Introgression of genetic material from primary synthetic hexaploids into an Australian bread wheat (*Triticum aestivum* L.)

A thesis submitted in fulfilment of the requirements for the degree of Master of Agricultural Science at the University of Adelaide

By

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March 2011

Abbreviations

CIMMYT : International Maize and Wheat Improvement Center
MPBCRC : Molecular Plant Breeding Cooperative Research Centre
AGT : Australian Grain Technologies
GRDC : Grains Research and Development Corporation
SARDI : South Australian Research and Development Institute
AWCC : Australian Winter Cereals Collection
BC : Backcross
BLUP : Best linear unbiased prediction
DArT : Diversity Array Technology™
SSR : Simple sequence repeats
AFLP : Amplified fragment length polymorphisms
QTL : Quantitative trait loci

Table of Contents

List of Tables

List of Figures

List of Appendices

Abstract

Primary synthetic wheats, created by hybridising *Triticum turgidum* L. with *Aegilops tauschii* Coss., the evolutionary progenitors of bread wheat (*Triticum aestivum* L.), have shown potential value for use in Australian bread wheat breeding. This study investigated primary and derived synthetic wheats in three ways to further evaluate this value. To determine whether primary synthetic wheats could broaden genetic diversity in Australian bread wheats, genetic similarity between and among 44 primary synthetics and nine modern Australian bread wheats was investigated using Diversity Array Technology™ (DArT). Greater dissimilarity was observed between these germplasm groups than within these groups. The A and B genomes of the primary synthetics were most divergent from the genomes of the bread wheats. These primary synthetics therefore could broaden the genetic diversity in Australian bread wheats. To identify primary synthetic wheats that could improve grain yield of an Australian bread wheat in drought, grain yield and its major components (grain weight and grains per m^2) were measured in 27 BC_1 synthetic-derived families of lines in five drought stressed environments in southern Australia. Fourteen families included lines with significantly ($p<0.05$) higher grain yield than Yitpi (recurrent bread wheat parent). These lines produced grain yields up to 12.0 % higher than Yitpi in the highest yielding environments, where improved grain weights were responsible. In the lowest yielding environments, superior synthetic derivatives achieved grain yields up to 43.8 % higher than Yitpi, with more grains per m^2 commonly responsible. Therefore, many but not all of the primary synthetics assessed could improve grain yield of an Australian bread wheat in drought. To gain an understanding of synthetic allele introgression into the genetic background of an Australian bread wheat, DArT loci were assayed in two families of synthetic backcross lines. Approximately half of the same loci assayed in each family showed synthetic allele introgression. At regions on chromosome 2A in both families, synthetic alleles were positively associated with grain weight and grain per m^2. It was concluded that primary and derived synthetic wheats can have broad value to bread wheat breeding in Australia.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Stephen John Talbot
March 2011

Acknowledgements

I would like to thank my supervisors, Professor Diane Mather, Associate Professor Kenneth Chalmers and Dr Francis Ogbonnaya for their academic supervision. I greatly appreciated the teachings from Professor Andrew (Andy) Barr, who was my initial primary supervisor before a change in his work commitments.

I thank the trustees of the South Australian Grains Industry Trust (SAGIT) for providing the funding for my project through the Molecular Plant Breeding Cooperative Research Centre (MPBCRC). MPBCRC provided professional development during my candidature, which was in-part led by Dr Heather Bray, a friend and supporter during my studies.

I am indebted to the staff of Australian Grain Technologies (AGT), in particular Dr Haydn Kuchel, Dr Jason Reinheimer and Simeon Hemer, who provided much guidance, logistical help and expert knowledge during my studies. AGT provide in-kind field support that made my field endeavours possible.

The following people and organisations have been important contributors to my project:

- Paul Eckermann and Dr Bev Gogel (GRDC National Statistics Program) for their advice and support on trial design and analysis,
- Dr Harbans Bariana (Plant Breeding Institute, University of Sydney) for disease nursery and glasshouse screening support,
- Jim Lewis (SARDI) for encouragement and the use of equipment,
- Barley Program (University of Adelaide) for the use of equipment,
- Members of the Molecular Marker Lab (University of Adelaide), in particular Elysia Vassos and Dr Genet Mekuria for their friendship and encouragement.

I would like to thank my parents, Graham and Helen Talbot for encouraging me to always do my best, and my siblings, extended family and friends for their ongoing support.

Professional editorial advice was sought from Mrs Margaret Cargill (Adjunct Senior Lecturer, University of Adelaide) on spelling, grammar, sentence and paragraph structure and language clarity. This advice was restricted to standards D and E of the Australian Standards for Editing Practice (ASEP). Mrs Margaret Cargill’s current or former area of academic specialisation is not similar to that presented in this thesis.

