012.

Figure 36. XS BC_1F_6 plot segregating for chlorosis, June 2012. Ear-rows (each tracing different individual BC_1F_5 plants, but the same BC_1F_4 plant) appear to have normal (white arrow) or chlorotic (red arrow) leaves.

3.3.3.3.7 WGIN and LoLa trials

XS material was included in WGIN2 subcontractor trials investigating drought tolerance at University of Nottingham for the 2009-10, 2010-11 and 2011-12 seasons. The lines sent are outlined in Table 24. Material from 2009-10 (year 1) was retained after harvest and replanted in 2010-11 (year 2) without purification; similarly material from 2010-11 was retained after harvest and replanted in 2011-12 (year 3). Additional stocks were also sent for testing in years 2 and 3.

Table 24. XS material included in WGIN2 drought tolerance work

Material	Generation[a]	NIAB code	Years tested[b]
XS-036>C06-B	BC_1F_3 bulk	501	1, 2, 3
XS-051>B14-B	BC_1F_3 bulk	510	1, 2, 3
XS-052>B04-B	BC_1F_3 bulk	518	1, 2, 3
XS-052>B07-B	BC_1F_3 bulk	525	1, 2, 3
XS-061>B04-B	BC_1F_3 bulk	536	1, 2, 3
XS-063>A02-B	BC_1F_3 bulk	569	1, 2, 3
XS-100>C16-B	BC_1F_3 bulk	589	1, 2, 3
XS-100>E36-B	BC_1F_3 bulk	609	1, 2, 3
XS-109>B04-B	BC_1F_3 bulk	614	1, 2, 3
XS-109>B09-B	BC_1F_3 bulk	626	1, 2, 3
XS-144>A01-B	BC_1F_3 bulk	637	1, 2, 3
XS-144>F44-B	BC_1F_3 bulk	665	1, 2, 3
XS-159>B07-B	BC_1F_3 bulk	675	1, 2, 3
XS-159>C11-B	BC_1F_3 bulk	677	1, 2, 3
XS-170>B04-B	BC_1F_3 bulk	687	1, 2, 3
XS-159>C11-B	BC_1F_3 bulk	693	1, 2, 3
XS-170>B04-B	BC_1F_3 bulk	697	1, 2, 3
XS-159>C11-B	BC_1F_3 bulk	703	1, 2, 3
XS-170>B04-B	BC_1F_3 bulk	707	1, 2, 3
XS-217>D15-B	BC_1F_3 bulk	714	1, 2, 3
XS-217>E25-B	BC_1F_3 bulk	726	1, 2, 3
XS-217>F35-B	BC_1F_3 bulk	733	1, 2, 3
XS-218>D18-B	BC_1F_3 bulk	749	1, 2, 3
XS-218>D19-B	BC_1F_3 bulk	756	1, 2, 3
XS-264>A02-B	BC_1F_3 bulk	774	1, 2, 3
XS-409>I41-B	BC_1F_3 bulk	815	1, 2, 3
XS-441>B04-B	BC_1F_3 bulk	828	1, 2, 3
XS-036>C06-6-B	BC_1F_4 bulk	3	2, 3
XS-051>B14-3-B	BC_1F_4 bulk	7	2, 3
XS-052>B07-1-B	BC_1F_4 bulk	11	2, 3
XS-100>C14-1-B	BC_1F_4 bulk	35	2, 3
XPS-058-3-19-B	BC_1F_4 bulk	802	2, 3
XPS-181-1-3-B	BC_1F_4 bulk	812	2, 3
XS>052-B04-1	BC_1F_6 bulk	452	3
XS>052-B04-2	BC_1F_6 bulk	453	3

XS>052-B04-3	BC_1F_6 bulk	454	3
XS>052-B04-4	BC_1F_6 bulk	455	3
XS>217-E25-1	BC_1F_6 bulk	1115	3
XS>217-E25-2	BC_1F_6 bulk	1116	3
XS>217-E25-3	BC_1F_6 bulk	1117	3
XS>217-E25-4	BC_1F_6 bulk	1118	3
XS>217-E25-5	BC_1F_6 bulk	1119	3
XS>217-E25-6	BC_1F_6 bulk	1120	3
XS>217-E25-7	BC_1F_6 bulk	1121	3
XS>217-E25-8	BC_1F_6 bulk	1122	3
XS>218-D19-2	BC_1F_6 bulk	1258	3

[a] Generation of material originally sent for testing: material not purified before re-testing

[b] Years 1, 2, 3 correspond to 2009-10, 2010-11, and 2011-12, respectively

A summary of results received to date is shown in Tables 25 (2009-10) and 26 (2010-11). No data have yet been received from 2011-12 trials.

Table 25. Summary of results from 2009-10 WGIN2 trials (data courtesy of Dr J Foulkes).

Material	NIAB code	Height	Above-ground biomass[a] (%)	Grain yield[b] (%)	Harvest index
Xi-19		80.0	100.0	100.0	0.458
XS-036>C06-B	501	88.3	97.6	75.6	0.355
XS-051>B14-B	510	89.7	91.2	76.2	0.383
XS-052>B04-B	518	77.7	86.9	83.9	0.442
XS-052>B07-B	525	70.7	94.0	90.0	0.439
XS-061>B04-B	536	98.0	92.3	84.3	0.419
XS-063>A02-B	569	90.2	113.4	87.9	0.354
XS-100>C16-B	589	76.8	87.0	83.4	0.439
XS-100>E36-B	609	74.3	78.6	74.1	0.432
XS-109>B04-B	614	80.3	83.3	81.8	0.450
XS-109>B09-B	626	79.3	102.9	89.5	0.399
XS-144>A01-B	637	79.7	107.1	86.8	0.371
XS-144>F44-B	665	101.0	88.2	83.5	0.434
XS-159>B07-B	675	98.5	90.0	74.1	0.378
XS-159>C11-B	677	89.3	109.5	72.1	0.302
XS-170>B04-B	687	75.0	79.2	66.4	0.384
XS-216>A02-B	693	78.3	93.2	72.7	0.357

XS-216>B03-B	697	88.3	106.1	96.5	0.417
XS-217>B05-B	703	105.0	88.2	77.0	0.400
XS-217>B06-B	707	81.3	90.7	64.6	0.340
XS-217>D15-B	714	74.8	90.6	84.9	0.431
XS-217>E25-B	726	81.3	92.4	84.6	0.420
XS-217>F35-B	733	84.0	105.0	97.6	0.426
XS-218>D18-B	749	81.7	94.1	95.8	0.465
XS-218>D19-B	756	95.7	118.7	109.8	0.424
XS-264>A02-B	774	76.0	76.8	66.2	0.395
XS-409>I41-B	815	70.0	75.6	77.3	0.468
XS-441>B04-B	828	79.3	114.1	97.3	0.391

[a] Expressed as a percentage, relative to Xi-19 (100%=16.96 t/ha dry weight)

[b] Expressed as a percentage, relative to Xi-19 (100%=7.77 t/ha dry weight)

Table 26. Summary of results from 2010-11 WGIN2 trials (data courtesy of Dr J Foulkes).

Material	Generation[a]	NIAB code	Grain yield[b]
XS-036>C06-B	BC_1F_3 bulk	501	79.1
XS-051>B14-B	BC_1F_3 bulk	510	84.9
XS-052>B04-B	BC_1F_3 bulk	518	107.0
XS-052>B07-B	BC_1F_3 bulk	525	91.6
XS-061>B04-B	BC_1F_3 bulk	536	86.6
XS-063>A02-B	BC_1F_3 bulk	569	86.3
XS-100>C16-B	BC_1F_3 bulk	589	86.6
XS-100>E36-B	BC_1F_3 bulk	609	96.4
XS-109>B04-B	BC_1F_3 bulk	614	90.9
XS-109>B09-B	BC_1F_3 bulk	626	71.7
XS-144>A01-B	BC_1F_3 bulk	637	92.2
XS-144>F44-B	BC_1F_3 bulk	665	79.9
XS-159>B07-B	BC_1F_3 bulk	675	87.0
XS-159>C11-B	BC_1F_3 bulk	677	92.2
XS-170>B04-B	BC_1F_3 bulk	687	79.4
XS-159>C11-B	BC_1F_3 bulk	693	100.5
XS-170>B04-B	BC_1F_3 bulk	697	88.9
XS-159>C11-B	BC_1F_3 bulk	703	91.3
XS-170>B04-B	BC_1F_3 bulk	707	88.6
XS-217>D15-B	BC_1F_3 bulk	714	91.3
XS-217>E25-B	BC_1F_3 bulk	726	109.3

XS-217>F35-B	BC_1F_3 bulk	733	84.2
XS-218>D18-B	BC_1F_3 bulk	749	88.2
XS-218>D19-B	BC_1F_3 bulk	756	90.6
XS-264>A02-B	BC_1F_3 bulk	774	90.0
XS-409>I41-B	BC_1F_3 bulk	815	78.8
XS-441>B04-B	BC_1F_3 bulk	828	89.7
XS-036>C06-6-B	BC_1F_4 bulk	3	73.7
XS-051>B14-3-B	BC_1F_4 bulk	7	88.8
XS-052>B07-1-B	BC_1F_4 bulk	11	83.1
XS-100>C14-1-B	BC_1F_4 bulk	35	87.8
XPS-058-3-19-B	BC_1F_4 bulk	802	88.5
XPS-181-1-3-B	BC_1F_4 bulk	812	74.2

[a] Generation of material originally sent for testing: material not purified before re-testing

[b] Expressed as a percentage, relative to Xi-19 (100%=7.25 t/ha, 15% moisture)

There was little correlation in grain yield between lines tested in both seasons (Pearson product-moment correlation coefficient = 0.005).

Similarly, PaS material was harvested from 2010, 2011 and 2012 nurseries for inclusion in phenotyping trials as part of the BBSRC public sector wheat pre-breeding LoLa.
The material sent is shown in Table 27.

Table 27. Lines for inclusion in LoLa phenotyping trials

Material	2010-11		2011-12		2012-13	
	Generation	Code	Generation	Code	Generation	Code
PS-008-2-1-7	F_6	109	F_7	26	F_8	PAR-01
PS-051-1-6-1	F_6	135	F_7	31	F_8	PAR-06
PS-052-3-2-7	F_6	162	F_7	36	F_8	PAR-11
PS-052-9-1-1	F_6	170	F_7	38	F_8	PAR-12
PS-052-10-4-1	F_6	172	F_7	41	F_8	PAR-13
PS-052-18-6-1	F_6	174	F_7	42	F_8	PAR-14
PS-052-22-1-1	F_6	178	F_7	43	F_8	PAR-15
PS-066-11-2-1	F_6	190	F_7	46	F_8	PAR-21
PS-109-3-5-1	F_6	195	Stop - unstable			
PS-109-3-4-1	F_6	197	Stop - unstable			
PS-109-12-3-1	F_6	203	F_7	47	F_8	PAR-24
PS-109-13-6-1	F_6	205	F_7	48	F_8	PAR-25
PS-144-2-6-1	F_6	230	F_7	51	F_8	PAR-31

PS-218-2-2-1	F_6	290	F_7	59	F_8	PAR-39
PS-218-2-1-1	F_6	292	F_7	60	F_8	PAR-40
PS-236-4-6-7			F_7	64	F_8	PAR-43
PS-330-9-1-1	F_6	351	F_7	72	F_8	PAR-46
PS-330-12-1	F_6	353	Stop - thin plot			
PS-330-15-1-1	F_6	355	F_7	74	F_8	PAR-47
PS-370-2-1-1	F_6	371	F_7	76	F_8	PAR-50
PS-441-1-3-1	F_6	407	F_7	84	F_8	PAR-57
PS-441-7-6-1	F_6	413	F_7	85	F_8	PAR-58
PS-441-7-1-1	F_6	415	F_7	87	F_8	PAR-59
PS-008>2-6	BC_1F_6	429	BC_1F_7	90	BC_1F_8	PAR-04
PS-038>11-7	BC_1F_6	452	BC_1F_7	93	BC_1F_8	PAR-05
PS-052>5-1	BC_1F_6	467	BC_1F_7	94	BC_1F_8	PAR-16
PS-052>9-1	BC_1F_6	473	BC_1F_7	95	BC_1F_8	PAR-17
PS-063>3-2			BC_1F_7	97	BC_1F_8	PAR-18
PS-079>3-4			BC_1F_7	100	BC_1F_8	PAR-22
PS-080>3-6			BC_1F_7	102	BC_1F_8	PAR-23
PS-143>2-2			BC_1F_7	105	BC_1F_8	PAR-26
PS-176>2-1			BC_1F_7	106	BC_1F_8	PAR-34
PS-181>12-4	BC_1F_6	614	BC_1F_7	108	BC_1F_8	PAR-35
PS-181>23-9	BC_1F_6	632	BC_1F_7	112	BC_1F_8	PAR-37
PS-216>8-1			BC_1F_7	114	BC_1F_8	PAR-38
PS-218>19-1	BC_1F_6	682	Stop - unstable			
PS-218>32-4	BC_1F_6	696	BC_1F_7	120	BC_1F_8	PAR-41
PS-218>33-12	BC_1F_6	704	BC_1F_7	121	BC_1F_8	PAR-42
PS-370>13-3	BC_1F_6	724	Stop - unstable			
PS-372>2-13	BC_1F_6	726	BC_1F_7	123	BC_1F_8	PAR-51
PS-372>3-2	BC_1F_6	728	BC_1F_7	124	BC_1F_8	PAR-52
PS-441>22-1	BC_1F_6	786	BC_1F_7	139	BC_1F_8	PAR-61
PS-441>22-12	BC_1F_6	790	Stop - sterility/ergot			
PS-144>5-12	BC_1F_6	842	BC_1F_7	147	BC_1F_8	PAR-27
PS-144>34-3	BC_1F_6	888	BC_1F_7	153	BC_1F_8	PAR-30
PS-330>10-11	BC_1F_6	945	BC_1F_7	160	BC_1F_8	PAR-48
PS-330>13-4	BC_1F_6	961	Stop - bad leaf			

Plot yields are shown from 2010-11 trials at Rothamsted Research (Figure 37, courtesy Dr M Hawkesford) and Sutton Bonington (Figure 38, courtesy Dr J Foulkes).

Figure 37. Grain yields (t/ha) of material grown at Rothamsted under high N (200 kg/ha) and low N (50kg/ha).

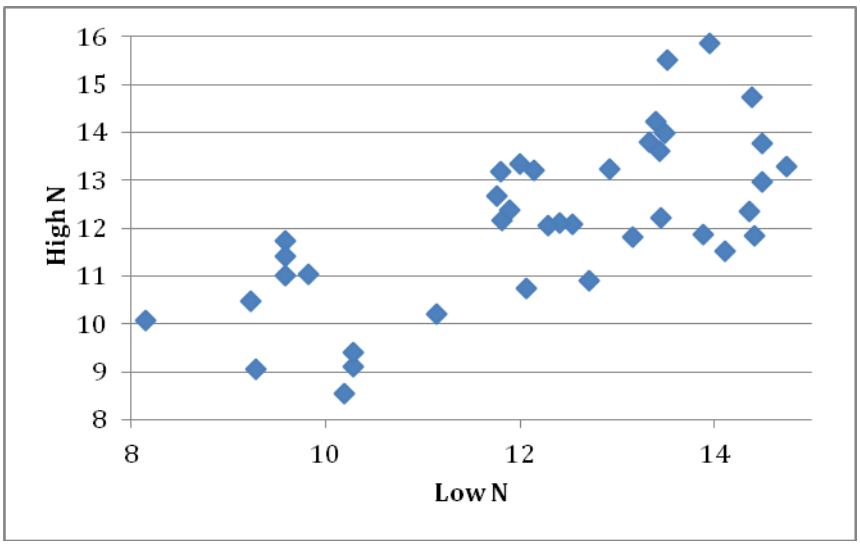
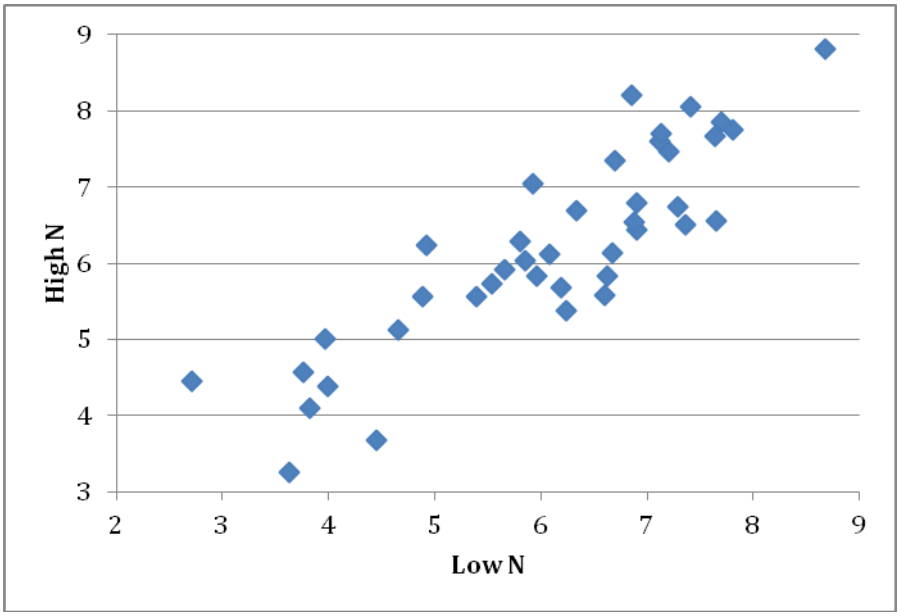


Figure 38. Grain yields (t/ha) of material grown at Sutton Bonington under high N (180 kg/ha) and low N (40kg/ha.



There were strong, positive correlations between yields when all treatments and sites were compared in each pair wise combination (Table 28).

Table 28. Coefficients of correlation between high and low nitrogen treatments and between trials sites at the University of Nottingham (Sutton Bonington) and Rothamsted

	High N RRes	Low N RRes	High N SB
Low N RRes	0.695		
High N SB	0.724	0.603	
Low N SB	0.811	0.597	0.872

3.3.4. Backcross programme based on CIMMYT breeding lines derived from synthetic hexaploid wheat

A total of 84 BC_2F_5 NILs consisting of 42 homozygous full sib allele pairs in the Paragon genetic background were produced (Table 29). Genomic regions from three chromosomes were introgressed; 3B and 7B from the CIMMYT elite durum cultivar CROC_1 and 4D from the *Ae.*

tauschii accession WX224. Regions for introgression were selected on the basis of previous work by Zhang *et al*. (2005) which suggested that SHW alleles detected at specific SSR loci (Table 29) conferred a selective advantage relative to the corresponding bread wheat alleles in CIMMYT SHW-derived varieties. The original intention was test BC_2-derived homozygous full sib pairs of the above introgressions in both the Paragon and Xi-19 genetic backgrounds, however, severe hybrid necrosis greatly reduced the success rate of crosses involving Xi-19 and too few NILs were produced to provide adequate numbers for statistically valid trials.

Table 29. Summary table of BC_2 germplasm developed and field tested from a backcross programme involving the introgression of synthetic wheat derived genomic blocks from CIMMYT germplasm. Genomic blocks for introgression were identified from work published by Zhang *et al.* (2005).

	CIMMYT SHWD donor							
Chrom	**Code**	**CID**	**SID**	**Pedigree**	**SHW donor**	**Genomic block**	**Markers**	**No. allele pairs**
3B	SHWD-5	72726	532	CROC_1/AE.SQUARROSA (224)//OPATA	CROC_1	3B2b	Xgwm0705	4
4D	SHWD-5	72726	532	CROC_1/AE.SQUARROSA (224)//OPATA	*Ae. tauschii* 224	4D1a_1b	Wmc457; Wmc331	4
4D	SHWD-5	72726	532	CROC_1/AE.SQUARROSA (224)//OPATA	*Ae. tauschii* 224	4D1b	Wmc331	6
4D	SHWD-4	72726	531	CROC_1/AE.SQUARROSA (224)//OPATA	*Ae. tauschii* 224	4D1c	Dupw041; Dupw278	4
4D	SHWD-1	3E+05	56	ALTAR 84/AE.SQUARROSA (224)//2*YACO/3/BABAX	*Ae. tauschii* 224	4D1d	Wmc457; DuPw041	10
7B	SHWD-4	72726	531	CROC_1/AE.SQUARROSA (224)//OPATA	CROC_1	7B3a	Xgwm0577	10
7B	SHWD-4	72726	531	CROC_1/AE.SQUARROSA (224)//OPATA	CROC_1	7B3a_4D1c	Xgwm0577; DuPw041	4
							Total allele pairs	**42**

3.3.4.1. Marker-assisted backcrossing

Genomic blocks were positioned on consensus SSR maps (Somers *et al*., 2005; Röder *et al*., unpublished) and flanking markers used to track them through the backcross generations (Figures 39-41). The 4D genomic block (4D1d) was a large introgression of at least 38 cM (it was not possible to define the distal breakpoint) positioned on the long arm, distal to *Rht-D1*. Three sub-fragments, 4D1a, 4D1b, 4D1c, were defined subtended by Wmc457, Wmc331, DuPw041 and DuPw278 (Table 29 & Figure 39). On a telomeric region on the long arm of 3B, two fragments were identified (3B2a and 3B2b) associated with Xgwm4010 and Xgwm0705 respectively; however, insufficient NILs carrying 3B2a were identified for a statistically significant analysis to be carried out (Figure 40). The exact breakpoints were not defined; however, it is likely that fragments are less than 5 cM. On 7B, two telomeric fragments (7B3a and 7B3b) were identified on the long arm associated with Xgwm0577 and Xgwm3119, respectively. Unfortunately, insufficient NILs carrying 7B3a were identified for a statistically significant analysis to be carried out (Figure 41). The exact breakpoints were not defined; however, it is likely that fragments are less than 5 cM.