Chapter 1
General Introduction

Since the initial investigations by McFadden and Sears (1944) into the origins of bread wheat (*Triticum aestivum* L.), it has been known that the genetic diversity of this important food crop plant has the potential to extend beyond its own genetic base to also include *Triticum turgidum* L. and *Aegilops tauschii* Coss. Bread wheat is an allohexaploid and has evolved from multiple but limited hybridisation events between *T. turgidum* and *Ae. tauschii* (McFadden and Sears 1946; Dvorak *et al.* 1998; Talbert *et al.* 1998). These progenitor species are more genetically diverse than bread wheat (Lubbers *et al.* 1991; Reif *et al.* 2005; Chabane *et al.* 2007), and possess genetic variation for traits that would be desirable in modern bread wheat cultivars (van Ginkel and Ogbonnaya 2007). The progenitor species of bread wheat are not readily used by breeders to develop new bread wheat cultivars. Modern breeding programs exploit adapted bread wheats as primary sources of germplasm. This is done to maintain characters within breeding pools that confer adaptation and high end use quality. Genetic introgressions from unadapted cultivars (secondary germplasm source) and *T. turgidum* and *Ae. tauschii* selections (tertiary germplasm sources) may express deleterious traits in target environments. However, limited trait variation in adapted germplasm and limited access to unadapted sources with known agronomic performance is increasingly inhibiting breeders from developing improved cultivars, for changing environments and for the requirements of growers and consumers (Gollin *et al.* 2000; van Ginkel and Ogbonnaya 2007). The introgression of genetic material from *T. turgidum* and *Ae. tauschii* into the background of adapted bread wheats may therefore be an option to introduce valuable variation for breeding new cultivars.

Several strategies exist to develop bridging lines that facilitate the introgression of generic material from *T. turgidum* and *Ae. tauschii* into bread wheat. Such bridging lines can be produced from 'direct crossing' strategies between bread wheat and *T. turgidum* or *Ae. tauschii*, however these strategies commonly require embryo rescue and several generations of backcrossing and self pollination to produce genetically stable lines (Reinhold *et al.* 1983; Gill and Raupp 1987; Cox *et al.* 1991; Reader and Miller 1991; Cox *et al.* 1995; Knott *et al.* 2005). These activities can be time and resource consuming. An alternative strategy is to hybridise *T. turgidum* with *Ae. tauschii*, producing bridging lines called 'primary synthetic wheat'. This strategy does not require multiple generations of

backcrossing or self pollination to make primary synthetic wheat genetically stable, but only the possible use of embryo rescue and/or a simple colchicine treatment for chromosome doubling (Liu *et al.* 2002; Matsuoka and Nasuda 2004; Mujeeb-Kazi *et al.* 2008). As primary synthetic wheats can be readily crossed with bread wheat (Lange and Jochemsen 1992), they can act as practical breeding tools for simultaneously exploiting the genomes of selected *T. turgidum* and *Ae. tauschii* cultivars and wild accessions.

In Australia primary synthetic wheats have the potential to improve the adaptation of local bread wheat cultivars. Primary synthetics have been reported to express genetic variation for traits that would be desirable in Australian bread wheat cultivars, which include drought tolerance (Reynolds *et al.* 2007), heat stress tolerance (Yang *et al.* 2002) and enhanced grain yield components (Villareal *et al.* 1994a; Villareal *et al.* 1994b). However some primary synthetics exhibit deleterious traits, such as poor threshability and excessive height (Pritchard *et al.* 2002; Tyagi *et al.* 2004; van Ginkel and Ogbonnaya 2007). Backcrossing and selection strategies can be used to introgress genetic material from primary synthetic wheat into deficient bread wheat cultivars. Using primary synthetic wheat as the donor parent and an Australian bread wheat cultivar as the recipient and recurrent parent can produce enhanced synthetic backcross lines. Genetically these lines would mainly constitute the recurrent bread wheat parent with introgression segments from the primary synthetic parent (Allard 1960; Tanksley and Nelson 1996).

In light of the breeding potential of primary synthetic wheats to develop improved Australian bread wheat cultivars there are three key research areas that need investigating, which include 1. genetic diversity, 2. grain weight and grain yield under drought stressed environments, and 3. introgression of synthetic alleles. To elaborate, Australian bread wheats are more genetic diverse than those from western Europe, the United Kingdom and the United States of America (Akbari *et al.* 2006; White *et al.* 2008), however investigations into whether primary synthetic wheats can be a useful resource to broaden the genetic diversity of modern Australian bread wheats have yet to be performed. Under a range of Australian environments, synthetic derived material, namely synthetic backcross lines, have achieved superior grain yields compared to their recurrent Australian bread wheat parent (Gororo *et al.* 2002; Dreccer *et al.* 2007; Ogbonnaya *et al.* 2007). The studies by Dreccer *et al.* (2007) and Ogbonnaya *et al.* (2007), which investigated a large number of synthetic backcross lines, did not report the major grain yield component(s) responsible for these superior grain yields, nor the influence of different primary synthetic parents on

these components. The potential of primary synthetic wheats to increase yield of an Australian bread wheat in drought stressed environments in southern Australia has also not been assessed before. Molecular marker analysis of synthetic backcross lines evaluated in environments outside of Australia has identified introgressed primary synthetic alleles responsible for improving the major grain yield components (Narasimhamoorthy *et al.* 2006; Röder *et al.* 2008), and grain quality parameters (Kunert *et al.* 2007). This type of molecular analysis has not been performed on synthetic backcross lines evaluated in Australian environments, which could identify primary synthetic alleles with positive effects on grain weight and grain yield. Further, the comparison of genetic introgression patterns from crossing more than one primary synthetic wheat separately to a common bread wheat has also not been investigated.

The main aim of this study was to investigate the value and potential use of primary and derived synthetic wheat in Australian bread wheat breeding programs. The evaluation of many primary synthetic wheats and associated families of synthetic backcross lines was performed in an Australian context, using Australian bread wheat cultivars and other reference germplasm. As outlined in Table 1, this study will review relevant literature (Chapter 2), and report on and discuss experimental methods and results. This study evaluated families of synthetic backcross lines in field experiments in Australian environments, to assess their grain yield performance and its major components (grain weight and grains per m^2) (Chapter 5). Molecular marker assays formed the basis of genetic similarity investigations between primary synthetic wheat and modern Australian bread wheat cultivars (Chapter 4), and also examinations of synthetic allele introgression within families of synthetic backcross lines (Chapter 6). Two final chapters will provide a general discussion (Chapter 7) and state the conclusions of the research (Chapter 8).

Table 1. Thesis structure.

Chapter	Content
1	General introduction
2	Review of literature on the creation and breeding advantages of primary synthetic wheat, their global use and achievements made with them in Australia
3	Plant material used in the investigations
4	Comparison of genetic similarity between primary synthetic wheat and modern Australian bread wheat cultivars
5	Assessment of grain yield and grain yield-components of synthetic backcross lines in diverse drought stressed environments of southern Australia
6	Identification of introgressed synthetic wheat alleles and their linkage to improved grain yield components
7	General discussion
8	Conclusions

Chapter 2

Review of literature: Synthetic wheat

2.1 Introduction

Primary synthetic wheats are bridging lines used to simultaneously introgress genetic material from the progenitor species of bread wheat into modern bread wheat cultivars. These bridging lines are evaluated for their potential to develop synthetic derivatives suitable for practical breeding and cultivar development. This review will cover the evolution and domestication of bread wheat, genetic diversity within bread wheat, the rationale for using primary synthetic wheat as a genetic bridge, grain yield and component performance of synthetic backcross lines, and the introgression of diverse genetic material into modern bread wheat cultivars. An Australian perspective will be covered in these subject areas.

2.2 Bread wheat, an allohexaploid

2.2.1 Evolution

Bread wheat (*Triticum aestivum* L.; genome AABBDD; 2n=6x=42) arose from the natural hybridisation of two wild grass species, *Triticum turgidum* L. (genome AABB; 2n=4x=28) and *Aegilops tauschii* Coss. (syn. *Ae. squarrosa*; genome DD; 2n=2x=14), which are found native on the Eurasian sub-continent (McFadden and Sears 1946; Harlan and Zohary 1966; Zohary and Hopf 1988). *Triticum turgidum* is itself a hybrid formed from two wild grass species, *Triticum urartu* Tumanian *ex* Gandilyan (genome AA) and a currently unspecified B-genome progenitor (Figure 1) (Zohary *et al.* 1969; Huang *et al.* 2002; Dvorak and Akhunov 2005). *Aegilops speltoides* Tausch (genome SS) is the closest known relative to this possibly extinct B genome progenitor (Feldman 2001). Unlike *T. turgidum* which has modern domesticated forms, including durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) Husn.), *Ae. tauschii* is often considered a weed in cultivated fields throughout its natural distribution, which spans from the Black and southern Caspian Seas to western China (Figure 2) (McFadden and Sears 1946; Zohary *et al.* 1969; Dvorak *et al.* 1998). The D genome of *Ae. tauschii* (historically called the C genome) (McFadden and Sears 1944) is genetically diverse, which is reflected in the many morphological forms this species exhibits. *Aegilops tauschii* occupies varied environments within this geographical zone, from “rain-soaked temperate hyrcanic forests” to the margins of hot plains (Zohary *et al.* 1969).