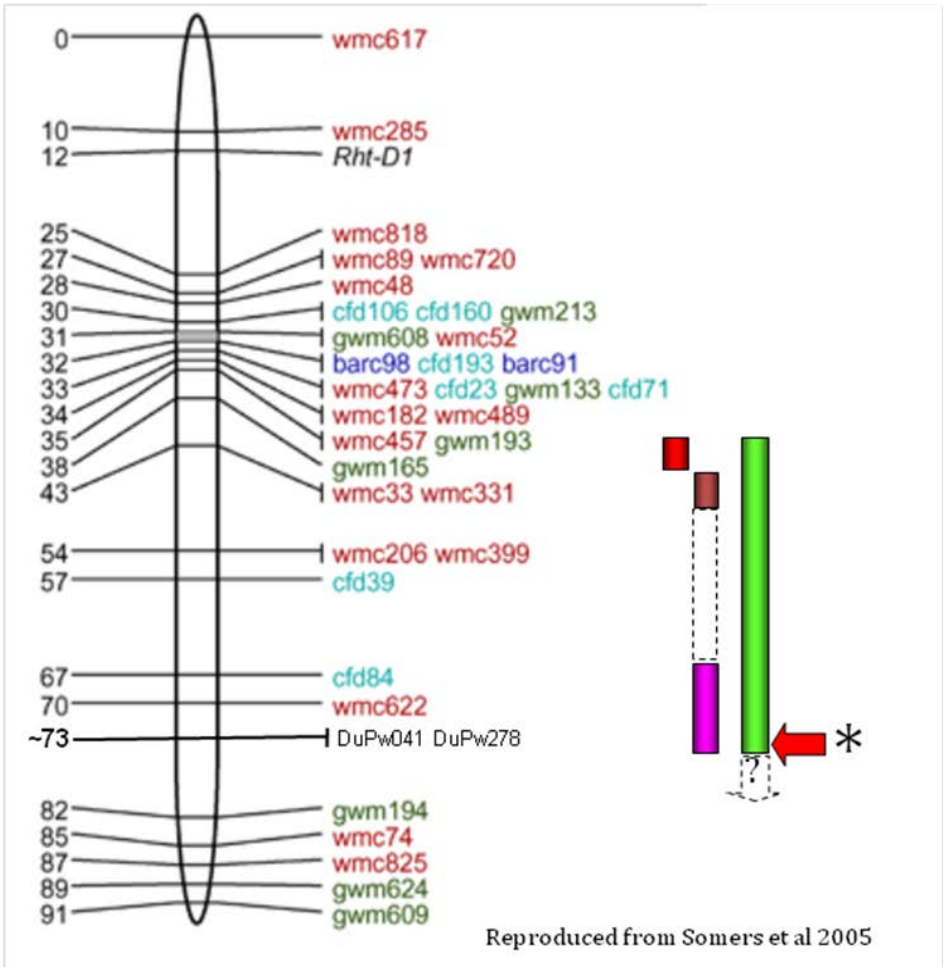


Figure 39. Composite image with chromosome 4D genomic block inherited from *Ae. tauschii* WX224 positioned on the consensus wheat map published in Somers *et al*. (2005). Synthetic wheat derived (SHWD) lines carrying portions of the 4D insertion are presented in the key. Asterisk identifies the position of markers used in backcrossing.

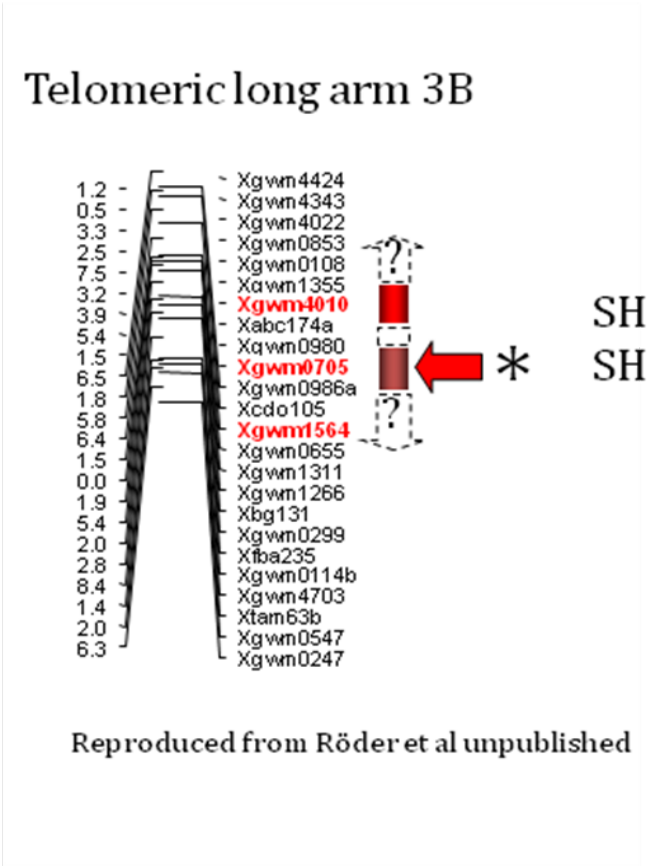


Figure 40. Composite image with chromosome 3B genomic block inherited from *T. turgidum* positioned on the consensus wheat map published in Röder *et al*. (unpublished). Synthetic wheat derived (SHWD) lines carrying portions of the 3B insertion are presented in the key. Asterisk identifies the position of markers used in backcrossing.

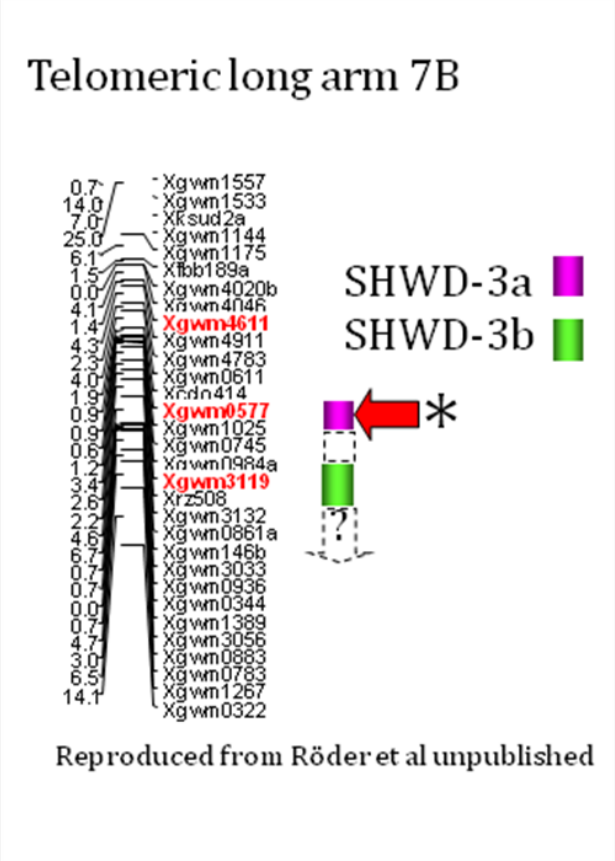


Figure 41. Composite image with chromosome 7B genomic block inherited from *T. turgidum* positioned on the consensus wheat map published in Röder *et al*. (unpublished). Synthetic wheat derived (SHWD) lines carrying portions of the 7B insertion are presented in the key. Asterisk identifies the position of markers used in backcrossing.

3.3.4.2. Yield trial of BC_2F_5 SHW-D NILs at NIAB

In 2012, a two replicate yield trial (randomised incomplete block design) was carried out at NIAB. In the analysis, data from allele pairs was grouped by genomic block and CIMMYT donor cultivar. Within genomic block group, relative to the corresponding recurrent parent (BW) introgression, ANOVA indicated that the small reduction in yield associated with the block inherited from the CIMMYT donor cultivar (SHWD) was non-significant ($P>0.05$) (Figure 42). However, significant differences in yield were observed between the NILs and their CIMMYT donor cultivars. For example, NILs carrying 4D1c and 7B3a inherited singly and in combination from SHWD-4 (Table 29) were significantly ($P<0.01$) higher yielding than the SHWD-4 parent (Figure 42). Similarly, introgression NILs carrying 3B2b and 4D1b inherited from SHWD-5 (Table 29) were significantly ($P<0.001$) higher yielding than the SHWD-5 parent (Figure 42). NILs which were +/- 4D1b inherited from SHWD-5 significantly outperformed Paragon, the recurrent parent (Figure 42).

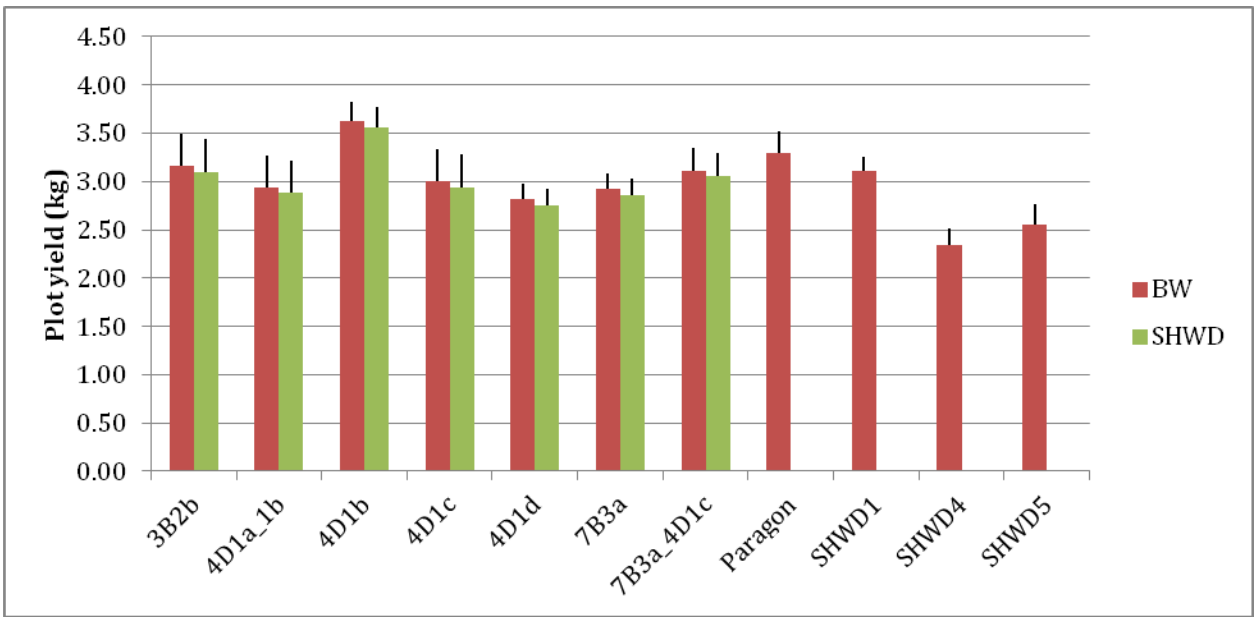


Figure 42. Analysis of mean yield for introgression blocks inherited in the Paragon background from CIMMYT cultivars derived from synthetic wheat. For paired NIL sets, BW refers to NILs carrying the bread wheat allele from the recurrent parent Paragon, whereas SHWD refers to NILs carrying the corresponding introgression from the donor CIMMYT cultivar.

4. DISCUSSION

4.1.1. Experiments with the Ppd *allelic series*

The development of an allelic series of *Ppd* BC_2F_4 lines has provided the opportunity to comprehensively characterise the flowering time effects of a range of PI and putative PS alleles in multiple genetic backgrounds. In the current study, BC_2-derived lines carrying various *Ppd-1* alleles on 2A, 2B and 2D, some of which were previously uncharacterised, were developed and their flowering time effect determined in SP, NP and EP to assess relative allele potency, and the degree of photoperiod (in)sensitivity conferred. Numerous previous authors (Law *et al*., 1978; Worland *et al*., 1996; Dyck *et al*., 2004; Tanio and Kato, 2007) have reported striking reductions in flowering time conditioned by *Ppd-D1a*. In the current study, *Ppd-D1a* was introgressed from two sources: the French cultivar 'Soissons' and the Mexican cultivar 'Ciano67'. The 'Soissons' *Ppd-D1a* allele had a potent flowering time-reducing effect across backgrounds and photoperiod treatments, whilst the same allele from 'Ciano67' had a similar effect in 'Alchemy' in NP (NIAB) but was significantly less potent in all other treatments, especially in the 'Robigus' recipient background. It is possible that the 'Ciano67' source carries tightly linked gene(s) or unexplored functional variation at *Ppd-D1* that specifically interact with loci in 'Robigus' to retard flowering, a phenomenon that has not been previously reported for this variety. This could be investigated by intercrossing 'Robigus (Ciano67 *Ppd-D1a*)' with 'Robigus (Soissons *Ppd-D1a*)'.

The *Ppd-A1a* allele from SHW_131 and SHW_173 ('GS-105' type) gave a consistent early flowering time phenotype in SP and NP. There are no previous reports on the effect of this durum-derived allele in bread wheat under field conditions, although Bentley *et al*. (2011) showed BC_4F_2 NILs for the 'GS-100' *Ppd-A1a* allele (not assessed in the current study) in the spring wheat 'Paragon' to have an intermediate effect on flowering compared to the stronger *Ppd-D1a* and weaker *Ppd-B1a* alleles in a controlled SP (10 hr). The 'GS-105' *Ppd-A1a* allele (introgressed in the current study) was reported to have a flowering time effect approximately equal to the 'Timstein' type *Ppd-B1a* allele and weaker than the 'GS-100' *Ppd-A1a* allele (Bentley *et al*., 2011), a result consistent with observations in durum wheat (Wilhelm *et al*., 2009; Maccaferri *et al*., 2008). The performance of the 'GS-105' *Ppd-A1a* allele across photoperiod treatments in the current study indicates that it is closer in strength to the *Ppd-D1a* allele than to *Ppd-B1a* in a hexaploid wheat background. Furthermore, in the NP (KWS) comparison, SHW_131 *Ppd-A1a* lines (2 observations only) were significantly earlier to reach GS39 than *Ppd-D1a* lines, suggesting a potentially different mode of action i.e. enabling a relative increase in the length of the construction phase (from GS31) of ear development. Again, this is a question that can be investigated further using the developed germplasm. *Ppd-A1a* is, therefore, a potent novel source of earliness for hexaploid bread wheat and it is interesting to consider whether this allele (and also the 'GS-100' *Ppd-A1a* allele) offers a flowering and/or yield advantage over existing PI alleles on 2B and 2D,

either individually or in combination. The availability of an additional strong PI allele may provide previously unavailable flexibility in manipulating flowering time. The proximity of the reduced height gene *Rht-8* gene to *Ppd-D1a* means that earliness is often achieved in combination with reduced height (Sip *et al*., 2010). Although the two loci are reportedly 20 cM apart, this linkage is rarely broken in varieties (Gasperini, 2010). The availability of a PI allele of comparable strength on a different chromosome could be utilised in situations where *Rht-8* is not required. For example, Sip *et al*. (2010) reported that when *Rht-8* is combined with other *Rht* genes, the resultant reduction in stature can be too severe in areas that experience heat stress during ear emergence, resulting in a reduction in spike fertility. *Ppd-A1a* could also be used in situations where earliness from *Ppd-B1a* is not suitable because of linkage with undesirable alleles of other genes on 2BS such as the hybrid necrosis gene *Ne2* (Chu *et al*., 2006), the *Vir* gene controlling viridescence (Simmonds *et al*., 2008) or resistances to orange wheat blossom midge (Thomas *et al*., 2005), soil-borne cereal mosaic virus (Bayles *et al*., 2007) or leaf rust (McCartney *et al*., 2005).

In the current study, the *Ppd-B1a* alleles from 'Chinese Spring' (4 x *Ppd-B1* haploid copy number) and 'Timstein' (3 x *Ppd-B1* haploid copy number) conditioned significant reductions in flowering time in both backgrounds, although their effect was not as potent as that of *Ppd-D1a* and *Ppd-A1a*. This is in agreement with previous observations (Diaz *et al*., 2012; Bentley *et al*., 2011; Worland, 1996). In the SP experiment in the 'Alchemy' background, the 'Chinese Spring' *Ppd-B1a* allele (x 4 copy number) had a significantly stronger effect on reducing flowering time compared to the 'Timstein' *Ppd-B1a* allele (x 3 copy number) which contrasts with the results of Diaz *et al*. (2012), suggesting a background effect. In the EP experiment, the 'Chinese Spring' *Ppd-B1a* lines significantly reduced time to GS59 but not to GS39. Diaz *et al*. (2012) observed differences in *Ppd-B1* expression affecting flowering time in copy number variants including 'Chinese Spring' and it is possible that the expression of PI is activated only once the plant has initiated double-ridge formation. The lack of earliness in the NP treatment suggests that while the allele confers insensitivity in SP and EP, it may have additional sources of latent sensitivity. Gonzalez *et al*. (2005) has previously reported *Ppd-B1a* NILs as having a reduced measurable effect on flowering in natural photo-thermal conditions. Despite this, the *Ppd-B1a* alleles assessed in the current study are a robust source of moderate earliness for use in fine-tuning flowering time.

In the NP and EP experiments, all BC_2F_4 lines without a PI allele were sensitive to photoperiod, that is, they flowered earlier in EP than in NP. Lines with a PI allele also flowered earlier in EP, with a further reduction conditioned by the specific PI allele, indicating they show a reduced sensitivity compared to the wild type, or, alternatively, there could be added effects of PS alleles on the other genomes when the photoperiod is extended. These findings contrast with those of Gonzalez *et al*. (2005) who reported that *Ppd-D1a* NILs were totally unresponsive when extended day length (+6 hr) was applied from the terminal spikelet to anthesis stage. However, the same authors reported

that the length of the late reproductive phase was reduced in both *Ppd-B1a* and wild-type plants under extended day-length as was found in the current study with 'Timstein' *Ppd-B1a* BC_2F_4 lines. These contrasting results could be explained by differences in the timing of day-length extension; day-length was extended in this study right through the period of short days from mid-winter, but only at the late reproductive phase in the Gonzalez study. Therefore, it is possible that *Ppd-D1* is sensitive to day-length earlier in development, e.g. prior to the terminal spikelet stage. Additionally, the 'Chinese Spring' *Ppd-B1a* PI allele lines in 'Alchemy' had reduced sensitivity only after GS39. This is in contrast to the work of Tanio and Kato (2007) who reported both *Ppd-B1a* and *Ppd-D1a* alleles accelerating initiation of the double-ridge stage and subsequent spike development (Tanio and Kato, 2007). It is possible that a difference at the double ridge stage is most readily detected for strong alleles compared to weaker (i.e. *Ppd-B1a*) alleles.

The lines created in this study will facilitate further study of the control of photoperiod (in)sensitivity at specific developmental stages with a view to enabling fine-tuning pre- and post-anthesis development to cater to specific environments.

The influence of PI alleles in combination was not examined in this study but parallel work in a spring wheat background shows that combining *Ppd-1a* alleles on different genomes enhances the early flowering phenotype (Shaw *et al*., 2012). This makes it of interest to test *Ppd-1a* combinations further. Previous reports (Hanocq *et al*., 2004) on the earliness conferred by 'Récital' (*Ppd-B1a* + *Ppd-D1a*) have described a highly epistatic but incomplete relationship between the two alleles which should be considered in future NIL development. It has previously been anecdotally observed that 'Soissons' (*Ppd-D1a* only) flowers earlier under UK field conditions than 'Récital' (*Ppd-B1a* + *Ppd-D1a*), suggesting there are other factors in the background of 'Récital' that delay flowering, or that suppression factor(s) similar to those hypothesised for 'Ciano67' retard the effect of *Ppd-D1a* on flowering. Clearly, the effects of individual and combined *Ppd-A1a*, *Ppd-B1a* and *Ppd-D1a* alleles should be assessed in more detail, and this will benefit from the germplasm resources developed in the current study. Stacking of the alleles through intercrossing BC_4 NILs will allow further characterisation of flowering phenotypes and other traits of interest, particularly yield, across environments and production systems.

Ppd-D1a, *Ppd-A1a* and *Ppd-B1a* alleles have been shown to reduce flowering time in SP, NP and EP in the current study. Although the magnitude of the response could be broadly summarised as *Ppd-D1a>Ppd-A1a>Ppd-B1a,* this varied according to allele donor, recipient background, photoperiod treatment and growth stage assessed. There was also evidence of additional effects modifying the effectiveness of *Ppd-1a*. As a result, it is necessary to consider background effects in order to regulate flowering and adaptation as effectively as possible. The phenotyping data and germplasm developed in this study will allow for additional precision in breeding hexaploid wheat

with specific flowering time adaptation.

The assessment of flowering time in both 2001 and 2012 provided the opportunity to assess the genotype-by-environment component of variance for each gene allele. ANOVA indicated that variance attributable to G x E effects was non-significant indicating the flowering response of each NIL set was highly reproducible over years. Authors of previous field studies of *Ppd-D1* near iso-genic lines have reported the gene effects to be highly reproducible over years (Worland & Sayers, 1995; Börner *et al*., 2006); however, less is known about the reproducibility of PI allele effects on the A and B-genomes. The current study also compared different donor sources of *Ppd* alleles on all three genomes (Soissons & Ciano-67 for *Ppd-D1a*; Chinese Spring & Timstein for *Ppd-B1a* and SHW-173 & SHW-131 for *Ppd-A1a*) finding that in all cases, the different sources were comparable in effect within and between years indicating that variants on all three genomes condition a similar effect over multiple genetic backgrounds.