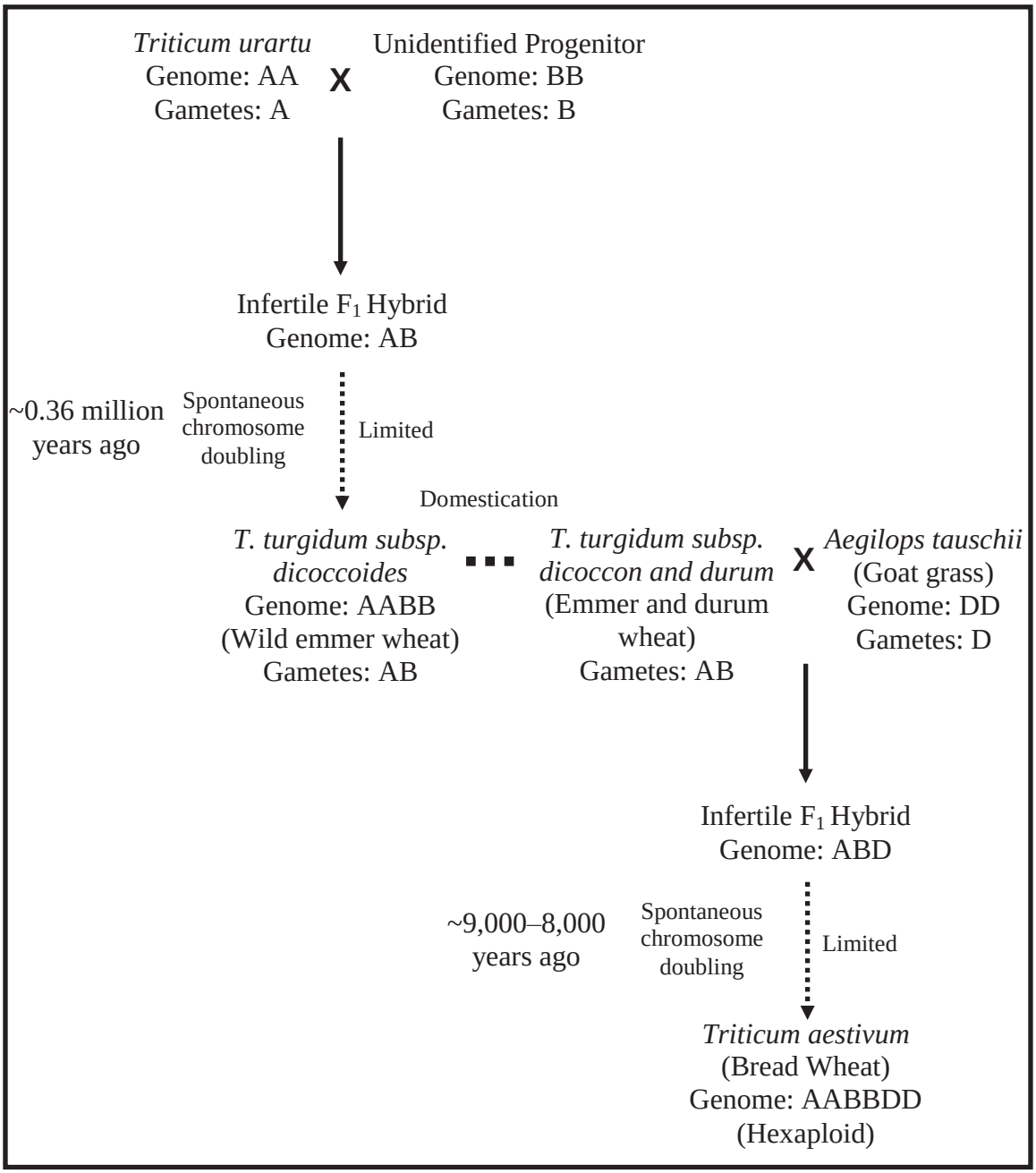

Figure 1. Evolutionary events that formed bread wheat (Kihara 1944; McFadden and Sears 1946; Zohary and Hopf 1988; Araus *et al.* 2001; Salamini *et al.* 2002; Dvorak and Akhunov 2005).