In 2012 at NIAB, plot yields were recorded on the same replicated trial used to assess flowering-time. The majority of NILs carrying the PI allele (with the exception of *Ppd-D1a* donated from Recital in the Alchemy background, *Ppd-B1a* donated by Chinese Spring and Timstein and *Ppd-A1a* donated by synthetic wheat line SHW-131 in the Robigus background) significantly out-yielded lines carrying the corresponding wild-type alleles from the recipient. Focussing on NILs where a significant yield difference was observed, PI alleles donated from Soissons, Ciano-67, Chinese Spring, Timstein and SHW-173 all conditioned significant reductions in flowering-time relative to the recurrent parents (see above), therefore, it can be concluded that reduced yield in this instance is associated with the later-flowering, wild-type allele. This finding contrasts with results from previous studies in which the shorter life cycle conferred on cultivars carrying the PI allele at *Ppd-D1* was reported to incur a yield penalty in north-western European environments where later flowering genotypes are better able to exploit the longer growing season (Worland & Sayers, 1995; Snape *et al*., 2001). In view of the existing strong evidence that genotypes with PS alleles at *Ppd-D1* and *Ppd-B1* tend to out-yield genotypes with PI alleles, it can be concluded that contrary evidence from the current study may have resulted from unusual weather conditions during the 2011-12 growing season. The relatively high yield of early types compared to late types was also characteristic of variety trials across the UK in 2011-12 (HGCA, 2012).

Analysis of publicly available meteorological data for this period from the weather station at NIAB (http://www.metoffice.gov.uk/climate/uk/stationdata/) indicated that whilst average monthly temperature ({max + min} / 2) appeared to be broadly similar to the 1981-2010 average, rainfall from sowing through to March was significantly below average followed by unusually high rainfall particularly during April, June and July (Figures 43 & 44). Dry conditions during the early construction phase of crop development and subsequent heavy rainfall during flowering and grain

fill may have favoured PI NILs over their PS counterparts. In addition to a dry early season, reduced light densities accompanying persistent rainfall later in the season may also have favoured early-flowering ideotypes since they would have been able to complete grain-fill early, reducing the impact on yield.

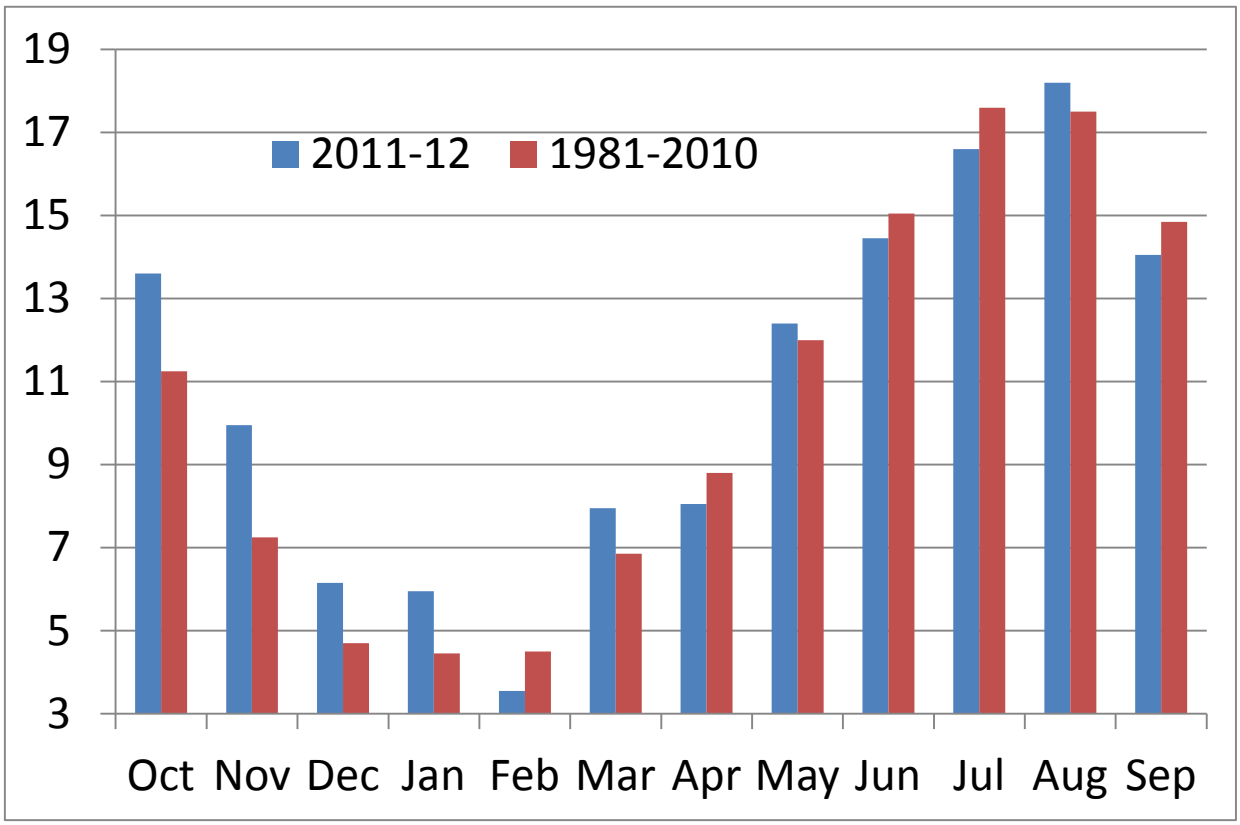


Figure 43. Comparison of 2011-12 and long-term average temperatures (°C; data obtained from the UK Meteorological Office).

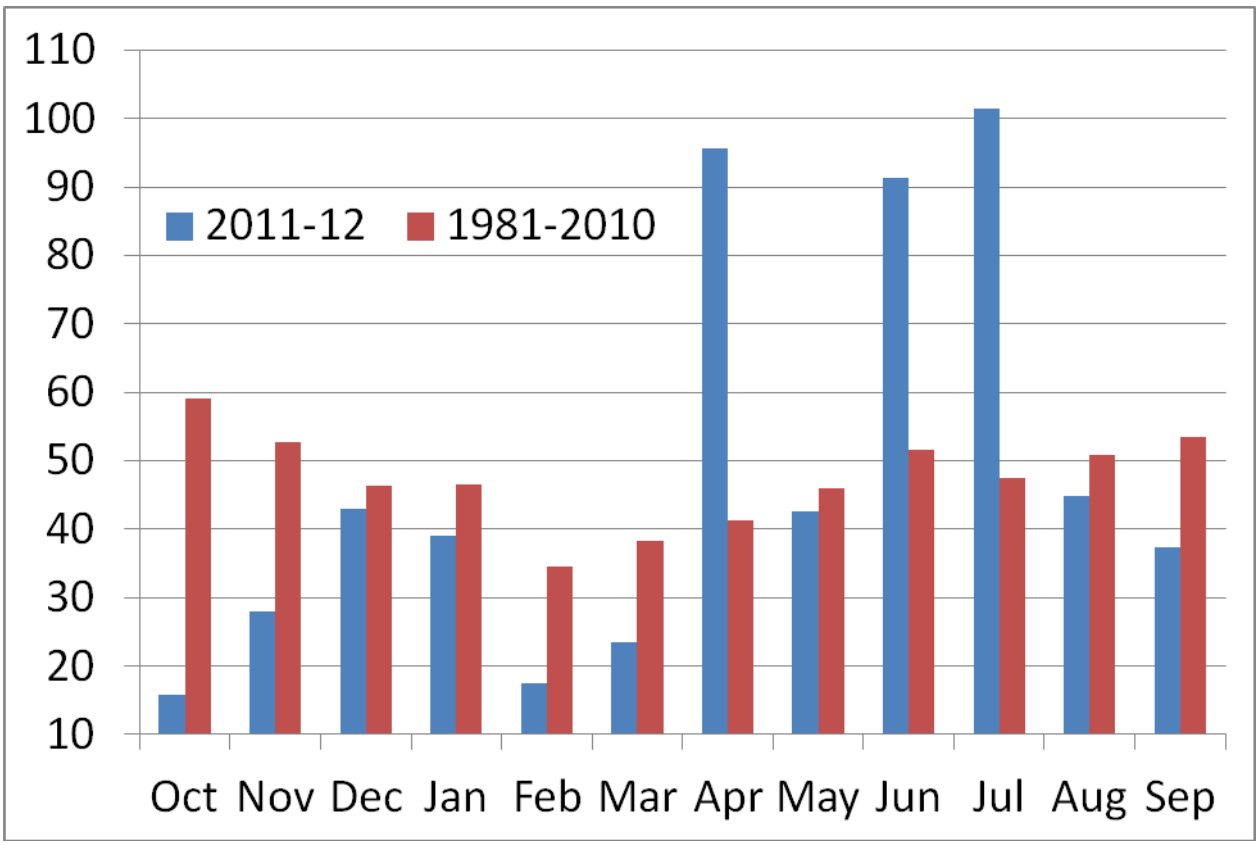


Figure 44. Comparison of 2011-12 and long-term rainfall (mm; data obtained from the UK Meteorological Office).

4.1.2. Experiments with Eps *QTL-NILs*

All experiments to determine flowering time (FT) effects of earliness *per se* (*Eps*) QTL were carried out on BC_2 derived near iso-genic lines (NILs). In a similar approach to that used for the *Ppd* allelic series (see above), up to eight NILs carrying contrasting early and late alleles were developed for each of ten *Eps* QTL. Five NILs were developed from each of two doubled haploid populations, Charger x Badger (CB prefix) and Spark x Rialto (SR prefix). Regression analysis of replicated sets of NIL pairs was used to identify and remove variance associated with genetic background interaction. BC_4 NILs have since been developed, and are available for further analyses beyond the end of the project.

QTL were identified using data from previous studies carried out by project partners at the John Innes Centre in Norwich (reviewed in Snape *et al*., 2001; meta-QTL analysis by Griffiths *et al*., 2009). Although the accepted definition suggests that *Eps* loci reduce the time to floral transition regardless of prevailing conditions (reviewed in Cockram *et al*., 2007) it appears from previous studies carried out by project partners that environment has a significant influence on the expression of *Eps* QTL over experimental years. Such fluctuation in relative potency has led some authors to question whether the influence of *Eps* on developmental rate is truly independent of environmental influence (Colasanti & Coneva, 2009). In contrast, *Eps* effects on FT in the current

study were found to be relatively reproducible over experiments and years with a consensus emerging as follows (derivative doubled haploid population followed by chromosomal location of QTL): SR-1D>SR-3A>SR-7A>SR-3B>SR-6B>[CB-3A, CB-3B, CB-6A, CB-6B & CB-7A]. In the current study, there has been no attempt to dissect the developmental phenology, e.g. leaf production rate (phyllochron), final leaf number or record developmental phase transitions occurring at primordia. Such in-depth assessments have been reserved for future experiments on BC_4 NILs where the influence of genetic background effects is less likely to mask subtle differences.

In lieu of detailed phenological assessment, differences in expression at the phenotype level were investigated in field and controlled environment experiments by recording FT at three floral growth stages, GS39 (flag leaf fully emerged), GS55 (spike half emerged from flag leaf) and GS61 (start of flowering). Authors of previous studies of photoperiod response (*Ppd*) NILs observed a differential in sensitivity to photoperiod associated with *Ppd-D1* and *Ppd-B1* loci by recording FT at early and late during floral development (Gonzalez *et al*., 2005). With *Eps*, no photoperiod sensitivity was expected, however, the intention was to identify whether different *Eps* loci might be increasing developmental rate during the vegetative or floral stages, or both. In addition to being day-length neutral, *Eps* loci condition relatively small reductions in developmental rate of between 1-3 days (Griffiths *et al*., 2009). However, it has been reported that exposing plants to continuous long days (16hr photoperiod) can significantly increase the FT differential associated with *Eps* (Lewis *et al*., 2008). With the above factors in mind, three experimental approaches were deployed to investigate the effect of *Eps* loci on FT at three different floral growth stages, (1) extended photoperiod (EP) in controlled environments, (2) extended photoperiod under field conditions and (3) field experiments under natural photoperiod (NP).

Prior to intensive field assessment, an initial glasshouse experiment was carried out under EP to, (1) valorise NILs by ensuring that QTL effects had been retained during backcrossing, (2) determine the maximal FT reducing effect of individual *Eps* loci and (3) to confirm whether loci identified in previous field-based QTL mapping experiments had the same relative potency in controlled environments. Compared to QTL discovered in the Charger x Badger doubled haploid population (that were found to have little or no influence on FT) with the exception of SR-3B, Spark x Rialto derived QTL all had a highly significant FT reducing effect. Relative FT effects (at GS55) of individual SR NILs in a subsequent growth chamber experiment under EP corresponded well with results from the glasshouse confirming SR-1D as the most potent effect followed by either SR-7A (growth chamber) or SR-3A (glasshouse). Relative potency of SR loci in field experiments (at GS55) in 2011 and 2012 for SR loci also correlated well between years with SR-1D>SR-3A>SR-7A; although, SR-3B and SR-6B were only significant in 2011. The influence of Charger x Badger derived loci was found to be much more variable. In the glasshouse experiment and in the 2012

field experiment, no FT reducing effect was observed. However, the 2011 field experiment, CB-3A reduced FT by 2 days with CB-3B and CB-6B having a borderline effect. From these results, one can conclude that SR QTL have a stronger FT reducing effect than CB. Interestingly, when the FT of parental cultivars are compared with their corresponding NILs, regardless of allele, several NILs flower significantly later, e.g. CB-3A and CB-3B. Since differences in *Ppd* allele can be discounted (all parents carry the wild type allele), later flowering in these NILs is probably the result of a linked locus conditioning late flowering inherited from the late allele parent. Unfortunately, such linkage drag may not be overcome through continued backcrossing if the locus is very tightly linked.

For SR loci, FT effects were observed at different floral growth stages in the growth chamber and field. In the growth chamber, with the exception of SR-3B (which had a non-significant effect at all growth stages) the greatest effect was observed at GS55 for SR-1D, SR-3A and SR-7A. The difference was highly significant for SR-3A and SR-7A, but marginal for SR-1D, suggesting that SR-1D was active during both vegetative and floral growth stages. This result goes some way to explain why SR-1D has the most consistent and significant effect on FT of the loci included in current study. In the field, the GS55 FT effect was consistently the most significant; although much less pronounced overall, SR-1D was again found to have a significant FT reducing effect at all three growth stages. These results suggest qualitative and/or quantitative differences in the influence on FT of different *Eps* loci. *Eps* loci are numerous and highly dispersed around the wheat genome, suggesting that they are likely be structurally heterogeneous (Griffiths *et al*., 2009). Therefore, it is not surprising that their mode of action on FT is also variable.

At KWS, an EP experiment was carried out on all ten QTL NILs. Surprisingly, in view of similar experiments in controlled environments, no FT effect was observed in either EP or NP treatments. One explanation for this could be attributed to the necessarily small scale of this experimental setup due to space constraints under the lighting system. Previous controlled environment experiments confirmed that EP stretches out the FT effects of *Eps*; however, under field conditions, *Eps* effects are significantly reduced overall. In the KWS experiments, NILs were sown as single rows with two replicates. In many cases, the small number of plants in each replicate made it difficult to determine FT since plant-plant spacing was high, possibly reducing the magnitude of potential FT differences.

In 2012, plot yield was recorded on *Eps* NILs (Figure 7). Results from this trial carry a significant caveat however, since the plot size was small ($1m^2$) and the sowing rate was not uniform across plots. Bearing this in mind, some potentially interesting effects were observed. For all NILs, both SR and CB derived, the late allele was associated with a slightly reduced yield of borderline significance. This runs against the accepted convention that the late types tend to out-yield the early types in temperate environments like the UK that lack the terminal drought experienced in

southern Europe (Worland, 1996; Worland & Sayers, 1995). However, as previously noted, the relatively high yield of early types compared to late types was a characteristic of variety trials across the UK in 2011-12 (HGCA, 2012). Looking across and between SR and CB derived NIL sets, a more statistically significant pattern of differences was observed. All CB derived NILs carried early alleles from Charger backcrossed into the genetic background of Badger: no significant yield differences were observed between lines or between NILs and Badger. In contrast, significant differences between NILs were observed for SR derived lines. However, this can easily be explained by the yields of Spark (lower) and Rialto (higher). For example, all NILs carrying an early allele from Rialto backcrossed into a Spark genetic background had a similar yield to Spark. Similarly, the two NILs carrying the early allele from Spark backcrossed into a Rialto genetic background had a similar yield to Rialto. This observation does, however, have a negative impact on the validity of cross comparisons of yield, and possibly FT, effects of potentially allelic *Eps* loci identified in both populations and for assessments of relative potency of different *Eps* loci.

4.1.3. CIMMYT synthetic wheat backcross programme

This programme of work represents a systematic evaluation of the breeding potential of SHWs from the CIMMYT collection, particularly focused on the intensive crop production systems common in the UK. In this respect, the input from leading UK-based commercial wheat breeders has been invaluable. Some of the pre-breeding material developed has already passed into breeders' own programmes, as potential varieties or crossing parents. A collaborative project has been funded through the BBSRC Follow-On Fund (BB/K020269/1) which will explore Xi-19 / SHW selections through a matrix of yield trials, disease nurseries, end-use quality evaluation and other tests, in order to identify the very best lines, and enter these into National List trials with a view to eventual commercialisation.

The two recurrent parents, Paragon and Xi-19, are both Group 1 (high quality) breadmaking varieties. Paragon is a leading spring breadmaking wheat, and a number of mapping populations, near-isogenic lines, mutant collections and other genetic resources have been developed in this background arising from work within the DEFRA Wheat Genetic Improvement Network (http://www.wgin.org.uk/, Derkx *et al*., 2012) and other studies (Al-Kaff *et al*., 2008, Diaz *et al*., 2012). Xi-19 is a facultative or alternative wheat which came through HGCA RL trials as an autumn-sown variety. Whilst it is no longer widely grown, it represents a key link in the UK pedigree as the parent of several current RL varieties, mainly within the breadmaking class. Whilst true winter types could be regarded as being more relevant to UK commercial wheat breeding, the use of spring or facultative varieties facilitated more rapid advancement through early generations.

There is a wealth of literature outlining the value of lines derived from CIMMYT SHWs, especially in the low-input cropping systems of Africa, Asia, and Australia (reviewed by van Ginkel and

Obgonnaya, 2007). In particular, SHW-derivatives appear to perform well under abiotic stresses such as drought (Reynolds *et al*., 2007), heat (Yang *et al*., 2002), salinity (Obgonnaya *et al*., 2005), and waterlogging (Villareal *et al*., 2001), in addition to conferring high yields *per se* (Villareal *et al*., 1994). In China, breeding with the CIMMYT SHWs began in 1995, resulting in improvements to yield components (grain size and ear weight) and yellow rust resistance. The first high-yielding SHW-derived variety, 'Chuanmai 42', was released in Szechuan province in 2003; SHW derivatives now take a significant proportion of the Chinese wheat acreage (Yang *et al*., 2009). In Europe, the CIMMYT SHW-derivative 'Carmona' was registered in 2003, as a short-cycle type well-suited to zero-tillage, low input situations in Southern Spain (CIMMYT, 2004).

At the outset, it was clear that the full collection of 448 SHWs was too unwieldy to work with in detail. The full set was sown and multiplied up, and a subset of 50 SHWs which appeared to be representative of the diversity within the full set was selected, largely based upon information gathered using a set of informative SSR markers. This set of 50 sampled the diversity clusters from the dendrogram generated using the SSR data, with at least one SHW drawn from each cluster. Some clusters were investigated in more detail: for example, SHW-060, -061, -062 and -063 all share the same pedigree (Croc_1 / Wx224); and SHW-216, -217, -218 and -219 similarly all come from a single cross (Ceta / Wx895). Differences between SHWs within each of these two clusters might reflect heterogeneity in the tetraploid and diploid progenitors (with different alleles being transmitted to the resulting SHWs), changes in gene expression arising from genomic shock (McClintock, 1984), outcrossing in later generations, or a combination of all three. Some of our results are suggestive of mistakes in the pedigree records, whilst others indicate the unexpected presence of known bread wheat alleles at *Ppd-D1* (Appendix 2) and *Glu-1* loci (Appendix 3), which strongly suggest outcrossing with bread wheat. Similarly, the dendrograms produced from screening SHWs with DArT markers indicate that many SHW accessions are closer genetically to bread wheat than we expected (Figures 9 & 10).

Several unusual features became apparent during crossing with the CIMMYT SHWs. The SHWs were clearly very different to elite UK varieties: they tended to flower very early, were nearly all awned, and were often very tall, with ears that were extremely difficult to thresh because of tenacious glumes. Compared to standard intervarietal crosses, seed set was generally slower, and fewer seeds were produced per crossed ear. Some SHW characters were useful in helping to visually confirm the hybridity of F_1 plants: the extreme non-glaucous character appears to be a dominant trait, whilst hairy glumes were semi-dominant, and awned / awnless heterozygotes can also usually be identified. Consistent use of Paragon and Xi-19 as maternal parents during crossing (both of which are relatively glaucous, medium-tall, with smooth glumes and no awns) meant that accidental selfs could be readily identified and quickly eliminated from the programme.