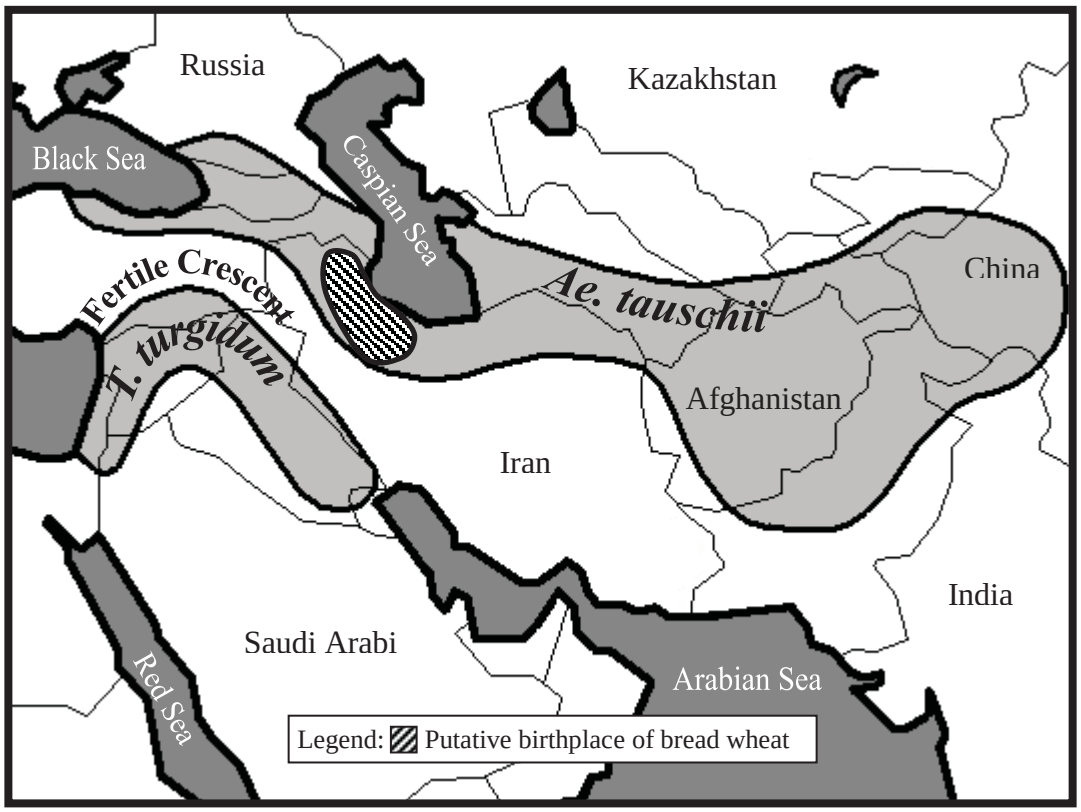

Figure 2. The putative birthplace of bread wheat relative to the Fertile Crescent (the domestication region of *T. turgidum*), and the distribution of collected *Ae. tauschii* accessions (Harlan and Zohary 1966; Dvorak *et al.* 1998).

It is widely accepted that the hybridisation events that led to the formation of bread wheat first began some 8,000-9,700 years ago (Figure 1), southwest of the Caspian Sea, a region outside of the Fertile Crescent (Figure 2) (Zohary *et al.* 1969; Kerber and Rowland 1974; Salamini *et al.* 2002). The Fertile Crescent is a horseshoe shaped region in the Near-East that encompasses ancient Mesopotamia where subspecies of *T. turgidum* evolved (Figure 2). It is thought that early farmers introduced and grew domesticated forms of *T. turgidum* outside of the Fertile Crescent, bringing them in close proximity with other plant species, including *Ae. tauschii* (for review, Harlan and Zohary 1966; Zohary *et al.* 1969; Zohary and Hopf 1988; Perrino *et al.* 1995; Dvorak *et al.* 1998; Matsuoka *et al.* 2008). This facilitated natural hybridisation events between forms of *T. turgidum* and *Ae. tauschii* to occur, resulting in the formation of sterile F_1 hybrids (genome ABD) (Figure 1). In the events that led to the formation of bread wheat, these F_1 hybrids are thought to have spontaneously set fertile hexaploid seed (genome AABBDD) (Kihara and Lilienfeld 1949). This increase in ploidy level made bread wheat more adaptable than the tetraploid wheats, enabling this new species to be cultivated into "higher latitudes and drier climates" (Zohary *et al.* 1969). The D genome from *Ae. tauschii* brought "profound changes in the adaptation" and abilities of wheat to "thrive under agricultural conditions" (Zohary *et al.* 1969). It is interesting to note that bread wheat has no wild forms (Kihara 1944).

Bread wheat has evolved from multiple hybridisation events between *T. turgidum* and *Ae. tauschii*, but this undefined number is considered to be limited (Dvorak *et al.* 1998; Talbert *et al.* 1998). Zohary *et al.* (1969) outlined a theory, which suggests that *T. aestivum* resulted from "recurrent hybridisations on numerous occasions with a variety of races and forms" of *Ae. tauschii* and *T. turgidum*. Zohary *et al.* (1969) also claimed that these hybridisations still occur. Later investigations supported these statements, however, debate continues over the actual number of hybridisations that have occurred, the identity of the parental sub-species involved, and the exact geographical location(s) in which they took place (Nishikawa *et al.* 1980; Talbert *et al.* 1998; Lelley *et al.* 2000; Matsuoka and Nasuda 2004).

2.2.2 Domestication

Early farmers initiated the domestication process of bread wheat by applying 'unconscious selection' to hulled and free-threshing forms. In conjunction with natural selection this facilitated the development of primitive landrace cultivars (syn. traditional cultivars) that were adapted to localised environments, as discussed by Reif *et al.* (2005) and Darwin

(1868). Over time farmers applied a level of 'active selection', most likely based on germination vigour, simultaneous ripening, general aesthetics, threshability, grain yield and taste (Heiser 1988).

2.2.3 Genetic diversity in bread wheat

2.2.3.1 Implications of evolution and domestication

Genetic diversity within the bread wheat gene pool has arguably been restricted by the limited number of hybridisation events that form this species, and from early domestication activities (Dvorak *et al.* 1998; Talbert *et al.* 1998). Zohary *et al.* (1969) reported that "crossing between *Ae. squarrosa* (syn. *Ae. tauschii*) and both tetraploid and hexaploid cultivated wheat can be detected in Iran at the present time". However, Lubbers *et al.* (1991) reported *Ae. tauschii* to contain "more genetic variability for disease and insect resistance, isozymes, and seed storage proteins than the D genome of *T. aestivum*." Lelley *et al.* (2000) later reported that average gene diversity is greater in *Ae. tauschii* than in modern bread wheat cultivars. A study by Reif *et al.* (2005), which used DNA fingerprinting to analyse the D genome of landrace bread wheat and *Ae. tauschii* accessions also showed a loss of genetic diversity from the *Ae. tauschii* accessions to the landrace selections.

2.2.3.2 Implications of modern breeding

2.2.3.2.1 Global trends

The genetic effects of modern breeding practices on the global gene pool of bread wheat and other crop species have been well documented (Frankel 1970; Ullstrup 1972; Tanksley and McCouch 1997; Manifesto *et al.* 2001; Khlestkina *et al.* 2004; Roussel *et al.* 2005; Tian *et al.* 2005; White *et al.* 2008). These studies indicate that changes to genetic diversity in bread wheat vary among wheat growing regions. The genetic base and the incorporation of diverse germplasm over time within these regions (North and South America, Europe, Asia and Australia for example) have influenced this. However, key events and the activities of international breeding programs have had global impacts.

An important worldwide breeding phenomenon, the 'green revolution' took place in the mid to late 1900's. It led to the replacement of standard-height cultivars, including landrace forms, by semi-dwarf cultivars (Frankel 1970; Smale *et al.* 2002). The term 'genetic erosion' was used at the time to capture the concept of this transition (as reviewed by Frankel 1970; Harlan 1972). Stemming from Norin 10, a Japanese variety that

contained the *Rht1* (*Rht-B1b*) and *Rht2* (*Rht-D1b*) dwarfing genes, semi-dwarf bread wheats are now the standard form developed by modern breeding programs. Under changing agricultural regimes that included the use of artificial fertilisers, semi-dwarf cultivars outperformed their landrace counterparts. Semi-dwarf wheat had improved spikelet fertility and efficiency of accumulating assimilates for grain development rather than for straw (as reviewed by Worland and Snape 2001; Smale *et al.* 2002). Smale *et al.* (2002) estimated that in 1997, only 3% of the area sown to spring bread wheat in the developing world, including China, was to landrace cultivars.

2.2.3.2.2 Influence of CIMMYT

The International Maize and Wheat Improvement Center (CIMMYT), based in Mexico, is a key organisation in world wheat breeding efforts. CIMMYT develops wheat germplasm for developing countries to provide yield stability, genetic enrichment and environmental protection (CIMMYT 2004). Changes in the genetic diversity of the germplasm output from such an organisation can have global implications. In 1997, CIMMYT germplasm was present in the pedigree of 86% of all spring wheat sown by the developing world, excluding China (Smale *et al.* 2002). Such wheat germplasm has been referred to as 'CIMMYT-related'. Studies carried out by Reif *et al.* (2005) into the genetic diversity of spring wheat and its relation to the international breeding efforts of the past 50 years, found that there was a "narrowing of genetic diversity among major CIMMYT modern wheat cultivars" between 1950 and 1981. This occurred despite CIMMYT obtaining genetic material during this period from locations in Turkey, Russia, the Middle East, Africa, Spain, the United States of America (USA), eastern Europe and South America (Villareal 1994). The influence of individual breeders must also be considered. Breeding practices of an individual at the Plant Breeding Institute in the United Kingdom (UK) involved the use of limited germplasm that contained the *Rht* dwarfing genes. This led to a reduction of genetic diversity within the respective breeding pool (Warburton *et al.* 2006). As CIMMYT has a pivotal role in the maintenance of global genetic diversity, in the 1970's and 1980's changes were made in CIMMYT breeding programs, which led to an increase in the genetic diversity of cultivars released between 1982 and 1997 (Villareal 1994). These changes involved exploitation of both winter and spring wheat gene pools by intercrossing, incorporating new plant material from Brazilian breeders and access to new Chinese gene pools via the Chinese Academy of Agricultural Science (Villareal 1994). Since then genetic diversity within CIMMYT breeding material has stabilised, which has

had flow-on effects to countries using CIMMYT-related germplasm in their own breeding programs (Smale *et al.* 2002; Warburton *et al.* 2006).

Despite CIMMYT actively broadening the genetic base of its bread wheat breeding pools, a gap still exists between the genetic diversity of its modern bread wheat cultivars and their landrace counter-parts. Molecular marker analyses of the A and B genomes of landrace bread wheat and CIMMYT bread wheat cultivars released after 1950 has shown slightly less gene diversity in the latter, with more unique alleles per locus present in the landrace forms (Reif *et al.* 2005). Dreisigacker *et al.* (2005) reported similar findings, stating that landrace bread wheat had "considerable genetic diversity." Other analyses reported by Reif *et al.* (2005), which focused on the D genomes of landrace and modern bread wheat showed that there were no significant differences in gene diversity between these two D genome pools.