The most striking aspect of crossing with CIMMYT SHWs was the high proportion of combinations with Xi-19 that showed extreme hybrid necrosis. Subsequent crosses between CIMMYT SHWs and a range of winter and spring varieties has shown that hybrid necrosis is relatively common in the resulting F_1s (Table 13). Closer scrutiny of this list shows that the only F_1s without consistent hybrid necrosis symptoms arose from crosses involving Cortez, Paragon, Robigus, Tybalt and their derivatives. Cortez, Tybalt (both bred by Wiersum Zelder, Netherlands) and Robigus (selected by KWS UK from Wiersum Zelder germplasm) are all known to be derivatives of the tetraploid wheat, wild emmer (*Triticum dicoccoides)*. However, other known *T. dicoccoides* derivatives such as Shamrock, Stigg (both bred by Limagrain UK), Timber and Glasgow (both bred by Saaten Union) gave necrotic reactions. Of the Robigus derivatives tested, most F_1s were also not necrotic; notably, those of both the high-yielding variety Oakley and its successor KWS Santiago were necrotic.

The hybrid necrosis reaction is thought to result from reactive oxygen molecules causing oxidative stress within the leaves (Dalal and Khanna-Chopra, 2001) and has even been proposed as post-zygotic barrier to gene flow between species, preventing interspecific hybridisation (reviewed by Bomblies and Wiegel, 2007). Hybrid necrosis in wheat is controlled by two complementary dominant genes *Ne1* and *Ne2*. Combinations of different alleles at *Ne1* and *Ne2* can cause symptoms which range from no necrosis to complete lethality. They have been mapped relative to microsatellite markers: *Ne1* on 5BL (2.0 cM from *Xbarc*74) and *Ne2* on 2BS (3.2 cM from *Xbarc*55) in segregating populations derived from CIMMYT SHWs (Chu *et al*., 2006).

Most crosses between elite varieties, which are the mainstay of commercial breeding programmes, do not show any hybrid necrosis. Prior knowledge of which varieties show necrotic reactions with the CIMMYT SHWs will help breeders to design future crosses that allow the integration of SHW-derivatives whilst avoiding the risk of hybrid necrosis. Indeed, this knowledge has already helped to optimise experiments within the BBSRC public sector wheat pre-breeding programme (http://www.wheatisp.org/). As part of this project, NIAB will generate thousands of new pre-breeding lines derived from crosses between hexaploid wheat and two sources: 1) novel SHWs, generated at NIAB by hybridising tetraploid wheat with a range of diploid *Aegilops tauschii* accessions not previously used by CIMMYT or others for resynthesis; and 2) a range of wild and cultivated tetraploids. Both sources have the potential for hybrid necrosis, so we have chosen Paragon and Robigus to be the two hexaploid backgrounds used for all crosses.

Pathology tests showed some interesting leads for future work. The 2010 yellow rust seedling tests identified a number of SHW lines which appeared to be resistant to the ‘Solstice / Oakley’ race, a virulent form of the disease which was just beginning to make an impact in the field. A follow-up experiment was sown in the field the following autumn to evaluate the more important adult plant

resistance phenotype. Whilst this was inoculated with three different isolates, the Solstice / Oakley race was the dominant pathotype established in the resulting epidemic. Several SHWs appeared to be resistant at both the seedling and adult plant stages; similarly, several lines were very susceptible in both tests, including the susceptible elite variety, Oakley. Although a number of lines were resistant in one test but not another or susceptible in one test but not another, every line which was very susceptible in the field was also very susceptible in the seedling test (Appendix 5). Within our pre-breeding material, it was possible to identify susceptible and resistant recombinants in the field. However, as both Paragon and Xi-19 show good field resistance themselves, it was not possible to identify or tell whether the resistance of the best lines was inherited from their SHW donor or the Paragon or Xi-19 background.

The fusarium experiments identified some SHW lines with type I resistance (to initial point infection) and others with type II resistance (to spread of disease from initial infection), shown in Appendix 4. SHW-003 was of particular interest: it was one of the most susceptible lines to point inoculation, but one of the most resistant lines to spray inoculation, suggesting that it had an unusual combination of good type I resistance, but poor type II resistance (once infected, disease spread rapidly). Unfortunately, the Paragon / SHW-003 material appeared to be a maternal self (=Paragon) and the Xi-19 / SHW-003 material was lost to hybrid necrosis. However, the cross with Paragon has been repeated and taken to BC_1, so there is potential to investigate this potential valuable resistance further. Another line, SHW-144, was amongst the most resistant lines to point inoculation, suggesting good type II resistance, and large numbers of recombinants had already been developed from this donor. Unfortunately, the positive results observed from SHW-144 in polytunnel tests could not be replicated in a large Paragon / SHW-144 BC_1F_6 population when grown in inoculated field trials in 2011 (P Nicholson, unpublished data).

When fieldwork commenced on SHW-derivatives, with the 2008 F_2 nursery, it quickly became apparent that the SHW donors could bring genuine potential for yield improvement. The advantage of a pre-breeding approach was also clear: whilst many selections showed some promise, it would have been very difficult to justify their retention within a commercial programme, alongside better-adapted material from typical elite x elite crosses. Having a pre-breeding mindset allowed the identification of types which were interesting for particular traits, but also had some glaring faults in adaptation, architecture or disease resistance which would make them undesirable within a commercial breeding programme. A fuller understanding of their faults, as well as their advantages, is required if breeders are to properly integrate these exotic types into their own crosses, and this will be a primary objective of the BBSRC Follow-On Fund project.

Most UK wheat breeding is based on pedigree selection, classically set out at the Plant Breeding Institute by Biffen (1905). Typically, promising individual plants are harvested from within F_2

populations, and then re-sown as F_3 ear-rows (each row made up of F_3 siblings originating from a single F_2 plant). Six ears are harvested from an F_3 ear-row (one ear each from six different superior plants) and re-sown the next season as F_4 ear-row families (a plot of six ear-rows: each ear-row represents F_4 siblings from a single F_3 plant, and all six ear-rows trace back to the same F_2 plant). This continues with larger F_5 families (ear rows), F_6 sub-families (plant progenies) and so on, with increased multiplication at each step, and yield-testing beginning typically at F_5. Following intense purification at F_7, involving rigorous plant-by-plant checks for those botanical characters used to characterise varieties during the Distinctness, Uniformity and Stability (DUS) tests that from part of the National Listing system (Fera, 2010), varieties are usually uniform enough to consider entry into official National List trials at F_8.

For high-value crosses, inbreeding in early generations can be accelerated through SSD in a modification of the pedigree selection system (Brim, 1966). Whilst this defers the intensity of selection until later generations, it reduces the timescale of the breeding cycle and thus increases the rate of genetic gain. A higher risk approach, but with even shorter timescales, is to breed F_1-derived doubled haploids (DH), which should be 100% homozygous from the outset: here selection is a case of identifying the best recombinants, testing and multiplying them up as quickly as possible. Clearly, a DH approach was out of the question for SHW pre-breeding, even putting aside the problem of hybrid necrosis: the significant costs of producing each DH line and relative lack of recombination make this technique poorly suited for wide crosses.

It quickly became clear that the SHW-derived material didn't fit into conventional breeding schemes. The expectation for the 2009 BC_1F_2 field nurseries was that selection would occur on a row-by-row basis, with six ears being taken forwards from the most promising rows (as described above for conventional F_3 ear-row nurseries). However, there was still such a high degree of segregation for gross morphological characters such as height, glaucosity, presence/absence of awns and flowering time, that it was instead decided to go through the nurseries plant-by-plant, as for a conventional F_2 nursery. It seemed marginally easier to spot good rows in Xi-19 material (selections were taken from 98 BC_1F_2 rows: 45% of these included at least 3 selected plants, 12.2% with at least 6 selected plants) than Paragon material (selections taken from 197 BC_1F_2 rows: 36% of rows with at least 3 selected plants, 3.6% with at least 6 selected plants). At BC_1F_3 there was still a high level of gross segregation within progenies, making it again difficult to spot good rows, but laborious to spot good plants. Even at later generations (e.g. F_7, BC_1F_6) breeders have still commented on the apparent instability of the material and prevalence of mixed types. Again, this all underlined how a pre-breeding approach was required, divorced from the commercial pressures faced by breeding companies.

Another feature of commercial breeding programmes is the way in which selections are made. Typically, breeders grow early generations without fungicides or PGRs and with artificially high foliar disease pressure, to establish the epidemics necessary for the identification of genuinely resistant germplasm. Selection in these early generations is largely visual, based upon a combination of disease resistance, agronomic characters (height, straw stiffness, growth habit, flowering time) and yield components (ear size, tiller number, grain size, spikelet fertility), relative to the parents and other reference varieties. Attrition rates are high: typically only 10% of the material planted is carried forwards to the next generation. At later generations, effort is also put into testing yield, quality and varietal purity, although the other characters are still assessed and used in making selections.

With guidance from the breeders, our selection criteria for the SHW-derived germplasm have been to largely ignore deficiencies in agronomic characters and disease resistance, and to concentrate primarily on yield components, including high biomass. Selection was generally less aggressive than in commercial situations, with a philosophy more of “eliminate the worst” than “select the best”, in an attempt to maintain as much diversity as possible. This was especially the case for the 2011 selection of XS BC_1F_5 lines to progress into yield trials: of the 2847 progenies sown in spring 2011, 1401 (49%) were taken forwards, with only 22/252 BC_1 lineages eliminated completely.

The SHW-derived material has now been tested in the field for up to five successive seasons (2008-2012), with contrasting weather conditions. Of particular note were the high early-season temperatures and drought stress of spring / early summer 2010 (PaS F_5 and BC_1F_3 selections, XS BC_1F_3 selections plus previously unselected PaS BC_1F_5) and the largely dull, cool and wet late spring and summer of 2012 (selections from all sources, but in particular the 1401 XS BC_1F_6 lines, including 1000 in yield trials).

The 2010 F_5 trial grown by Limagrain UK was the first indication that these SHW-derivatives displayed real yield potential (Figure 19). The highest-yielding lines in this trial yielded over 120% of the control mean (100% = the mean yield of 24 plots: eight plots each of three check varieties). This was a single-replicate trial, so the results should be treated with caution, but these high yields were still 5-8% above the highest individual control plot. It is likely that the early drought and high temperatures affected this trial, with tolerant types favoured over sensitive types. These stresses will certainly have also had an impact on the selections made within the hitherto unselected PaS BC_1F_5 material during 2010. For example, 24% of progenies (6/25) were taken forwards as six ears from the combination PS-080, and over 10% of progenies were similarly selected from a further eight donors (Table 19). Tolerance to drought / heat stress was clearly segregating in at least some of these combinations (Figure 25). Whilst three of the larger populations (PS-144, PS-330, PS-144) contained a relatively high proportion of lines with good tolerance, this was not the

case for two other large populations (PS-181: 19 selections from 252 lines sown, PS-218: 26/326; Table 19). SHWs have a reputation for improved drought tolerance and SHW-derived lines have been shown to outperform their bread wheat parents under drought conditions, largely due to increased rooting at depth (Reynolds *et al*., 2007).

The late sowing of the 2010 nurseries revealed that some SHW donors clearly carried cryptic 'winter' alleles: whilst both the SHW parent and Xi-19 came through to flowering as expected, in some cases recombinants displayed the characteristic tussocky appearance of plants which have received insufficient vernalisation (Figure 23). These indicate that the SHW donors of these lines probably carried complementary 'winter' alleles at *Vrn* loci to Xi-19, and in certain combinations this gave rise to plants with a higher vernalisation requirement than either parent.

The contrasting 2012 harvest season provided very different selection pressures. High plant populations and leafy plots led to widespread, but differential, lodging from June onwards (Figure 27a). The lush canopies and high rainfall gave rise to high foliar disease pressure whilst the weather also prevented fungicide treatments being applied at the optimum timings, leading to disease symptoms in susceptible material even though the trials and nurseries received a full fungicide programme. There was also considerable chlorosis in many plots (Figure 28) which may reflect the inheritance of weak alleles for hybrid necrosis. However, this was also seen in many other wheat lines arising from elite x elite crosses, and was reported widely in trials and on farm during the 2011-12 growing season, suggesting that other mechanisms were also at work. Anecdotal explanations of this have included de-waxing caused by pesticide applications, oxidative stress caused by leaves which emerged in dull conditions being unable to cope with subsequent strong sunlight, and the hypersensitive response sometimes seen in resistant plant/pathogen interactions. Some of the XS BC_1F_6 ear-row families grown in 2011-12 included instances where single ear-rows appeared uniformly chlorotic whilst adjacent ear-rows (tracing back to the same BC_1F_4 plant) showed no chlorosis (Figure 36). Several of these contrasting types have been taken forwards as pairs of near-isogenic lines.

On farm, UK wheat yields for 2011-12 were 13% lower than the previous year (DEFRA, 2012) despite a slight increase in cropped area, reflecting a yield-building period characterised by high disease pressure, high rainfall and generally dull, cool conditions. This was also reflected in HGCA Recommended List Trials, where the average control yield of 8.8t/ha for harvest 2012 was 13.7% lower than the five-year average (HGCA, 2012). Within RL trials, there was a general trend of earlier varieties performing well relative to later varieties, and trials grown on light land out yielding those grown on heavier land, both of which are the opposite of what is expected in "normal" seasons. Certainly the earliness effect was seen within the XS yield trials in 2011-12, with many of the top yields coming from early-flowering types.

A danger for plant breeders in these extreme seasons is that they simply follow the pulls and pushes of the season, taking the highest yielding lines in isolation of other factors. For example, a dry season tends to favour taller types over shorter types, but in a wet season these taller types are at risk of lodging. It is not enough to simply select the highest yielding material, there needs to be an understanding of why the yield is high and, crucially, whether this high yield is likely to be stable (shown over years and across different locations). A concerted effort was made at harvest 2012 not just to select the highest yielding material, but to select the highest yielding early lines, medium lines, later lines etc within the XS recombinants.

Improvements in grain yield per unit area can be broken down into a number of interacting yield components: increased grain number, increased grain size, or both. Increased grain number can in turn arise from higher tillering (more ears per unit area), larger ears (more spikelets per ear), higher spikelet fertility (more grains per spikelet), or various combinations of these traits (reviewed by Foulkes *et al*., 2011). Large ears and / or high spikelet fertility are relatively simple traits to select for visually in early generation material, and can certainly be found within the SHW-derived material (Figures 18, 24 & 26). Similarly, many SHW-derivatives have been selected with high thousand grain weight (TGW), which seems to reflect physically large grains, with very high positive correlations between TGW and grain area, length, width and factor form density (Table 17, Figure 21). Similar results were seen in a meta-analysis of several biparental mapping populations (Gegas *et al*., 2010) which detected distinct QTLs for grain size and grain shape largely on the A and B genomes. Figure 22 indicates that individuals with high TGW do have physically larger grains. The expectation was that grain sizes within low and high TGW samples would follow similar distributions but that the mean would simply be shifted along, as appears to be the case for PS-218 material (Figure 22c). However, for the other three examples, the distributions in grain size appear to be different. This may indicate different ear morphologies or varying spikelet fertility. There is a danger in selecting for high TGW in isolation from other yield components. Varieties with “blind” grain sites (often caused by sterility, abortion of the developing grain or attack by orange wheat blossom midge) can compensate by diverting photosynthate into those grains which have set, thus increasing thousand grain weights. In this way, a superficially positive yield component is actually an indication of poor yield stability.

Another recurring theme in selections made within the SHW-derivatives is that of high above-ground biomass. This can be considered as a production trait in its own right, in the hope that through successive rounds of crossing and selection, breeders can eventually turn this into increased grain yield by improving the harvest index. It is also a trait which appears in the drought-tolerance ideotype (van Ginkel *et al*., 1998). SHW-derivatives have shown higher biomass, poorer harvest index, and higher yields than their recurrent parent in both irrigated and drought situations

in CIMMYT trials (Reynolds, 2007). In 2010 WGIN trials, line 756 similarly had higher biomass, poorer harvest index but higher grain yields than Xi-19, its recurrent parent (Table 27).

The inclusion of PaS germplasm in phenotyping trials within the BBSRC public sector pre-breeding LoLa has been extremely valuable. Data from 2010-11 trials strongly suggests that not only can SHW-derivatives give relatively high yields under high nitrogen (as typically used in commercial practice) but that much of this yield is maintained under lower nitrogen (Figures 37 & 38). A low-nitrogen screen has been included in the BBSRC Follow-On Fund proposal, to further investigate this in XS selections.

The red-grained / white-grained character is controlled by the *R* genes, which are found on the long arm of the group three chromosomes (McIntosh *et al*., 1998) and encode *Myb*-like transcription factors (Himi and Noda, 2005). Red grain is dominant to white grain, and grain colour is conferred by the maternal genotype: plants must be homozygous for white alleles at all three *R* genes in order to produce white grain, and just a single red allele is sufficient for the seed to appear red grained. We have seen a considerable number of white-grained types within some PaS combinations, but none within Xi-19 progenies of the same SHW donors. This is consistent with a hypothesis that Xi-19 carries red alleles at all three *R* genes, whilst Paragon carries cryptic white alleles at one or more *R* genes. Whilst the white-grained character is desirable for many end-uses, in UK conditions it is often associated with high levels of pre-harvest sprouting and unacceptably low Hagberg Falling Number. Despite this, there is a small, niche market for white-grained varieties such as Zircon (KWS), Heroldo (RAGT) and even old varieties such as Recital (Syngenta), and breeders are often interested in testing new white-grained material.

In the SHW-derivatives tested here, delayed senescence has been associated with extreme non-glaucous types (Figure 20). If canopy longevity can be increased by delaying senescence, this should extend the grainfill period and thus increase yield. Derkx *et al*. (2012) screened a collection of induced mutants in Paragon, and identified some lines with delayed senescence and others with accelerated senescence. When mutant lines with similar anthesis dates but contrasting senescence were investigated in detail, delayed senescence consistently increased yield above accelerated senescence. In pot experiments, a stay-green mutant had comparable yield to wild-type Paragon, with increases in tiller number and grain number balanced by decreased TGW. The stay-green trait is an important factor in the continued rise in the yields of maize (Duvick 2005) and sorghum (Borrell *et al*., 2000).

Glaucosity refers to the degree and type of crystalline wax deposited on the surface of the leaf, and has been associated with drought-tolerance traits (Richards *et al*., 1986). The glaucous leaf character is controlled by a dominant glaucous gene *W1* and a dominant inhibitor of glaucosity *Iw1*,

which are both found on chromosome 2BS, and their homoelogues *W2* and *Iw2* on 2DS (Tsunewaki and Ebana, 1999). The effect of the dominant inhibitor alleles *Iw1* and *Iw2* appears not to be to prevent the production of cuticular wax, but rather to alter the way in which waxes are deposited on the leaf surface.

Simmonds *et al*. (2008) detected a locus on the short arm of chromosome 2B, which they called *Vir*. Segregation at this locus in a Shamrock x Shango DH population explained the contrast between the viridescent vivid green colour typical of Shamrock and the almost blue colour of the very glaucous variety Shango. It was observed that viridescent types displayed prolonged canopy duration in the field. Across the DH population, the grainfill period (the time between the onset of anthesis and the onset of senescence) was one day longer for viridescent types than for glaucous types. Furthermore, across 5 location-years of replicated trials, viridescent types out-yielded glaucous types by 2.4-5.6%. This yield increase was expected to be due to increased grain filling, but no appreciable difference in grain size was noted. Based upon the pedigree of Shamrock and the SSR alleles amplified at the closest marker on 2BS, *Xgwm614*, an unknown *Triticum dicoccoides* source was proposed as the origin of the Shamrock *Vir* allele.

A mapping experiment in tetraploid wheats investigated this 2BS locus relative to SSR markers (Yoshiya *et al*., 2011). It concluded that the Shamrock *Vir* locus was equivalent to a *T. dicoccoides* allele, $Iw1^{DIC}$, which co-segregated with *Xgwm614,* although a cross between the glaucous tetraploid line and Shamrock failed due to hybrid necrosis in the F_1 plants.

F_2 and F_3 progeny from a cross between a non-glaucous CIMMYT SHW and a glaucous wheat line were assessed for glaucosity and screened with molecular markers (Liu *et al*., 2007). F_2 segregation data suggested that the non-glaucous types were dominant, and that a single gene was segregating in the cross, which was termed *Iw3672*. This was mapped to the distal region of wheat chromosome 2DS, close to SSR marker *Xbarc124* and rice BAC-derived markers *Xwe6* and *Xte6*, and was assumed to be the inhibitor of glaucosity, *Iw2*.

From observations in the PS-144 F_2 population, we have established that the non-glaucous phenotype is dominant and appears to be controlled by a single gene. Several non-glaucous SHWs (including SHW-144) have been crossed with Shamrock, but all resulting F_1s have been necrotic, so it has not been possible to evaluate segregating F_2s for glaucosity. Close observation has also identified three phenotypes within the SHW-derivatives (Figure 32): glaucous ear and leaf (e.g. Xi-19, Paragon and many other varieties), non-glaucous ear, glaucous leaf (e.g. recent RL variety Stigg) and non-glaucous ear and leaf (e.g. Shamrock and current RL variety Crusoe, a Shamrock derivative). Unfortunately, crosses between non-glaucous SHWs and Stigg also gave necrotic F_1s.

Near-isogenic lines for glaucosity in the Paragon background have been developed through consecutive selection cycles during inbreeding. Firstly, heterozygosity was maintained by selecting segregating rather than fixed progenies. At later generations, true-breeding glaucous and non-glaucous progenies were selected and multiplied. Contrasting material for ear glaucosity has also been developed (non-glaucous ear and leaf versus non-glaucous ear, glaucous leaf). Yield trials were grown in 2012 (Figure 34), although yields were generally very low due to the weather conditions, and no significant differences were found between glaucous and non-glaucous types (Table 23). These lines will be retested in further trials, together with material developed in the Xi-19 background.

Additional NILs have been developed by selecting contrasting types from within segregating progenies at late generations. These can be used to test hypotheses about specific attributes. For example, the presence of hairy glumes (as in the recent RL variety Gatsby) is anecdotally associated with the spread of powdery mildew from the leaf up onto the ear; we have selected NILs contrasting for hairy and smooth glumes which can be tested in high mildew situations. Other characters include NILs for the presence or absence of awns, and NILs for chlorotic versus normal leaves (Figure 36).