2.2.3.3 Genetic diversity in Australian bread wheat

Australian bread wheat cultivars and breeding material have been found to be genetically more diverse than those from western Europe, the UK and the USA (Akbari *et al.* 2006; White *et al.* 2008). This has been attributed to Australian breeders using varieties and breeding material from many sources, which includes programs in the UK, USA, Canada, South Africa, India and Italy (O'Brien *et al.* 2001). The exploitation of germplasm from CIMMYT has also provided improvement to Australian bread wheats at a national level (Brennan and Fox 1998). Molecular marker analysis of the A, B and D genomes of Australian bread wheat cultivars released since 1901 and historically important breeding material, found the genetic similarity among older cultivars to be higher than among modern cultivars (Paull *et al.* 1998; Parker *et al.* 2002). On the other hand investigations reported by White *et al.* (2008), showed that the genetic similarity (converse of genetic distance) of Australian bread wheats has fluctuated since the early 1900's. The use of different molecular marker systems and genotypes to represent Australian bread wheat germplasm by these studies may have influenced the reported results, however none of these reports stated that modern breeding activities in Australia have significantly reduced genetic diversity in local bread wheat pools.

2.2.3.4 Germplasm resources for bread wheat breeders

Germplasm resources that can maintain or increase the genetic diversity of bread wheat gene pools in countries such as Australia need not only be genetically diverse but also

express agronomically desirable traits. Landrace bread wheats are known to be a germplasm resource for important traits (as reviewed by Moghaddam *et al.* 1997), however, there are few reports of their deliberate use within modern breeding programs. Theoretical modelling for the exploitation of traits possessed by landrace accessions, which are stored in seed banks provides some explanation for this (Gollin *et al.* 2000). Gollin *et al.* (2000) reported that for many accessions of landrace bread wheats held by seed banks there is a lack of detailed information about their origin, ancestry and performance. This situation has to some extent inhibited breeders from using these materials to develop improved cultivars for changing environments, and for the requirements of growers and consumers (van Ginkel and Ogbonnaya 2007). *Triticum turgidum* and *Ae. tauschii* are alternative sources of diverse germplasm, which can be used in 'pre-breeding'. These progenitor species of bread wheat are well known sources of important traits for modern cultivars. For example, resistance to Fusarium head blight and cereal rusts are expressed by *T. turgidum* subsp. *dicoccoides* (Nevo 2001; Oliver *et al.* 2007), with resistance to stem rust, powdery mildew and tan spot being identified in *Ae. tauschii* (Cox *et al.* 1992; Yang *et al.* 2003a).

2.3 Exploiting the progenitors of bread wheat

2.3.1 Direct hybridisations with T. turgidum

Bread wheat can be directly hybridised with *T. turgidum* (Figure 3). This strategy can transfer desirable alleles from the A and B genomes of *T. turgidum* into the complementary A and B genomes of modern bread wheat cultivars. These direct hybridisations typically use *T. turgidum* as the female parent, with bread wheat as the male parent. Genes conferring resistance to stem, leaf and stripe rust, and powdery mildew have been introgressed into the A and B genomes of bread wheat in this way (Reinhold *et al.* 1983; Reader and Miller 1991; Knott *et al.* 2005). However, direct hybridisations between *T. turgidum* and bread wheat can lead to genetically unstable hybrids. Non-hexaploid hybrids are initially produced from this strategy, with backcrossing to the recurrent bread wheat parent and self fertilisation required to return to a stable hexaploid state (Figure 3). During this crossing strategy hybrids can revert back to being tetraploid (Knott *et al.* 2005).

2.3.2 Direct hybridisations with Ae. tauschii

Direct hybridisations between *Ae. tauschii* and bread wheat can be achieved as the D genome of this species readily recombines with the D genome of bread wheat (Figure 3).