In summary, we have shown that the CIMMYT SHWs represent a valuable resource for UK wheat breeders, for the improvement of disease resistance, drought tolerance and most importantly, yield itself.

4.1.4. Backcrossing with CIMMYT SHW-derived lines

In the current study, marker-assisted backcross selection was used to identify and introgress specific genomic regions derived from the parental components of SHWs (*T. turgidum* (AABB) and *Ae. tauschii* (DD)) that had previously been reported to be inherited preferentially over corresponding bread wheat regions during pedigree selection at CIMMYT in Mexico (Zhang *et al*., 2005). Synthetic-derived (SHWD) germplasm, SHW parental components (CROC_1 durum (AABB) and WX224 *Ae. tauschii* (DD)), genomic regions for introgression and subtending SSR markers were identified from work published by Zhang *et al*. (2005). The corresponding germplasm was supplied by CIMMYT and three genomic regions on chromosomes 3B (CROC_1), 4D (WX224) and 7B (CROC_1) were introgressed into Paragon and Xi-19 (Table 29). In order to compare and contrast trait value, full sib, paired near iso-genic lines (NILs) were developed in which bread wheat and the corresponding SHW-derived introgressions could be compared directly in identical bread wheat genetic backgrounds. Although severe hybrid necrosis prevented the development of a sufficient number of Xi-19 lines for analysis, 84 BC_2 lines in Paragon were developed. No selection was practised during single seed descent (SSD) and multiplication; therefore all 84 lines were included in a BC_2F_5 replicated yield trial at NIAB in 2012. In lieu of

specific traits with which to evaluate individual SHW derived introgressions, plot yield under a standard treated yield trial regime was used as a proxy for breeding value under UK conditions.

At BC_2, it is expected that a significant amount of genetic heterozygosity remains. To determine the amount of variation attributable to genetic background effects, trials of a minimum of four, and up to ten, full sib pairs were assessed per introgression. Although trials data generated in a single year must be treated with caution, analysis of the mean of pooled data identified a consistent, but non-significant, yield reduction associated with the SHW source of each introgression. Although at BC_2, segregating loci at other genomic locations may potentially mask the true effect on yield, these data suggest that whilst individual SHWD blocks do not appear to have a grossly detrimental effect on yield, they certainly don't confer any significant breeding value under UK conditions. Comparing pooled data from NIL pairs with CIMMYT donor cultivars and Paragon, however, reveals some significant differences. For example, NILs carrying 4D1c and 7B3a inherited singly or in combination from the SHWD-4 parent significantly out-yielded SHWD-4, suggesting that background Paragon alleles may have counterbalanced negative effects on yield inherited from the non-adapted SHWD CIMMYT parents. For introgressions inherited from CIMMYT line SHWD-5, a similar pattern was observed for NILs carrying 3B2b, however, 4D1b NILs (whether carrying the SHW-D or Paragon alleles at 4D1b) also out-performed Paragon suggesting that these lines harbour background yield-promoting loci originating from SHWD-5. In view of this, further investigation of the yield effects of 4D1b NILs is warranted. It is possible that 4D1b corresponds to a synthetic derived genomic block identified in a study by Li *et al*. (2011), who reported a region on 4DL associated with the SSR marker Barc1183 that conditioned positive effects on several yield components. It would be interesting to discover whether Barc1183 is also associated the 4D1b genomic block identified in the current study.

4.2. References

Al-Kaff, N., Knight, E., Bertin, I., Foote, T., Hart, N., Griffiths, S., Moore, G. (2008). Detailed Dissection of the Chromosomal Region Containing the *Ph1* Locus in Wheat *Triticum aestivum*: With Deletion Mutants and Expression Profiling. Ann Bot 101: 863-872

Beddington, J (2009) available online at http://www.dius.gov.uk/assets/goscience/docs/p/perfect-storm-paper.pdf

Biffen, R.H. (1905). Mendel's laws of inheritance and wheat breeding. Journal of Agricultural Science, Cambridge 1: 4–48.

Bomblies, K., Wiegel, D. (2007). Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. Nat Rev Genet. 8:382-93 .

Borrell, A.K., Hammer, G.L., Henzell, R.G. (2000). Does Maintaining Green Leaf Area in Sorghum Improve Yield under Drought? II.Dry Matter Production and Yield. Crop Sci. 40:1037–1048.

Brim, C. A. (1966) A modified pedigree method of selection in soybeans. Crop Sci., 6: 220.

Bayles, R., O'Sullivan, D., Lea, V., Freeman, S., Budge, G., Walsh, K., Henry, C. (2007) Controlling soil-borne cereal mosaic virus in the UK by developing resistant wheat cultivars. In: HGCA, Project Report 418.

Beales, J., Turner, A., Griffiths, S., Snape, J.W., Laurie, D.A. (2007) A Pseudo-Response Regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (Triticum aestivum L.). Theoretical and Applied Genetics 115: 721-733.

Bentley, A.R., Turner, A.S., Gosman, N., Leigh, F.J., Maccaferri, M., Dreisigacker, S., Greenland, A., Laurie, D.A.(2011). Frequency of the photoperiod-insensitive Ppd-A1a alleles in tetraploid, hexaploid and synthetic hexaploid wheat germplasm. Plant Breeding 130: 10-15.

Bryan, G., Collins, A., Stephenson, P., Orry, A., Smith, J., and Gale, M. (1997) Isolation and characterisation of microsatellites from hexaploid bread wheat. Theor. Appl. Genet. 94: 557–563.

Cockram, J., H. Jones, F.J. Leigh, D. O'Sullivan, W. Powell, D. A. Laurie, A. J. Greenland (2007). Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. J. Exp. Bot. 58: 1231-1244

Colasanti, J. and Coneva, V. (2009). Mechanisms of Floral Induction in Grasses: Something Borrowed, Something New. Plant Physiology 149: 56-62

Chu, C.G., Faris, J.D., Friesen, T.L., Xu, S.S. (2006). Molecular mapping of hybrid necrosis genes *Ne1* and *Ne2* in hexaploid wheat using microsatellite markers. Theor Appl Genet 112:1374-1381.

CIMMYT (2004) http://www.cimmyt.org/es/boletin/120-2004/387-wild-wheat-relatives-help-boost-genetic-diversity

Dalal, M., Khanna-Chopra, R. (2001). Differential response of anti-oxidant enzymes in leaves of necrotic wheat hybrids and their parents. Physiol Planta 111:297-304

DEFRA (2012) http://www.defra.gov.uk/statistics/files/defra-stats-foodfarm-landuselivestock-farmingstats-june-statsrelease-jun2012ukprovcrops-1210151.pdf

Derkx, A.P., Orford, S., Griffiths, S., Foulkes, M.J., Hawkesford, M.J. (2012). Identification of differentially senescing mutants of wheat and impacts on yield, biomass and nitrogen partitioning. J Integr Plant Biol. 54:555-66

Diaz, A., Zikhali, M., Turner, A.S., Isaac, P., Laurie, D.A. (2012). Copy number variation affecting the Photoperiod-B1 and Vernalisation-A1 genes is associated with altered flowering time in wheat (Triticum aestivum). PLoS ONE 7, e33234.

Dolferus, R., Xuemei, J. (2011). Abiotic stress and control of grain number in cereals. Plant Science 181(4): 4331–341

Dubcovsky, J., Dvorak, J. (2007). Genome plasticity a key factor in the success of polyploid wheat under domestication. Science 316: 1862-1866.

Duvick, D.N.(2005). The contribution of breeding to yield advances in maize (*Zea mays* L.). Adv. Agron. 86:84–145.

Dyck, J.A., Matus-Cadiz, M.A., Hucl, P., Talbert, L., Hunt, T., Dubuc, J.P., Nass, H., Clayton, G., Dobb, J., Quick, J. (2004). Agronomic performance of hard red spring wheat isolines sensitive and insensitive to photoperiod. Crop Science 44: 1976-1981.

Eujayl, I., Sorrells, M.E., Baum, M., Wolters, P. and Powell, W. (2002). Isolation of EST-derived

microsatellite markers for genotyping the A and B genomes of wheat. TAG. 104: 399-407.
Feldman, M. (2001) The origin of cultivated wheat. In: Bonjean, A.P., Angus, W.J. eds The World Wheat Book. Lavoisier Tech & Doc, Paris, pp. 3-56.

Feldman, M., Levy, A.A. (2005). Allopolyploidy – a shaping force in the evolution of wheat genomes. Cytogenet Genome Res, 109:250-258.

Fera (2010)
http://www.fera.defra.gov.uk/plants/plantVarieties/nationalListing/documents/nlGuideSept10.pdf

Fischer, R. A. and Edmeades, G.O. (2010). Breeding and Cereal Yield Progress Crop Science, 50: 85-98.

Foulkes, M. J., Scott, R.K. & Sylvester-Bradley, R. (2002). The ability of wheat cultivars to withstand drought in UK conditions: formation of grain yield. J. Ag. Sci. 138: 153-169

Foulkes, M. J.,Sylvester-Bradley,R., Worland, A.J. and Snape, J.W, (2004). Effects of a photoperiod-response gene Ppd-D1 on yield potential and drought resistance in UK winter wheat. Euphytica 135: 63-73.

Foulkes M.J., Sylvester-Bradley, R., Weightman, R., Snape, J.W. (2007). Identifying physiological traits associated with improved drought resistance in winter wheat. Field Crops Research 103: 11-24.

Foulkes, M.J, Slafer, G.A., Davies, W.J., Berry, P.M., Sylvester-Bradley, R., Martre, P., Calderini, D.F., Griffiths, S., Reynolds, M.P. (2011). Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. J Exp Bot 62: 469–486.

Fulton, Teresa, M., Julapark Chunwongse and Tanksley, S.D. (1995). Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Molecular Biology Reporter 13: 207-209

Gasperini, D. 2010. Genetic and physiological characterisation of Rht8 in hexaploid wheat, University of East Anglia/JIC, Norwich, 235.

Gonzalez, G. G., Slafer, G.A. & Miralles, D.J. (2005). Pre-anthesis development and number of fertile florets in wheat as affected by photoperiod sensitivity genes Ppd-D1 and Ppd-B1. Euphytica 146: 253–269.

Gororo, N.N., Eagles, H.A., Eastwood, R.F., Nicolas, M.E., Flood, R.G. (2002). Use of Triticum tauschii to improve yield of wheat in low-yielding environments. Euphytica 123: 241 – 254

Griffiths, S., Simmonds, J., Leverington, M., Yingkun Wang, Fish, L., Sayers, L., Alibert, L., Orford, S., Wingen, L., Herry, L., Faure, S., Laurie, D., Bilham, L., Snape, J. (2009). Meta-QTL analysis of the genetic control of ear emergence in elite European winter wheat germplasm. Theor Appl Genet (2009) 119:383–395

Gur, A., Zamir, D. (2004) Unused natural variation can lift yield barriers in plant breeding. PLoS Biol. 2: 1610-1615.

Hanocq, E., Niarquin, M., Heumez, E., Rousset, M., Gouis, J.L.(2004) Detection and mapping of QTL for earliness components in a bread wheat recombinant inbred lines population. Theoretical and Applied Genetics 110: 106-115.

HGCA RL trial update (2012)
http://www.hgca.com/document.aspx?fn=load&media_id=8124&publicationId=5624

Himi, E., Noda, K. (2005). Red grain colour gene (R) of wheat is a *Myb*-type transcription factor. Euphytica 143: 239–242

Kato, K., Yokoyama, H. (1992) Geographical variation in heading characters among wheat landraces, Triticum aestivum L., and its implication for their adaptability. Theoretical and Applied Genetics 84: 259-265.

Jones P.D., Lister D.H., Jaggard K.W., Pidgeon J.D. (2003). Future climate change impact on the productivity of sugar beet (Beta vulgaris L.) in Europe. Climatic Change 58: 93-108.

Kilian, A., Huttner, E., Wenzl, P., Jaccoud, D., Carling, J., Caig, V., Evers, M., Heller- Uszynska, K., Uszynski, G., Cayla, C., Patarapuwadol, S., Xia, L., Yang, S., Thomson, B. (2003) The fast and the cheap: SNP and DArT-based whole genome profiling for crop improvement. In: Tuberosa R, Phillips RL, Gale M, eds. Proceedings of the International Congress “In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution”. Bologna, Italy: Avenue media, 443-461.

Lage, J., Skovmand, S.B. Andersen (2002). Expression and suppression of resistance to Greenbug (Homoptera: Aphididae) in synthetic hexaploid wheats derived from T. dicoccum x Ae. tauschii crosses. J. of Economic Entomology 96: 202 – 206

Law, C.N., Sutka, J., Worland, A.J. (1978) A genetic study of day-length response in wheat. Heredity 41: 185-191.

Lewis, S., Faricelli, M.E., Appendino,M.L., Valarik, M. and Dubcovsky, J. (2008). The chromosome region including the earliness per se locus Eps-Am1 affects the duration of early developmental phases and spikelet number in diploid wheat. J. Exp. Bot. 85: 3595-3607.

Li, J., Wei, H.T., Hu, X.R., Li, C.S., Tang, Y.L., Liu, D.C., Yang, W.Y. (2011). Identification of a high-yield introgression locus from synthetic hexaploid wheat in Chuanmai-42. Acta Agron. Sin. 37: 255-262.

Liu, Q., Ni, Z., Peng, H., Song, W., Liu, Z., Sun, Q. (2006). Molecular mapping of a dominant non-glaucousness gene from synthetic hexaploid wheat (*Triticum aestivum* L.). Euphytica 155:71–78

Maccaferri, M., Sanguineti, M.C., Corneti, S., Ortega, J.L.A., BenSalem, M., Bort, J., DeAmbrogio, E., delMoral, L.F.G., Demontis, A., El-Ahmed, A., Maalouf, F,, Machlab, H., Martos, V., Moragues, M., Motawaj, J., Nachit, M., Nserallah, N., Ouabbou, H., Royo, C., Slama, A., Tuberosa, R. (2008). Quantitative trait loci for grain yield and adaptation in durum wheat (Triticum durum Desf.) across a wide range of water availability. Genetics 178: 489-511.

Mackay, I., Horwell, A., Garner, J., White, J., McKee, J., Philpott, H. (2010) Re-analyses of the historical series of UK variety trials to quantify the contributions of genetic environmental factors to trends and variability in yield over time. Theor. Appl. Genet., published online on 21 Sept. 2010

McCartney, C.A., Somers, D.J., McCallum, B.D., Thomas, J., Humphreys, D.G., Menzies, J.G., Brown, P.D. (2005) Microsatellite tagging of the leaf rust resistance gene Lr16 on wheat chromosome 2BSc. Molecular Breeding 15: 329-337.

McIntosh, R.A., Yamazaki, Y., Devos, K.M., Dubcovsky, J., Rogers, W.J., Appels, R. (2003). Catalogue of Gene Symbols for Wheat. Available at: http://wheat.pw.usda.gov/ggpages/wgc/2003/

Mujeeb-Kazi A., Fuentes-Davila, G., Villareal, R.L., Cortes, A., Roasas, V., Delgado, R. (2001). Registration of ten synthetic wheat and six bread wheat germplasms resistant to karnal bunt. Crop Science 41: 1652 – 1653

Reynolds, M., Manes, Y., Izanloo, A., Langridge, P. (2009) Phenotyping approaches for physiological breeding and gene discovery in wheat. Annals of Applied Biology 155(3): 309-320

Pánková, K., Milec, Z., Simmonds, J., Leverington-Waite, M., Fish, L., .Snape, J.W. (2008). Genetic mapping of a new flowering time gene on chromosome 3B of wheat. Euphytica (2008) 164: 779–787

Pfeiffer, W. H., R. M. Trethowan, M. van Ginkel, M. I. Ortiz, and S. Rajaram, (2005) Breeding for abiotic stress tolerance in wheat, pp. 401-489 in Abiotic Stresses: Plant Resistance through Breeding and Molecular Approaches., edited by M. Ashraf, and P. J. C. Harris. The Haworth Press, Inc., NY

Richter G.M., Semenov M.A. (2005). Modeling impacts of climate change on wheat yields in England and Wales: assessing drought risks. Agricultural Systems 84: 77-97.

Reynolds, M., Dreccer, F., Trethowan, R. (2007). Drought-adaptive traits derived from wheat wild relatives and landraces. J. of Exp. Bot. 58: 177 – 186

Röder, M.S., Korzun, V., Gill, B.S., Ganal, M.W. (1998). The physical mapping of microsatellite markers in wheat. Genome 41(2): 278-283.

McIntosh, R.A., Hart, G.E., Devos, K.M., Gale, M.D., Rogers, W.J. (1998). Catalogue of gene symbols for wheat. In: Proceedings of the Ninth International Wheat Genetics Symposium, Vol. 5. University of Saskatchewan Extension Press, Canada.

McClintock, B. (1984) The significance of responses of the genome to challenge. Science 226: 792–801

Reynolds, M.P., Dreccer, F., Trethowan, R. (2007). Drought-adaptive traits derived from wheat wild relatives and landraces. J Exp Bot 58: 177–186.

Reynolds, M.P., Bonnett, D., Chapman, S., Furbank, R., Manes, Y., Mather, D., Parry, M. (2011). Raising yield potential of wheat: (I) overview of a consortium approach and breeding strategies. J Exp Bot. 62:439-52.

Richards, R.A., Rawson, H.M., Johnson, D.A. (1986) Glaucousness in wheat: its development and effect on water-use efficiency, gas exchange and photosynthetic tissue temperatures. Aust J Plant Physiol 13:465–473

Shaw, L.M., Turner, A.S., Laurie, D.A. (2012). The impact of photoperiod insensitive Ppd-1a mutations on the photoperiod pathway across the three genomes of hexaploid

wheat (Triticum aestivum). The Plant Journal 71: 71-84.

Shah, M. M., Baenziger, P.S., Yen, Y., Gill, K.S., Moreno-Sevilla, B. and Haliloglu, K. (1999). Genetic Analyses of Agronomic Traits Controlled by Wheat Chromosome 3A. Crop Science 39: 1016-1021.

Simmonds, J.R., Fish, L.J., Leverington-Waite, M.A., Wang, Y., Howell, P., Snape, J.W. (2008). Mapping of a gene (Vir) for a non-glaucous, viridescent phenotype in bread wheat derived from Triticum dicoccoides, and its association with yield variation. Euphytica 159: 333-341.

Snape, J. W., Butterworth, K., Whitechurch, E. & Worland, A.J. (2001). Waiting for fine times: genetics of flowering time in wheat. Euphytica, 119: 185-190.

Sip, V., Chrpova, J., Zofajova, A., Pankova, K., Uzik, M., Snape, J.W. (2010). Effects of specific Rht and Ppd alleles on agronomic traits in winter wheat cultivars grown in middle Europe. Euphytica: 172: 221-233.

Song, Q. J., Shi, J.R., Singh, S., Fickus, E.W., Costa, J.M., Lewis. J., Gill, B.S., Ward, R., Cregan, P.B. (2005). Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet. 110: 550–560

Tanksley, S.D., McCouch, S.R. (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science, 277: 1063-1066.

Tanio M, Kato K. (2007). Development of near-isogenic lines for photoperiodinsensitive genes, Ppd-B1 and Ppd-D1, carried by the Japanese wheat cultivars and their effect on apical development. Breeding Science, 57: 65-72.

Thomas, J., Fineberg, N., Penner, G., McCartney, C., Aung, T., Wise, I., McCallum, B. (2005) Chromosome location and markers of Sm1: a gene of wheat that conditions antibiotic resistance to orange wheat blossom midge. Molecular Breeding 15: 183-192.

Tsunewaki, K., Ebana, K. (1999) Production of near-isogenic lines of common wheat for glaucousness and genetics basis of the trait clarified by their use. Genes Genet Syst 74:33–41.

Wilhelm, E.P., Turner, A.S., Laurie, D.A. (2009) Photoperiod insensitive Ppd-A1a mutations in tetraploid wheat (Triticum durum Desf.). Theoretical and Applied Genetics 118: 285-294.

Worland, A.J. (1999) The importance of Italian wheats to worldwide varietal improvement. Journal of Genetics and Breeding 53: 165-173.

Worland, A.J. (1996). The influence of flowering time genes on environmental adaptability in European wheat. Euphytica, 89: 49-57.

Valkoun, J. J. (2001) Wheat breeding using wild progenitors. Euphytica, 119: 17-23.

van Ginkel, M.,Calhoun, D.S., Gebeyehu, G., Miranda, A., Tian-you, C., Pargas Lara, R., Trethowan, R.M., Sayre, K., Crossa, L., Rajaram, S. (1998). Plant traits related to yield of wheat in early, late, or continuous drought conditions. Euphytica 100:109–121.

van Ginkel M., Ogbonnaya F. (2007). Novel genetic diversity from synthetic wheats in breeding cultivars for changing production conditions. Field Crops Res 104: 86–94.

Villareal, R.L., Mujeeb Kazi, A., Del Toro, E., Crossa, J., Rajaram, S., (1994). Agronomic variability in selected Triticum turgidum x T. tauschii synthetic hexaploid wheats. J. Agron. Crop Sci. 173: 307–317.

Villareal, R.L., Sayre, K., Banuelos, O., Mujeeb-Kazi, A., (2001). Registration of four synthetic hexaploid wheat (*Triticum turgidum/Aegilops tauschii*) germplasm lines tolerant to waterlogging. Crop Sci. 41: 274.

Warburton, M. L., Crossa, J., Franco, J., Kazi, M., Trethowan, R., Rajaram, S.,Pfeiffer, W., Zhang, P., Dreisigacker, S., & van Ginkel, M. (2006). Bringing wild relatives back into the family: recovering genetic diversity in CIMMYT improved wheat germplasm. Euphytica 149: 289–301

Yang J., Sears R.G., Gill B.S., Paulsen G.M. (2002). Growth and senescence characteristics associated with tolerance of wheat-alien amphiploids to high temperature under controlled conditions. Euphytica 126: 185-193

Yang, W., Liu, D., Li, J., Zhang, L., Wei, H., Hu, X., Zheng, Y., He, Z., Zou, Y. (2009). Synthetic hexaploid wheat and its utilization for wheat genetic improvement in China. J Genet Gen 36: 539-546

Yoshiya, K., Watanabe, N., Kuboyama, T (2011) Genetic mapping of the genes for non-glaucous phenotypes in tetraploid wheat. Euphytica 177: 293-297.

Zadoks, J.C., Chang, T.T., Konzak, C.F. (1974) Decimal code for growth stages of cereals. Weed Research 14: 415-421.

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4.4. Appendices

Appendix 1. Synthetic hexaploid wheat nursery of 448 lines supplied by CIMMYT

Ent[a]	Cid	Sid	Cross	Sel_Hist
1	152417	1	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (164)	CIGM88.1
2	152416	3	CROC_1/AE.SQUARROSA (168)	CIGM87.2 0PR-0B
3*	159512	1	ALTAR 84/AE.SQUARROSA (178)	CIGM88.1
4	159513	0	ACO89/AE.SQUARROSA (178)	CIGM90.5
5*	159514	3	ALTAR 84/AE.SQUARROSA (188)	CIGM87.2 0PR-0B
6	152418	1	DOY1/AE.SQUARROSA (188)	CIGM88.1
7	159515	1	RABI//GS/CRA/3/AE.SQUARROSA (190)	CIGM88.1
8*	159516	3	ALTAR 84/AE.SQUARROSA (191)	CIGM87.2 0PR-0B
9	159516	3	ALTAR 84/AE.SQUARROSA (191)	CIGM87.2 0PR-0B
10	159517	1	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (191)	CIGM88.1
11	159518	1	SORA/AE.SQUARROSA (191)	CIGM88.1
12	88724	4	ALTAR 84/AE.SQUARROSA (192)	CIGM87.2 0PR-0B
13	159519	1	SORA/AE.SQUARROSA (192)	CIGM88.1
14	159519	1	SORA/AE.SQUARROSA (192)	CIGM88.1
15	159520	0	SORA/AE.SQUARROSA (192)	CIGM90.5
16	159520	0	SORA/AE.SQUARROSA (192)	CIGM90.5
17	159521	3	ALTAR 84/AE.SQUARROSA (193)	CIGM87.2 0PR-0B
18	159522	1	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (193)	CIGM88.1
19	152419	1	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (196)	CIGM88.1
20	88725	4	ALTAR 84/AE.SQUARROSA (198)	CIGM87.2 0PR-0B
21	159523	1	GAN/AE.SQUARROSA (201)	CIGM88.1
22*	62052	6	CROC_1/AE.SQUARROSA (205)	CIGM86.9 0B-0PR-0
23	159524	3	ALTAR 84/AE.SQUARROSA (205)	CIGM87.2 0PR-0B
24	152420	1	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (205)	CIGM88.1
25	159525	1	SORA/AE.SQUARROSA (207)	CIGM88.1
26	152421	1	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)	CIGM88.1
27	152421	1	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)	CIGM88.1
28	159526	1	SORA/AE.SQUARROSA (208)	CIGM88.1
29	159526	1	SORA/AE.SQUARROSA (208)	CIGM88.1
30	159527	3	CROC_1/AE.SQUARROSA (210)	CIGM87.2 0PR-0B
31	159527	3	CROC_1/AE.SQUARROSA (210)	CIGM87.2 0PR-0B
32	159527	4	CROC_1/AE.SQUARROSA (210)	CIGM87.2 0B
33	88726	4	ALTAR 84/AE.SQUARROSA (211)	CIGM87.2 0PR-0B
34	159528	1	D67.2/P66.270//AE.SQUARROSA (211)	CIGM88.1
35	159529	0	SORA/AE.SQUARROSA (211)	CIGM90.5
36*	159530	4	CROC_1/AE.SQUARROSA (213)	CIGM86.9 0B-0PR-0

37*	159531	1	D67.2/P66.270//AE.SQUARROSA (213)	CIGM88.1
38*	62061	5	DVERD_2/AE.SQUARROSA (214)	CIGM86.9 0B-0PR-0
39	159532	5	ROK/KML//AE.SQUARROSA (214)	CIGM86.9 1Y-0B-0PI
40	62054	5	CROC_1/AE.SQUARROSA (215)	CIGM86.9 0B-0PR-0
41	159533	1	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (215)	CIGM88.1
42	159534	0	SORA/AE.SQUARROSA (215)	CIGM90.5
43	159535	1	DOY1/AE.SQUARROSA (216)	CIGM88.1
44	159536	1	D67.2/P66.270//AE.SQUARROSA (217)	CIGM88.1
45	159537	0	YUK/AE.SQUARROSA (217)	CIGM90.5
46	159538	5	ARLIN_1/AE.SQUARROSA (218)	CIGM86.9 1Y-0B-0PI
47	159539	1	D67.2/P66.270//AE.SQUARROSA (218)	CIGM88.1
48*	62048	11	ALTAR 84/AE.SQUARROSA (219)	CIGM86.9 0B-0PR-0
49	159540	3	ALTAR 84/AE.SQUARROSA (220)	CIGM87.2 0PR-0B
50	159541	1	D67.2/P66.270//AE.SQUARROSA (220)	CIGM88.1
51*	62062	10	DVERD_2/AE.SQUARROSA (221)	CIGM86.9 1Y-0B-0PI
52*	88720	4	ALTAR 84/AE.SQUARROSA (221)	CIGM87.2 0PR-0B
53	159542	1	D67.2/P66.270//AE.SQUARROSA (221)	CIGM88.1
54*	154089	1	TK SN1081/AE.SQUARROSA (222)	CIGM88.1
55	154090	1	D67.2/P66.270//AE.SQUARROSA (222)	CIGM88.1
56	154089	1	TK SN1081/AE.SQUARROSA (222)	CIGM88.1
57	62051	4	ALTAR 84/AE.SQUARROSA (223)	CIGM87.2 0PR-0B
58*	154091	1	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (223)	CIGM88.1
59	159543	1	D67.2/P66.270//AE.SQUARROSA (223)	CIGM88.1
60*	62059	8	CROC_1/AE.SQUARROSA (224)	CIGM86.9 1Y-0B-0PI
61*	62059	8	CROC_1/AE.SQUARROSA (224)	CIGM86.9 1Y-0B-0PI
62*	62056	6	CROC_1/AE.SQUARROSA (224)	CIGM86.9 0B-0PR-0
63*	62056	6	CROC_1/AE.SQUARROSA (224)	CIGM86.9 0B-0PR-0
64*	62049	5	ALTAR 84/AE.SQUARROSA (224)	CIGM86.9 0B-0PR-0
65	62049	5	ALTAR 84/AE.SQUARROSA (224)	CIGM86.9 0B-0PR-0
66*	159544	3	ALTAR 84/AE.SQUARROSA (224)	CIGM86.9 0PR-0B
67	159545	4	ARLIN_1/AE.SQUARROSA (225)	CIGM86.9 0B-0PR-0
68*	159546	1	CETA/AE.SQUARROSA (230)	CIGM88.1
69	159547	1	GAN/AE.SQUARROSA (236)	CIGM88.1
70	159548	1	YAV_2/TEZ//AE.SQUARROSA (243)	CIGM88.1
71	159549	1	DVERD_2/AE.SQUARROSA (247)	CIGM88.1
72	159549	1	DVERD_2/AE.SQUARROSA (247)	CIGM88.1
73	159550	1	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/AE.SQUARROSA (254)	CIGM89.3

74	159551	1	AOS/AE.SQUARROSA (269)	CIGM88.1
75	159552	1	GARZA/BOY//AE.SQUARROSA (271)	CIGM88.1
76	159553	1	SCA/AE.SQUARROSA (279)	CIGM88.1
77	159554	0	ACO89/AE.SQUARROSA (282)	CIGM90.5
78	159555	1	GARZA/BOY//AE.SQUARROSA (286)	CIGM88.1
79*	159556	0	ACO89/AE.SQUARROSA (290)	CIGM90.5
80*	159557	3	ALTAR 84/AE.SQUARROSA (291)	CIGM87.20PR-0B
81	159558	1	GARZA/BOY//AE.SQUARROSA (307)	CIGM88.1
82	63026	4	LARU/AE.SQUARROSA (309)	CIGM87.20PR-0B
83	63026	4	LARU/AE.SQUARROSA (309)	CIGM87.20PR-0B
84	159559	0	ACO89/AE.SQUARROSA (309)	CIGM90.5
85	159559	0	ACO89/AE.SQUARROSA (309)	CIGM90.5
86	159560	1	GARZA/BOY//AE.SQUARROSA (311)	CIGM88.1
87	159561	0	ACO89/AE.SQUARROSA (315)	CIGM90.5
88	159562	1	68.111/RGB-U//WARD/3/AE.SQUARROSA (316)	CIGM88.1
89	159563	1	68.111/RGB-U//WARD/3/AE.SQUARROSA (321)	CIGM88.1
90	159564	1	68.111/RGB-U//WARD/3/AE.SQUARROSA (322)	CIGM88.1
91*	159565	1	SORA/AE.SQUARROSA (323)	CIGM88.1
92	159565	1	SORA/AE.SQUARROSA (323)	CIGM88.1
93*	159566	1	68.111/RGB-U//WARD/3/AE.SQUARROSA (325)	CIGM88.1
94	159567	1	68.111/RGB-U//WARD/3/AE.SQUARROSA (326)	CIGM88.1
95	159568	1	68.111/RGB-U//WARD/3/AE.SQUARROSA (328)	CIGM88.1
96	159569	1	ALTAR 84/AE.SQUARROSA (328)	CIGM88.1
97	159569	2	ALTAR 84/AE.SQUARROSA (328)	CIGM88.1
98	159570	1	68.111/RGB-U//WARD/3/AE.SQUARROSA (329)	CIGM88.1
99	159571	1	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (332)	CIGM88.1
100*	159572	1	ALTAR 84/AE.SQUARROSA (332)	CIGM88.1
101	159573	0	68112/WARD//AE.SQUARROSA (369)	CIGM88.1
102*	159573	0	68112/WARD//AE.SQUARROSA (369)	CIGM88.1
103	159573	0	68112/WARD//AE.SQUARROSA (369)	CIGM88.1
104	159573	0	68112/WARD//AE.SQUARROSA (369)	CIGM88.1
105	159573	0	68112/WARD//AE.SQUARROSA (369)	CIGM88.1
106	159573	0	68112/WARD//AE.SQUARROSA (369)	CIGM88.1
107	159574	1	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (411)	CIGM88.1
108	159575	1	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (412)	CIGM88.1
109*	159576	1	CHEN_7/AE.SQUARROSA (429)	CIGM89.4
110	159577	1	YUK/AE.SQUARROSA (434)	CIGM88.1
111	159578	1	SCOOP_1/AE.SQUARROSA (434)	CIGM88.1
112*	159579	0	GAN/AE.SQUARROSA (437)	CIGM90.5
113*	159580	1	SRN/AE.SQUARROSA (446)	CIGM88.1
114	159581	1	DOY1/AE.SQUARROSA (446)	CIGM88.1
115	159581	1	DOY1/AE.SQUARROSA (446)	CIGM88.1
116	159582	0	GAN/AE.SQUARROSA (446)	CIGM90.5
117	159583	1	DOY1/AE.SQUARROSA (447)	CIGM88.1
118	159584	1	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (449)	CIGM89.4
119*	154092	1	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)	CIGM88.1
120*	154092	1	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)	CIGM88.1
121	159585	1	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (477)	CIGM88.1
122	159585	1	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (477)	CIGM88.1
123	152422	1	DOY1/AE.SQUARROSA (488)	CIGM88.1
124	159586	1	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)	CIGM88.1
125	154093	1	YAR/AE.SQUARROSA (493)	CIGM89.4

126*	159681	0	SCA/AE.SQUARROSA (493)	CIGM90.5
127	154093	1	YAR/AE.SQUARROSA (493)	CIGM89.4
128	160184	1	DOY1/AE.SQUARROSA (510)	CIGM88.1
129	160185	1	DOY1/AE.SQUARROSA (511)	CIGM88.1
130*	160186	1	68.111/RGB-U//WARD/3/AE.SQUARROSA (511)	CIGM88.1
131	154094	0	DOY1/AE.SQUARROSA (515)	CIGM90.5
132	160187	1	ACO89/AE.SQUARROSA (521)	CIGM89.4
133	160188	0	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (521)	CIGM90.5
134	160189	1	GAN/AE.SQUARROSA (522)	CIGM88.1
135	160190	1	YAR/AE.SQUARROSA (524)	CIGM89.4
136	160192	1	6973/WARD.7463//74110/3/AE.SQUARROSA (35A)	CIGM88.1
137	160193	0	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (629)	CIGM90.5
138	160194	0	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (629)	CIGM90.5
139	160195	0	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (633)	CIGM89.5
140	160195	0	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (633)	CIGM89.5
141	160195	0	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (633)	CIGM89.5
142	160196	0	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (637)	CIGM90.5
143	160197	1	FGO/USA2111//AE.SQUARROSA (658)	CIGM89.5
144*	160198	1	CROC_1/AE.SQUARROSA (662)	CIGM89.5
145	160199	1	CROC_1/AE.SQUARROSA (725)	CIGM89.5
146*	160200	1	CETA/AE.SQUARROSA (742)	CIGM89.5
147	160201	1	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (781)	CIGM89.5
148	160202	1	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (783)	CIGM89.5
149	160203	0	CETA/AE.SQUARROSA (783)	CIGM90.5
150*	160204	0	YAR/AE.SQUARROSA (783)	CIGM90.6
151*	160205	1	CROC_1/AE.SQUARROSA (784)	CIGM89.5
152	160206	0	YUK/AE.SQUARROSA (784)	CIGM90.6
153	160207	0	YAR/AE.SQUARROSA (809)	CIGM90.7
154	160208	1	CETA/AE.SQUARROSA (819)	CIGM89.5
155	160209	1	CROC_1/AE.SQUARROSA (826)	CIGM89.5
156	160210	1	CETA/AE.SQUARROSA (850)	CIGM89.5
157*	160211	0	YUK/AE.SQUARROSA (864)	CIGM90.7
158	160212	1	CETA/AE.SQUARROSA (872)	CIGM89.5
159	160213	0	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	CIGM89.5
160	160213	0	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	CIGM89.5
161	160214	1	CROC_1/AE.SQUARROSA (879)	CIGM89.4
162	160215	1	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (882)	CIGM89.5
163	160216	0	SORA/AE.SQUARROSA (884)	CIGM90.5
164	160217	1	CROC_1/AE.SQUARROSA (886)	CIGM89.5
165	160218	1	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (890)	CIGM89.5
166	160219	0	RABI//GS/CRA/3/AE.SQUARROSA (891)	CIGM90.6
167	160219	0	RABI//GS/CRA/3/AE.SQUARROSA (891)	CIGM90.6
168	160220	0	RABI//GS/CRA/3/AE.SQUARROSA (895)	CIGM90.6
169	160220	0	RABI//GS/CRA/3/AE.SQUARROSA (895)	CIGM90.6
170*	160221	1	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (900)	CIGM89.5
171	160222	0	RABI//GS/CRA/3/AE.SQUARROSA (904)	CIGM90.6
172	160223	0	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (904)	CIGM90.6
173*	160224	1	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (905)	CIGM89.5
174	160225	0	RABI//GS/CRA/3/AE.SQUARROSA (914)	CIGM90.6
175	160226	0	SORA/AE.SQUARROSA (939)	CIGM90.5
176*	160227	1	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (948)	CIGM89.5
177	160228	1	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (949)	CIGM89.5
178	160229	1	CETA/AE.SQUARROSA (954)	CIGM89.5
179	160230	0	YAV_2/TEZ//AE.SQUARROSA (963)	CIGM90.6

180	160231	1	CETA/AE.SQUARROSA (976)	CIGM89.5
181*	160232	0	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/AE.SQUARROSA (518)	CIGM90.5
182	160233	2	CROC_1/AE.SQUARROSA (518)	CIGM86.9 0Y
183	152340	3	PBW114/AE.SQ	-0B-0PR-0
184	154033	3	RUFF/AE.SQ	-0B-0PR-0
185	160602	3	LARU/AE.SQUARROSA (TA2459)	CIGM87.2 0PR-0B
186	161076	3	ALTAR 84/AE.SQUARROSA(Y86-87 S401)	CIGM87.2 0PR-0B
187	161185	4	ALTAR 84/AE.SQUARROSA (JBANGOR)	CIGM86.3 0B-0PR-0
188	161077	1	YAV_2/TEZ//AE.SQUARROSA (249)	CIGM88.1
189	161077	1	YAV_2/TEZ//AE.SQUARROSA (249)	CIGM88.1
190	161077	2	YAV_2/TEZ//AE.SQUARROSA (249)	CIGM88.1
191	161077	3	YAV_2/TEZ//AE.SQUARROSA (249)	CIGM88.1
192	161077	1	YAV_2/TEZ//AE.SQUARROSA (249)	CIGM88.1
193	161077	4	YAV_2/TEZ//AE.SQUARROSA (249)	CIGM88.1
194	161077	3	YAV_2/TEZ//AE.SQUARROSA (249)	CIGM88.1
195	161077	1	YAV_2/TEZ//AE.SQUARROSA (249)	CIGM88.1
196	161077	4	YAV_2/TEZ//AE.SQUARROSA (249)	CIGM88.1
197	161077	3	YAV_2/TEZ//AE.SQUARROSA (249)	CIGM88.1
198	161077	1	YAV_2/TEZ//AE.SQUARROSA (249)	CIGM88.1
199	161077	2	YAV_2/TEZ//AE.SQUARROSA (249)	CIGM88.1
200	159573	1	68112/WARD//AE.SQUARROSA (369)	CIGM88.1
201	159573	2	68112/WARD//AE.SQUARROSA (369)	CIGM88.1
202	159573	2	68112/WARD//AE.SQUARROSA (369)	CIGM88.1
203	161078	1	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (809)	CIGM89.5
204	161078	1	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (809)	CIGM89.5
205	161078	2	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (809)	CIGM89.5
206	161078	2	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (809)	CIGM89.5
207	161078	0	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (809)	CIGM89.5
208	160213	1	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	CIGM89.5
209	160213	0	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	CIGM89.5
210	160213	2	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	CIGM89.5
211	160213	0	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	CIGM89.5
212*	160213	2	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	CIGM89.5
213	160213	3	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	CIGM89.5
214	160213	3	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	CIGM89.5
215	160213	1	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	CIGM89.5
216*	161079	1	CETA/AE.SQUARROSA (895)	CIGM89.5
217*	161079	2	CETA/AE.SQUARROSA (895)	CIGM89.5
218	161079	1	CETA/AE.SQUARROSA (895)	CIGM89.5
219*	161079	0	CETA/AE.SQUARROSA (895)	CIGM89.5
220	161080	0	LCK59.61/AE.SQUARROSA (173)	CIGM90.7
221	161005	0	GAN/AE.SQUARROSA (180)	CIGM90.7
222	161081	0	GAN/AE.SQUARROSA (257)	CIGM90.8
223	161577	0	D67.2/P66.270//AE.SQUARROSA (257)	CIGM90.8
224	161186	0	D67.2/P66.270//AE.SQUARROSA (308)	CIGM90.8
225	161187	0	LCK59.61/AE.SQUARROSA (308)	CIGM90.8
226	161188	0	ARLIN/AE.SQUARROSA (308)	CIGM90.8
227	161189	0	LCK59.61/AE.SQUARROSA (313)	CIGM90.8
228	161578	0	LCK59.61/AE.SQUARROSA (324)	CIGM90.8
229	161579	0	LCK59.61/AE.SQUARROSA (344)	CIGM90.8

230*	161190	0	CPI/GEDIZ/3/GOO//JO/CRA/4/AE.SQUARROSA (358)	CIGM90.8
231	161006	0	SRN/AE.SQUARROSA (358)	CIGM90.8
232*	161191	0	SCOOP_1/AE.SQUARROSA (358)	CIGM90.8
233	161580	0	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (381)	CIGM90.8
234*	161581	0	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (397)	CIGM90.8
235	161192	0	SCOOP_1/AE.SQUARROSA (407)	CIGM90.8
236*	154095	0	GAN/AE.SQUARROSA (408)	CIGM90.8
237*	161193	0	STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (431)	CIGM90.8
238	161582	0	YAV_2/TEZ//AE.SQUARROSA (435)	CIGM90.8
239	161583	0	YAV_2/TEZ//AE.SQUARROSA (437)	CIGM90.8
240	161584	0	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (443)	CIGM90.8
241	161585	0	RABI//GS/CRA/3/AE.SQUARROSA (457)	CIGM90.8
242*	161586	0	YAV_2/TEZ//AE.SQUARROSA (457)	CIGM90.8
243*	161082	0	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (457)	CIGM90.8
244*	161587	0	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (490)	CIGM90.8
245	161083	0	YAR/AE.SQUARROSA (513)	CIGM90.8
246	161588	0	SCA/AE.SQUARROSA (518)	CIGM90.8
247	161589	0	YAR/AE.SQUARROSA (518)	CIGM90.8
248	161084	0	TK SN1081/AE.SQUARROSA (519)	CIGM90.8
249	154096	0	SCA/AE.SQUARROSA (523)	CIGM90.8
250	161085	0	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (528)	CIGM90.8
251	161086	0	BOTNO/AE.SQUARROSA (617)	CIGM90.8
252	161087	0	BOTNO/AE.SQUARROSA (620)	CIGM90.8
253	161590	0	BOTNO/AE.SQUARROSA (625)	CIGM90.8
254	161088	0	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (628)	CIGM90.8
255	161089	0	CIT71/CPT//AE.SQUARROSA (629)	CIGM90.8
256	161194	0	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (629)	CIGM90.8
257*	161591	0	D67.2/P66.270//AE.SQUARROSA (633)	CIGM90.8
258	161091	0	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (633)	CIGM90.8
259	161592	0	SCOOP_1/AE.SQUARROSA (634)	CIGM90.8
260	161593	0	D67.2/P66.270//AE.SQUARROSA (646)	CIGM90.8
261	161594	0	D67.2/P66.270//AE.SQUARROSA (659)	CIGM90.8
262	154097	0	SCOOP_1/AE.SQUARROSA (659)	CIGM90.8
263	161595	0	CETA/AE.SQUARROSA (661)	CIGM90.8
264	161596	0	SCOOP_1/AE.SQUARROSA (662)	CIGM90.8
265	161092	0	6973/WARD.7463//74110/3/AE.SQUARROSA (665)	CIGM90.8
266*	161597	0	CETA/AE.SQUARROSA (665)	CIGM90.8
267	161195	0	ARLIN/AE.SQUARROSA (665)	CIGM90.8
268*	161598	0	BOTNO/AE.SQUARROSA (666)	CIGM90.8
269	161599	0	LCK59.61/AE.SQUARROSA (689)	CIGM90.8
270	161600	0	LCK59.61/AE.SQUARROSA (690)	CIGM90.8
271	161601	0	TK SN1081/AE.SQUARROSA (690)	CIGM90.8
272	161602	0	LCK59.61/AE.SQUARROSA (693)	CIGM90.8
273*	161603	0	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (700)	CIGM90.8
274	161604	0	TRN/AE.SQUARROSA (700)	CIGM90.8
275*	161605	0	LCK59.61/AE.SQUARROSA (783)	CIGM90.9
276	161007	0	CETA/AE.SQUARROSA (796)	CIGM90.9
277	161606	0	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSA (877)	CIGM90.9
278	161607	0	YAV_2/TEZ//AE.SQUARROSA (882)	CIGM90.9
279	161093	0	GAN/AE.SQUARROSA (890)	CIGM90.9
280	161608	0	GAN/AE.SQUARROSA (897)	CIGM90.9
281	161609	0	YAV_2/TEZ//AE.SQUARROSA (895)	CIGM90.9
282	161610	0	GARZA/BOY//AE.SQUARROSA (165)	CIGM92.1
283	161611	0	GARZA/BOY//AE.SQUARROSA (171)	CIGM92.1

284	161612	0	KAPUDE/AE.SQUARROSA (175)	CIGM92.1
285	161613	0	SCOT/MEXI_1//AE.SQUARROSA (186)	CIGM92.1
286	161614	0	GARZA/BOY//AE.SQUARROSA (195)	CIGM92.1
287	161615	0	GARZA/BOY//AE.SQUARROSA (232)	CIGM92.1
288	161616	0	GARZA/BOY//AE.SQUARROSA (233)	CIGM92.1
289	161617	0	GARZA/BOY//AE.SQUARROSA (240)	CIGM92.1
290	161618	0	GARZA/BOY//AE.SQUARROSA (241)	CIGM92.1
291	161619	0	GARZA/BOY//AE.SQUARROSA (265)	CIGM92.1
292	161620	0	GARZA/BOY//AE.SQUARROSA (270)	CIGM92.1
293	161621	0	GARZA/BOY//AE.SQUARROSA (276)	CIGM92.1
294	161622	0	GARZA/BOY//AE.SQUARROSA (278)	CIGM92.1
295	161623	0	GARZA/BOY//AE.SQUARROSA (280)	CIGM92.1
296	161624	0	GARZA/BOY//AE.SQUARROSA (281)	CIGM92.1
297	161625	0	GARZA/BOY//AE.SQUARROSA (283)	CIGM92.1
298	161626	0	ARLIN/AE.SQUARROSA (283)	CIGM92.1
299	161627	0	GARZA/BOY//AE.SQUARROSA (284)	CIGM92.1
300	161628	0	GARZA/BOY//AE.SQUARROSA (287)	CIGM92.1
301	161629	0	6973/WARD.7463//74110/3/AE.SQUARROSA (289)	CIGM92.4
302	161630	0	DOY1/AE.SQUARROSA (293)	CIGM92.1
303*	161631	0	GARZA/BOY//AE.SQUARROSA (294)	CIGM92.1
304	161632	0	ARLIN/AE.SQUARROSA (295)	CIGM92.1
305	161633	0	DVERD_2/AE.SQUARROSA (295)	CIGM92.1
306	161634	0	ROK/KML//AE.SQUARROSA (295)	CIGM92.1
307	161635	0	CROC_1/AE.SQUARROSA (298)	CIGM92.1
308	161636	0	CROC_1/AE.SQUARROSA (299)	CIGM92.1
309	161637	0	GARZA/BOY//AE.SQUARROSA (300)	CIGM92.1
310	161638	0	FALCIN/AE.SQUARROSA (312)	CIGM92.1
311	161639	0	RASCON/AE.SQUARROSA (312)	CIGM92.1
312	161640	0	RASCON/AE.SQUARROSA (314)	CIGM92.1
313	161641	0	KAPUDE/AE.SQUARROSA (314)	CIGM92.1
314	161642	0	SCOT/MEXI_1//AE.SQUARROSA (314)	CIGM92.1
315	161643	0	ARLIN/AE.SQUARROSA (317)	CIGM92.1
316	161644	0	AJAIA/AE.SQUARROSA (330)	CIGM92.1
317	161645	0	ARLIN_1/AE.SQUARROSA (333)	CIGM92.1
318	161646	0	ALTAR 84/AE.SQUARROSA (333)	CIGM92.1
319	161647	0	CROC_1/AE.SQUARROSA (333)	CIGM92.1
320*	161648	0	LARU/AE.SQUARROSA (333)	CIGM92.1
321	161649	0	DVERD_2/AE.SQUARROSA (333)	CIGM92.1
322	161650	0	ROK/KML//AE.SQUARROSA (333)	CIGM92.1
323	161651	0	DOY1/AE.SQUARROSA (333)	CIGM92.1
324	161652	0	KAPUDE/AE.SQUARROSA (341)	CIGM92.1
325*	161653	0	RASCON/AE.SQUARROSA (343)	CIGM92.1
326	161654	0	DOY1/AE.SQUARROSA (349)	CIGM92.1
327	161655	0	GARZA/BOY//AE.SQUARROSA (350)	CIGM92.1
328*	161656	0	GARZA/BOY//AE.SQUARROSA (366)	CIGM92.1
329	161657	0	RASCON/AE.SQUARROSA (367)	CIGM92.1
330*	161658	0	DOY1/AE.SQUARROSA (370)	CIGM92.1
331	161659	0	GARZA/BOY//AE.SQUARROSA (374)	CIGM92.1
332	161660	0	GARZA/BOY//AE.SQUARROSA (375)	CIGM92.1
333	161661	0	RASCON/AE.SQUARROSA (385)	CIGM92.1
334	161662	0	KAPUDE/AE.SQUARROSA (385)	CIGM92.1
335	161663	0	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/AE.SQUARROSA (389)	CIGM92.4
336	161664	0	FALCIN/AE.SQUARROSA (389)	CIGM92.1
337	161665	0	ARLIN/AE.SQUARROSA (410)	CIGM92.1

338	161666	0	DOY1/AE.SQUARROSA (415)	CIGM92.1
339*	161667	0	68.111/RGB-U//WARD/3/AE.SQUARROSA (426)	CIGM92.1
340	161668	0	GARZA/BOY//AE.SQUARROSA (427)	CIGM92.1
341	161669	0	DOY1/AE.SQUARROSA (428)	CIGM92.1
342	161670	0	GARZA/BOY//AE.SQUARROSA (433)	CIGM92.1
343*	161671	0	GARZA/BOY//AE.SQUARROSA (439)	CIGM92.1
344	161672	0	68112/WARD//AE.SQUARROSA (451)	CIGM92.4
345	161673	0	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/AE.SQUARROSA (451)	CIGM92.4
346	161674	0	68.111/RGB-U//WARD/3/AE.SQUARROSA (452)	CIGM92.1
347	161675	0	68.111/RGB-U//WARD/3/AE.SQUARROSA (454)	CIGM92.1
348	161676	0	68.111/RGB-U//WARD/3/AE.SQUARROSA (456)	CIGM92.1
349	161677	0	DOY1/AE.SQUARROSA (458)	CIGM92.1
350*	161678	0	68.111/RGB-U//WARD/3/AE.SQUARROSA (458)	CIGM92.1
351	161679	0	GREEN/AE.SQUARROSA (458)	CIGM92.1
352	161680	0	68.111/RGB-U//WARD/3/AE.SQUARROSA (463)	CIGM92.1
353	161681	0	GARZA/BOY//AE.SQUARROSA (467)	CIGM92.1
354*	161682	0	GARZA/BOY//AE.SQUARROSA (484)	CIGM92.1
355	161683	0	GARZA/BOY//AE.SQUARROSA (503)	CIGM92.1
356*	161684	0	ALTAR 84/AE.SQUARROSA (507)	CIGM92.1
357	161685	0	CROC_1/AE.SQUARROSA (507)	CIGM92.1
358	161686	0	LARU/AE.SQUARROSA (507)	CIGM92.1
359	161687	0	DVERD_2/AE.SQUARROSA (507)	CIGM92.1
360	161688	0	ROK/KML//AE.SQUARROSA (507)	CIGM92.1
361	161689	0	DOY1/AE.SQUARROSA (507)	CIGM92.1
362	161690	0	GARZA/BOY//AE.SQUARROSA (520)	CIGM92.1
363	161691	0	DOY1/AE.SQUARROSA (532)	CIGM92.1
364	161692	0	LCK59.61/AE.SQUARROSA (536)	CIGM92.4
365	161693	0	GAN/AE.SQUARROSA (163)	CIGM93.1
366	161694	0	CROC_1/AE.SQUARROSA (170)	CIMG93.1
367	161695	0	CETA/AE.SQUARROSA (170)	CIGM93.1
368*	161696	0	YAV_2/TEZ//AE.SQUARROSA (170)	CIGM93.1
369	161697	0	ALTAR 84/AE.SQUARROSA (174)	CIGM93.1
370*	161698	0	CETA/AE.SQUARROSA (174)	CIGM93.1
371	161699	0	STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (174)	CIGM93.3
372	161700	0	CROC_1/AE.SQUARROSA (177)	CIGM93.1
373	161701	0	DOY1/AE.SQUARROSA (177)	CIGM93.1
374	161702	0	GAN/AE.SQUARROSA (182)	CIGM93.1
375	161703	0	CROC_1/AE.SQUARROSA (231)	CIGM93.1
376	161704	0	SCA/AE.SQUARROSA (248)	CIGM93.2
377	161705	0	DOY1/AE.SQUARROSA (255)	CIGM93.2
378	161706	0	CROC_1/AE.SQUARROSA (256)	CIGM93.2
379	161707	0	CETA/AE.SQUARROSA (256)	CIGM93.2
380	161708	0	DOY1/AE.SQUARROSA (256)	CIGM93.2
381	161709	0	DOY1/AE.SQUARROSA (258)	CIGM93.2
382	161710	0	GAN/AE.SQUARROSA (259)	CIGM93.2
383	161711	0	DOY1/AE.SQUARROSA (264)	CIGM93.2
384	161712	0	GAN/AE.SQUARROSA (264)	CIGM93.2
385	161713	0	DOY1/AE.SQUARROSA (267)	CIGM93.2
386	161714	0	GAN/AE.SQUARROSA (267)	CIGM93.2
387	161715	0	GAN/AE.SQUARROSA (268)	CIGM93.2
388	161716	0	SCA/AE.SQUARROSA (272)	CIGM93.2
389	161717	0	CROC_1/AE.SQUARROSA (275)	CIGM93.2
390	161718	0	STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (277)	CIGM93.3
391	161719	0	DOY1/AE.SQUARROSA (285)	CIGM93.2

392	161720	0	GAN/AE.SQUARROSA (285)	CIGM93.3
393	161721	0	ALTAR 84/AE.SQUARROSA (304)	CIGM93.3
394	161722	0	SKARV_2/AE.SQUARROSA (304)	CIGM93.3
395	161723	0	DOY1/AE.SQUARROSA (318)	CIGM93.2
396	161724	0	DOY1/AE.SQUARROSA (322)	CIGM93.2
397	161725	0	CETA/AE.SQUARROSA (327)	CIGM93.2
398	161726	0	DOY1/AE.SQUARROSA (334)	CIGM93.2
399	161727	0	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (334)	CIGM93.3
400	161728	0	CROC_1/AE.SQUARROSA (362)	CIGM93.3
401*	161729	0	DOY1/AE.SQUARROSA (372)	CIGM93.2
402	161730	0	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (390)	CIGM93.2
403	161731	0	DOY1/AE.SQUARROSA (390)	CIGM93.3
404	161732	0	AAZ_3/AE.SQUARROSA (398)	CIGM93.3
405	161733	0	CROC_1/AE.SQUARROSA (406)	CIGM93.2
406	161734	0	SCA/AE.SQUARROSA (409)	CIGM93.2
407	161735	0	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (409)	CIGM93.3
408*	161736	0	GAN/AE.SQUARROSA (413)	CIGM93.2
409	161737	0	DOY1/AE.SQUARROSA (418)	CIGM93.2
410	161738	0	STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (418)	CIGM93.2
411	161739	0	CROC_1/AE.SQUARROSA (436)	CIGM93.2
412	161740	0	CROC_1/AE.SQUARROSA (444)	CIGM93.2
413*	161741	0	GAN/AE.SQUARROSA (459)	CIGM93.2
414	161742	0	CROC_1/AE.SQUARROSA (466)	CIGM93.2
415*	161743	0	CROC_1/AE.SQUARROSA (481)	CIGM93.2
416	161744	0	DOY1/AE.SQUARROSA (489)	CIGM93.2
417	161745	0	CETA/AE.SQUARROSA (499)	CIGM93.2
418*	161746	0	STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (502)	CIGM93.2
419	161747	0	ALTAR 84/AE.SQUARROSA (502)	CIGM93.3
420	161748	0	STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (502)	CIGM93.3
421	161749	0	CROC_1/AE.SQUARROSA (516)	CIGM93.2
422	161757	0	DOY1/AE.SQUARROSA (516)	CIGM93.2
423	161750	0	CROC_1/AE.SQUARROSA (517)	CIGM93.2
424	161751	0	DOY1/AE.SQUARROSA (517)	CIGM93.2
425	161752	0	CETA/AE.SQUARROSA (525)	CIGM93.2
426	161753	0	DOY1/AE.SQUARROSA (526)	CIGM93.2
427	161754	0	68.111//RGB-U/WARD/3/FG/4/RABI/5/AE.SQUARROSA (535)	CIGM93.2
428	161755	0	ARLIN_1/AE.SQUARROSA (536)	CIGM93.2
429*	161756	0	CETA/AE.SQUARROSA (540)	CIGM93.3
430	161758	0	CROC_1/AE.SQUARROSA (1008)	CIGM93.4
431*	161759	0	CETA/AE.SQUARROSA (1011)	CIGM93.2
432	161760	0	DOY1/AE.SQUARROSA (1011)	CIGM93.2
433	161761	0	ALTAR 84/AE.SQUARROSA (1012)	CIGM93.2
434	161762	0	DOY1/AE.SQUARROSA (1016)	CIGM93.2
435*	161763	0	DVERD_2/AE.SQUARROSA (1016)	CIGM93.4
436	161764	0	CETA/AE.SQUARROSA (1016)	CIGM93.4
437	161765	0	DVERD_2/AE.SQUARROSA (1022)	CIGM93.2
438	161766	0	CETA/AE.SQUARROSA (1022)	CIGM93.2
439	161767	0	CROC_1/AE.SQUARROSA (1023)	CIGM93.4
440	161768	0	CETA/AE.SQUARROSA (1024)	CIGM93.2
441*	161769	0	DOY1/AE.SQUARROSA (1024)	CIGM93.2
442	161770	0	CETA/AE.SQUARROSA (1025)	CIGM93.2
443	161771	0	DVERD_2/AE.SQUARROSA (1027)	CIGM93.3
444	161772	0	DOY1/AE.SQUARROSA (1027)	CIGM93.3
445	161773	0	CETA/AE.SQUARROSA (1027)	CIGM93.4

446*	161774	0	CETA/AE.SQUARROSA (1030)	CIGM93.3
447	161775	0	DOY1/AE.SQUARROSA (1030)	CIGM93.3
448	161776	0	CETA/AE.SQUARROSA (1042)	CIGM93.3

[a] Entries included in the set of 81 lines sent for DArT analysis shown by *

Appendix 2. Genotypes of CIMMYT synthetic hexaploid lines at the *Ppd* loci.

	Ppd-A1		Ppd-D1	
Ent	1027bp	1117bp	2kb deletion	16bp del ex.8
1	N	Y	415	Y
2	N	Y	454	N
3	N	Y	454	N
4	N	Y	454	N
5	N	Y	430	N
6	N	Y	430	N
7	N	Y	?	N
8	N	Y	415	N
9	N	Y	415	N
10	Y	N	415	N
11	N	Y	415	N
12	N	Y	415	N
13	N	Y	415	N
14	N	Y	415	N
15	N	Y	415	N
16	N	Y	415	N
17	N	Y	454	N
18	N	Y	454	N
19	N	Y	430	N
20	N	Y	415	N
21	N	Y	454	N
22	N	Y	415	N
23	N	Y	454	N
24	N	Y	454	N
25	N	Y	430 / 454	N
26	N	Y	430	N
27	N	Y	430	N
28	N	Y	454	N
29	N	Y	297 / 415	HET
30	N	Y	415	N
31	N	Y	415	HET
32	N	Y	415	N
33	N	Y	415	N
34	N	HET	415	N
35	N	N	415	N
36	N	Y	415	N
37	N	N	415	N
38	N	Y	430	N
39	Y	N	430	N
40	N	Y	415 / 430	N
41	N	Y	415	N
43	N	Y	430	N
44	N	N	430	N
45	N	Y	430	N
46	N	Y	415 / 430	N
47	N	HET	415 / 454	N
48	N	Y	454	N
49	?	Y	415	N
50	N	N	454	N
51	N	HET	297	HET
52	N	Y	297 / 454	N

53	N	N	454	N
54	Y	N	454	N
55	N	N	454	N
56	N	Y	430	N
57	?	Y	454	N
58	Y	N	454	N
59	N	N	297 / 454	N
60	N	Y	454	N
61	N	Y	454	N
62	N	Y	430 / 454	N
63	N	Y	454	N
64	?	Y	454	N
65	?	Y	415	N
66	N	Y	415	N
67	N	Y	415 / 454	HET
68	N	Y	415 / 454	HET
69	N	Y	415 / 454	N
70	N	Y	454	N
71	N	Y	454	N
72	N	Y	415 / 454	N
73	N	?	297	Y
74	N	HET	415 / 454	HET
75	N	N	454	N
76	N	Y	454	N
77	N	Y	454	N
78	N	N	454	N
79	N	Y	454	N
80	N	Y	454	N
81	N	N	297 / 415	N
82	N	Y	415	N
83	N	Y	415	N
84	N	Y	415	N
85	N	Y	415	N
86	N	N	415	N
87	N	Y	415	N
88	?	N	454	N
89	?	N	415	Y
90	N	Y	415	Y
91	N	Y	430	N
92	N	Y	430	N
93	N	N	454	N
94	N	N	454	N
95	N	N	415	Y
96	N	Y	415	N
97	N	Y	415	N
98	N	N	415	Y
99	N	Y	454	N
100	N	Y	415	N
101	N	Y	454	N
102	N	Y	454	N
103	N	Y	454	N
104	N	Y	454	N
105	N	N	415	Y
106	N	Y	454	N

107	N	Y	454	N
108	N	Y	454	N
109	N	Y	454	N
110	N	Y	454	N
111	N	Y	454	N
112	N	Y	415	Y
113	N	Y	415	N
114	N	Y	454	N
115	N	Y	415	N
116	N	Y	415	N
117	N	Y	415	Y
118	N	HET	297	HET
119	N	Y	415	Y
120	N	Y	415	N
121	N	Y	415	N
122	N	Y	415	N
123	N	Y	415	HET
124	N	Y	415	N
125	N	HET	415	Y
126	N	Y	415	N
127	N	Y	415	N
128	N	Y	415	N
129	N	Y	415	N
130	N	Y	430	N
131	N	Y	430	N
132	N	HET	454	N
133	Y	N	415 / 454	N
134	Y	N	415	N
135	N	Y	430	N
136	N	N	454	N
137	N	Y	454	N
138	N	Y	HET	N
139	N	Y	454	N
140	N	Y	415	HET
141	N	Y	454	N
142	N	Y	415	N
143	N	Y	415	N
144	N	Y	415	Y
145	N	Y	?	N
146	N	Y	454	N
147	N	Y	430	N
148	N	Y	430	N
149	N	Y	430	N
150	N	Y	430	N
151	N	Y	430	N
152	N	Y	415	HET
153	N	Y	415	Y
154	N	HET	454	N
155	N	Y	HET	N
156	N	Y	HET	N
157	N	HET	HET	N?
158	N	Y	415 / 454	N?
159	Y	N	415	N
160	HET	N	415	HET

161	N	?	415	N
162	N	N	415	N
163	Y	N	297	HET
164	N	Y	454	N
165	N	Y	430	N?
166	N	Y	415	N
167	N	Y	415	N
168	N	Y	297	Y
169	N	N	297	HET
170	Y	N	454	N
171	N	Y	454	N
172	N	HET	?	N
173	Y	N	415	Y
174	N	Y	415	Y
175	N	Y	454	N
176	Y	N	415	N
177	Y	N	415	N
178	N	Y	454	N
179	N	Y	454	N
180	N	HET	454	N
181	N	Y	415	N
182	Y	N	430	N
183	Y	N	415	Y
184	N	Y	415	N
185	N	Y	430	N
186	N	Y	HET	N
187	N	Y	430	N
188	N	HET	454	Y
189	N	Y	454	N?
190	N	N	454	N
191	N	Y	454	N
192	N	Y	454	N
193	N	Y	454	N
194	N	Y	454	N
195	N	Y	454	N
196	N	Y	454	N
197	N	Y	454	?
198	N	Y	454	N
199	N	Y	454	N
200	N	N	454	N
201	N	Y	?	N
202	N	Y	454	N
203	Y	N	415	Y
204	Y	N	415	Y
205	N	N	297	Y
206	Y	N	415	Y
207	Y	N	415	Y
208	Y	N	415	N
209	N	N	297	HET
210	N	N	297	Y
211	Y	N	415	N
212	Y	N	415	N
213	N	N	415	N
214	Y	N	297	Y

215	Y	N	415	N
216	N	HET	454	N
217	N	N	454	N
218	N	N	297	Y
219	N	N	297 / 413	HET
220	N	N	415	N
221	N	Y	430	N
222	N	Y	454	N
223	N	N	415	N
224	N	N	415	N
225	N	N	415	N
226	N	N	415	N
227	N	N	430	N
228	N	N	454	N
229	N	N	454	N
230	N	Y	415	N
231	N	Y	415	N
232	N	Y	415	N
233	N	Y	415	N
234	N	Y	413 / 454	N
235	N	Y	430	N
236	N	Y	430	N
237	N	Y	430 / 454	N
238	N	HET	413 / 454	N
239	N	Y	415	Y
240	N	Y	415	N
241	N	Y	415	N
242	N	Y	415	N
243	N	Y	413 / 430	N
244	N	Y	415	N
245	N	N	415	Y
246	N	Y	454	N
247	N	Y	454	N
248	Y	N	454	N
249	N	HET	454	N
250	N	Y	415	N
251	N	N	430	?
252	N	N	430	?
253	N	HET	430	N
254	N	Y	297 / 413	HET
255	N	N	454	N
256	N	Y	454	N
257	N	N	454	HET
258	N	HET	454	N
259	N	Y	454	N
260	N	N	415	N
261	N	N	415	N
262	N	Y	415	N
263	N	Y	454	N
264	N	Y	415	Y
265	N	Y	415	Y
266	N	Y	413 / 454	HET
267	N	N	415	Y
268	N	N	430	N

269	N	N	430	N
270	N	N	415	N
271	HET	Y?	415	N
272	N	N	415	HET
273	N	Y	415	N
274	N	HET	415	N
275	N	N	454	N
276	N	HET	413 / 454	HET
277	N	Y	415	N
278	N	HET	415	HET
279	Y	Y?	413 / 454	N
280	N	Y	415	N
281	N	Y	454	N
282	N	HET	297 / 454	N
283	N	N	454	N
284	N	Y	454	N
285	N	Y	454	N
286	N	N	454	N
287	N	Y?	454	N
288	N	N	454	N
289	N	N	454	?
290	N	N	415 / 454	HET
291	N	N	454	N
292	N	HET	454	?
293	N	N	454	N
294	N	N	454	N
295	N	N	415 / 454	?
296	N	N	454	N
297	N	N	454	N
298	N	Y	454	N
299	N	N	454	N
300	N	N	454	N
301	N	N	454	N
302	N	Y	454	N
303	N	N	454	N
304	N	Y	454	N
305	N	Y	454	N
306	N	Y	454	N
307	N	Y	454	N
308	N	Y	454	N
309	N	N	454	N
310	N	Y	415	N
311	N	Y	454	N
312	N	Y	454	N
313	N	HET	297	HET
314	N	Y	454	HET?/N?
315	N	Y	415 / 454	Y
316	N	Y	415	N
317	N	Y	454	N
318	N	Y	454	N
319	N	Y	454	N
320	N	Y	454	N
321	N	Y	454	N
322	Y	N	454	N

323	N	Y	454	N
324	N	Y	415	N
325	N	Y	415	N
326	N	HET	454	HET
327	N	N	454	N
328	N	N	454	N
329	N	Y	415	HET
330	N	N	415	Y
331	N	N	454	N
332	N	N	415 / 454	N
333	N	Y	415	N
334	N	Y	415	N
336	N	Y	454?	N
337	N	Y	454?	N
338	550bp band?	Y	454?	N
339	N	Y	415	N
340	N	N	HET	HET
341	N	Y	415	N
342	N	N	454	N
343	N	Y	454	N
344	N	N	454	N
345	N	HET	415	N
346	N	Y?	415	N
347	N	Y	415	N
348	N	Y	415 / 454	N
349	N	Y	415	N
350	Y	N	415	Y
351	N	Y	415	Y
352	N	HET	415	N
353	N	N	454	N
354	N	N	454	N
355	N	N	454	N
356	N	Y	454	N
357	N	Y	454	N
358	N	Y	454	N
359	Y	HET?	454	N
360	Y	HET?	454	N
361	N	Y	454	N
362	N	N	454	N
363	N	Y	454	N
364	N	N	454	N
365	N	Y	454	N
366	N	Y	454	N
367	N	Y	454	N
368	N	Y	454	N
369	N	Y	415	N
370	N	Y	415	N
371	N	Y	415	N
372	N	Y	454	N
373	N	Y	454	N
374	N	Y	454	N
375	N	Y	454	N
376	N	Y	454	N
377	N	Y	454	N

378	N	Y	297	HET
379	N	Y	454	N
380	N	Y	297 / 454	N
381	N	HET	297	HET
382	N	Y	454	N
383	N	Y	454	N
384	N	Y	454	N
385	N	Y	454	N
386	N	Y	454	N
387	N	Y	454	N
388	N	Y	454	N
389	N	Y	454	N
390	N	Y	454	N
392	N	HET	297 / 454	HET
393	N	Y	415	N
394	N	Y	415	N
395	N	HET	297 / 415	?
396	N	HET	HET	HET
397	N	Y	454	N
398	N	Y	415	Y
399	N	HET	297 / 415	Y
400	N	Y	297	Y
401	N	Y	454	N
402	N	Y	415	N
403	N	Y	297 / 415	N
404	N	HET	430	HET
405	N	Y	454	N
406	N	Y	454	N
407	?	HET	454	N
408	N	Y	297 / 454	N
409	N	N	454	N
410	N	N	454	N
411	N	Y	415	N
412	N	Y	415	N
413	N	Y	454	N
414	N	Y	454	N
415	N	Y	454	N
417	N	N	454	N
418	N	Y	415	N
419	N	Y	454	N
420	N	Y	415	N
421	N	Y	415	N
422	N	Y	415	N
423	N	Y	415	N
424	N	Y	415	N
425	N	Y	454	N
426	N	Y	454	N
428	?	Y	454	N
429	N	Y	415	N
430	N	Y	454	N
431	N	Y	454	N
433	N	Y	454	N
434	N	Y	415	N
435	N	Y	415	N

436	N	N	415	N
437	N	Y	415	N
438	N	Y	415	N
439	N	Y	454	N
440	N	Y	454	N?
441	N	N	415 ./ 454	N
442	N	Y	454	N
443	N	Y	454	N
444	N	Y	454	N
445	N	Y	454	N
446	N	Y	454	N
447	N	Y	?	N
448	N	Y	454	N
Paragon	N	N	415	Y
Xi19	N	N	415	Y

Key

Ppd-A1

1027bp deletion

1117bp deletion

Intact Ppd-A1

Heterozygote

? Not determined

Ppd-D1

Wild-type intact (2kb deletion) = sensitive

Wild-type intact+24bp+15bp insertion (2kb deletion) = sensitive

Wild-type intact+15bp insertion (2kb deletion) = sensitive

Mutant (2kb deletion) = insensitive

No 16bp deletion = *Ae. tauschii* type

16bp deletion = cultivated type (outcrossed)

Heterozygote

? Not determined

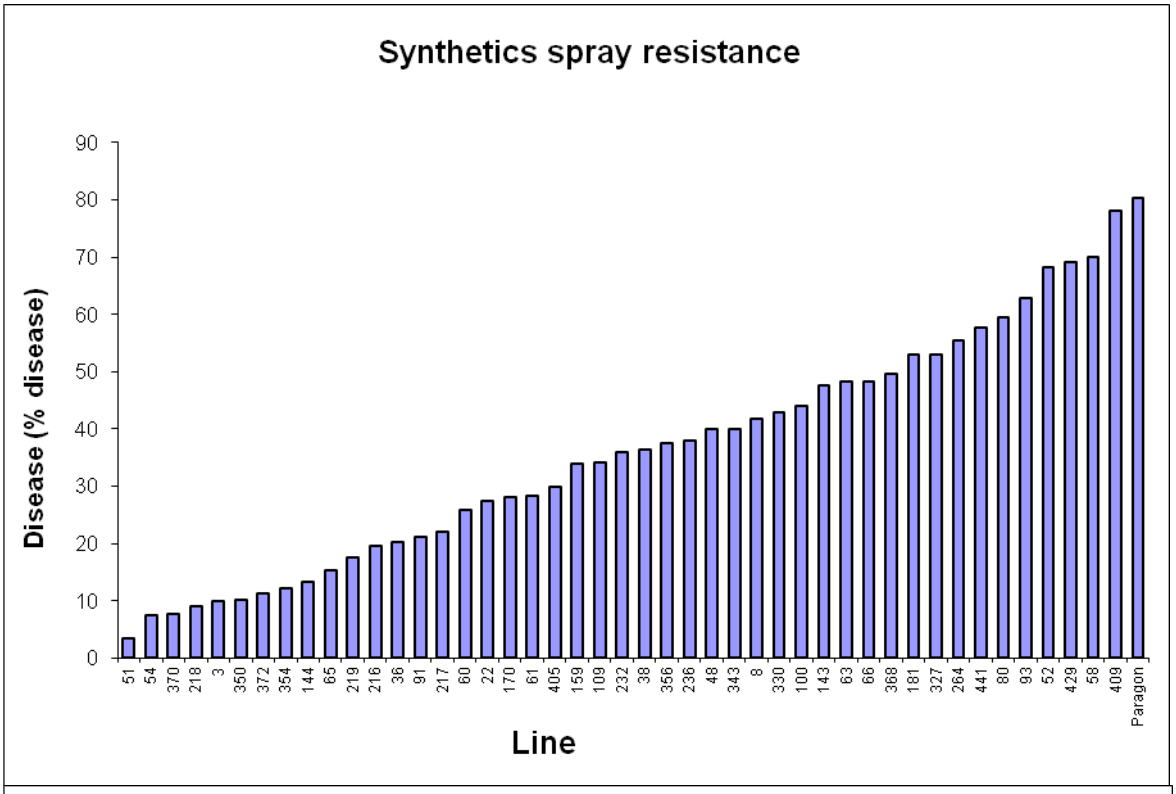
Appendix 3: High molecular weight glutenin sub-unit profiles of the CIMMYT SHWs used in crosses

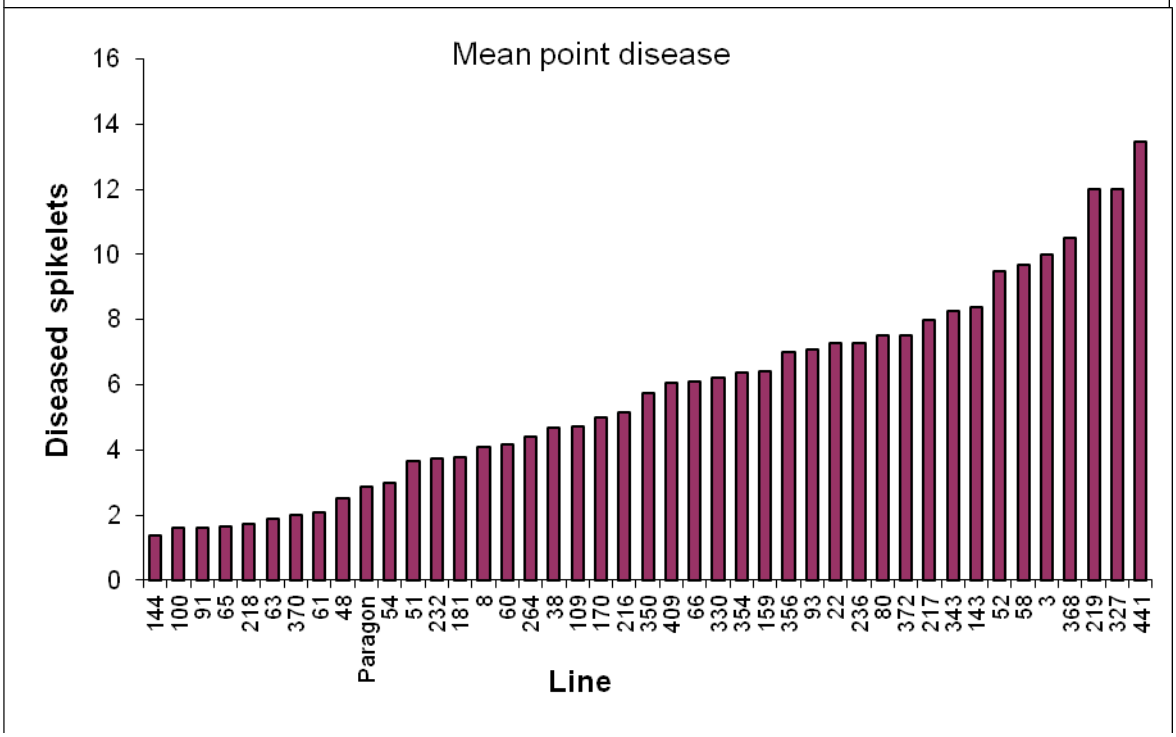
SHW	1A	1B	1B segregant	1D	1D segregant	Notes
SHW-003	N	7+8		3+10		
SHW-008	21*?	7+8		2+10?		
SHW-022	N	7+8		2+10?		
SHW-036	N	7+8		2+10?		
SHW-038	N	7+8		2+10?		
SHW-048	21*?	7+8		3+10?		
SHW-051	N	7+8		2+12		
SHW-052	N	7+8		2+12		
SHW-054	21*?	6+8		3+10?		
SHW-058	N	17+18		2+12		
SHW-060	N	7+8	20?	2+12	?	

SHW-061	N	7+8		2+12	3+12	
SHW-062	21*?	7+8		2+12		
SHW-063	21*?	7+8		2+12		
SHW-065	N	7+8		2?+10	2?+12	2 looks a bit high
SHW-066	21*?	7+8		2+10		
SHW-079	N	7+8		3+10		
SHW-080	N	7+8		3+10		
SHW-091	N	7+8		2?+11		2 looks a bit high
SHW-093	N	20	9	2+12		
SHW-100	N	20		5+10		
SHW-109	N	7+8		2?+11		
SHW-120	N	6+8		2?+10		
SHW-126	N	20		2+10		
SHW-143	N	6+8		2+11		
SHW-144	21*?	7+8		3+10		
SHW-159	N	6+8		2+10		
SHW-170	21*?	6+8		2+10		
SHW-173	N	6+8	20	5+10		
SHW-176	N	6+8		2+10		
SHW-181	21*?	7+8		3+?		band much lower than 12
SHW-216	N	14+15		2?+11	3+11?	
SHW-217	N	14+15		2?+11	3+11?	
SHW-218	N	14+15		2?+11	3+11?	
SHW-219	2*?	6+8	14+15	5+10	2+10?	2+10 or 2*
SHW-232	N	6+8		2?+10		
SHW-236	N	6+8		2?+10		
SHW-237	N	20		2?+10		
SHW-264	21*?	6+8		3+10		
SHW-330	21*?	6+8		3+10		
SHW-339	N	6+8		2+12		
SHW-343	N	6+8		3+10		
SHW-350	21*?	6+8		2+10		
SHW-354	N	6+8		3+10		
SHW-356	N	7+8		3+10		
SHW-368	N	7+8		3+10		
SHW-370	N	20		5+10		

SHW-372	N	7+8		2+10		
SHW-405	N	7+8		2+10		
SHW-409	N	7+8	20	2+11		
SHW-429	N	7+8		2?+11		
SHW-441	**Grain sample not tested**					

Appendix 4. Fusarium head blight resistance, point and spray inoculation, of CIMMYT synthetic hexaploids used in backcrossing





Appendix 5. Yellow rust disease scores of SHW lines

SHW	Seedling test			Adult field test YR%				
	14 dpi	18 dpi	**Mean**	27/5	2/6	8/6	14/6	**Mean**
SHW-003	3.0	3.0	**3.0**	0	1	1	3	**1.3***
SHW-008	0	0	**0.0**	0	0	0	0	0.0*
SHW-022	2.3	2.7	**2.5**	0.1	0.1	0	5.1	**1.3***
SHW-036	1.4	1.9	**1.7**	0.1	0.1	0.1	*	**[0.1]***
SHW-038	1.3	0.9	**1.1**	0	0	0.1	0.6	**0.2***
SHW-048	0.6	0.6	**0.6**	0	10	10	10	**[7.5]***
SHW-051	0	0	**0.0**	1.5	6	7.5	10	**6.25**
SHW-052	1.7	2	**1.9**	0.1	0.1	1.1	3.5	**1.2**
SHW-054	2.1	2.9	**2.5**	0.1	0.1	0	0	**0.1***
SHW-058	0	0	**0.0**	0.1	0.1	0.1	0.1	0.1
SHW-060	0.3	1.1	**0.7**	1.1	5.1	5.1	8	**4.8***
SHW-061	1	1.1	**1.1**	1	2.5	2.5	3.5	**2.375**
SHW-062	0.6	0.6	**0.6**	0	0.1	0.1	0.5	**0.175**
SHW-063	1.5	2	**1.8**	0	0.5	2.5	2.5	**1.375**
SHW-065	0	0	**0.0**	0	0	0	0	0.0*
SHW-066	0	0	**0.0**	0	1	3.5	4.5	**2.25**
SHW-080	3	3	**3.0**	0	0	2	2	**[1.0]***
SHW-091	0	0	**0.0**	0.1	0.1	1	6	**1.8***
SHW-093	3	3	**3.0**	7.5	17.5	32.5	50	**26.875**
SHW-100	2.9	2.9	**2.9**	0.1	0.1	0.1	1	**0.325**
SHW-109	0	0	**0.0**	0.1	0.1	0	0.1	0.1
SHW-143	0	0	**0.0**	0.6	0.6	0.5	1	**0.675**
SHW-144	2.3	2.4	**2.4**	5	8.5	12.5	12.5	**9.625**
SHW-159	0.7	1.1	**0.9**	2.5	5	7.5	22.5	**9.375**
SHW-170	2.75	2.75	**2.8**	6	22.5	22.5	47.5	**24.625**
SHW-181	2.3	2.3	**2.3**	6	15	25	32.5	**19.625**
SHW-216	3	3	**3.0**	0.1	0.1	0	0	**0.05**
SHW-217	2.5	2.2	**2.4**	0.1	0.1	0	0.1	**0.075**
SHW-218	2	2	**2.0**	2.6	3.5	3.5	3.5	**3.275**
SHW-219	0.1	0.5	**0.3**	0	0.1	0.6	0.5	**0.3**
SHW-232	0.1	0.1	**0.1**	2	3.5	5	6	**4.125**
SHW-236	0.3	0.1	**0.2**	0	0	0.1	0	**0.025**
SHW-237	0	0	**0.0**	0.1	0.1	0.5	0.5	0.3
SHW-264	2.9	2.9	**2.9**	12.5	22.5	25	25	**21.25**
SHW-330	0	0	**0.0**	0.1	0.1	1	6.5	**1.925**
SHW-343	0	0	**0.0**	0.1	0.1	0.6	0.5	**0.325**
SHW-350	2.3	2.3	**2.3**	0.1	5	8.5	17.5	**7.8***

SHW-354	2	2.8	**2.4**	*	*	*	*	****
SHW-356	2.5	2.5	**2.5**	0	10	10	25	**[11.3]***
SHW-368	3	3	**3.0**	0	15	15	17.5	**11.9***
SHW-370	0.8	0.6	**0.7**	0	1	2	2	**1.3***
SHW-372	3	3	**3.0**	0	0	3	3.5	**1.6***
SHW-405	3	3	**3.0**	0.5	8.5	8.5	8.5	**6.5***
SHW-409	0	0	**0.0**	0.1	0.1	1	1	**0.55**
SHW-441	2.3	2.3	**2.3**	3.5	15	17.5	27.5	**15.875**
Paragon	1.1	1.3	**1.2**	0	0	0.1	1	**0.275**
Xi19	2.3	2.2	**2.3**	0	0	0.1	0.1	**0.1***
SOLSTICE	3.2	3.2	**3.2**	4	7.5	12.5	15	**9.75**
OAKLEY	3	3	**3.0**	5	15	20	22.5	**15.625**
ROBIGUS	3	3	**3.0**	5	17.5	22.5	25	**17.5**
VUKA	3.1	3.1	**3.1**	****	****	****	****	****
Brock				1	0.5	0.6	1.1	**0.8**
Alexandria				8.5	22.5	25	27.5	**20.875**
Timber				0.1	0.1	0.1	1.5	**0.45**
Napier				0.1	5	7	10	**5.525**