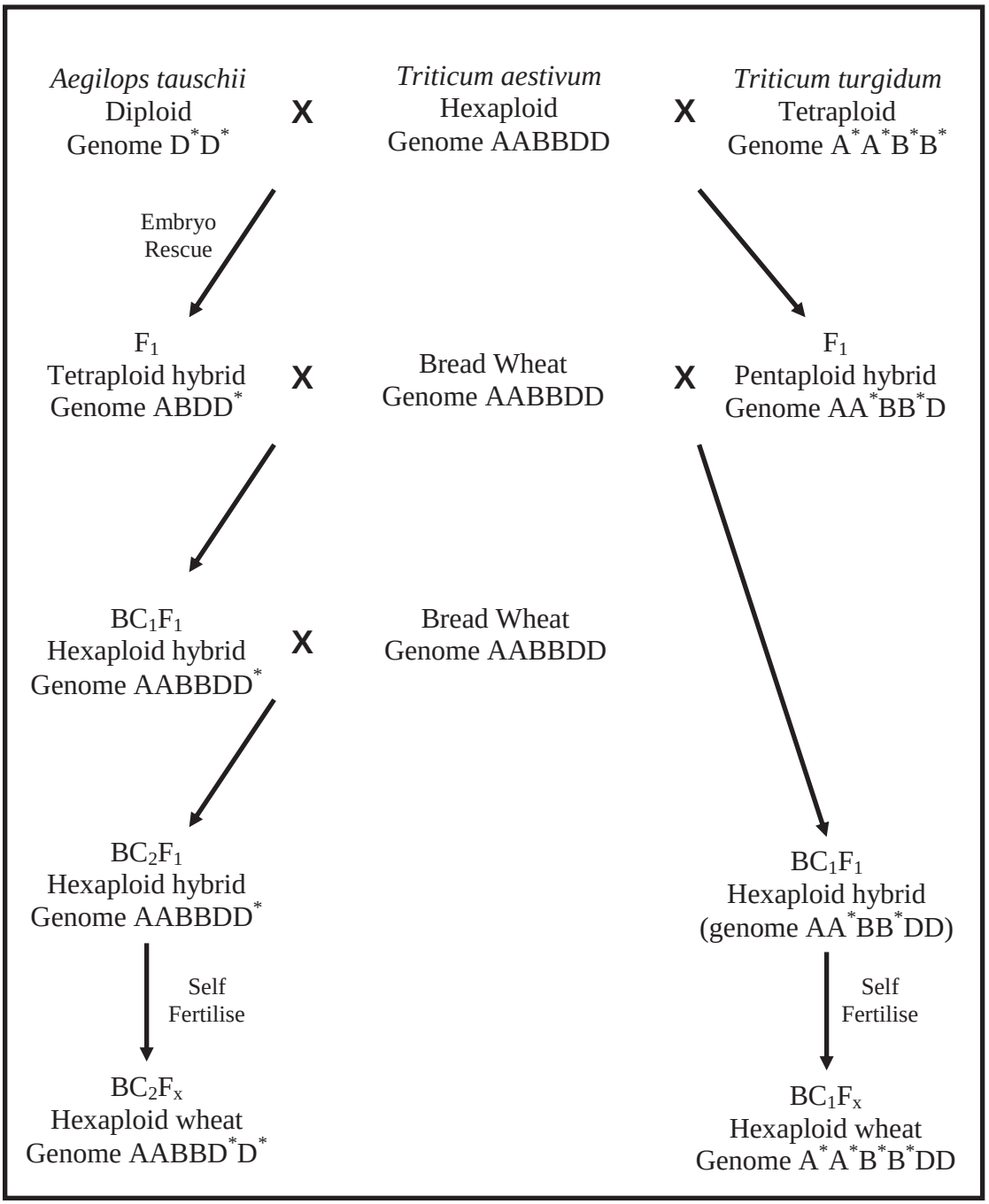

Figure 3. Direct hybridisation strategies used between *T. aestivum* (genome AABBDD) and progenitor species *Ae. tauschii* (genome D^*D^*) and *T. turgidum* (genome $A^*A^*B^*B^*$) (Gill and Raupp 1987; Reader and Miller 1991).

This direct crossing strategy has successfully introgressed quality characters and resistance to wheat spindle-streak mosaic virus, leaf rust and soil borne mosaic virus into bread wheat cultivars (Gill and Raupp 1987; Cox *et al.* 1994; Murphy *et al.* 1997; Yan *et al.* 2003). However, direct hybridisation between bread wheat and *Ae. tauschii* requires human intervention, commonly involving embryo rescue and up to four cycles of crossing to regain a stable hexaploid (Figure 3) (Gill and Raupp 1987; Cox *et al.* 1991). Cox *et al.* (1995) reported that 35% of BC_2F_1 individuals produced from this strategy were aneuploid. Seed abortion, lethality and sterility can occur in the F_1 hybrids (Gill and Raupp 1987). Suppression of traits donated from *Ae. tauschii* in bread wheat is also not uncommon. Investigations reported by Yang *et al.* (2003b) showed suppression of stripe rust resistance upon the direct hybridisation between resistant *Ae. tauschii* accessions and susceptible bread wheat. Gill and Raupp (1987) also reported a reduced level of leaf rust resistance in susceptible bread wheat derivatives compared to the *Ae. tauschii* donor.

2.3.3 Synthetic hexaploid wheat

2.3.3.1 Creation, advantages and history

Primary synthetic wheat (genome AABBDD; 2n=6x=42) is the hybrid from crossing between the evolutionary progenitors of bread wheat, *T. turgidum* and *Ae. tauschii* (McFadden and Sears 1944; McFadden and Sears 1946). Creating primary synthetic wheat involves screening subspecies and accessions of *T. turgidum* and *Ae. tauschii* for characters desired in the resulting hybrid (Figure 4) (Reynolds *et al.* 1999). Spikes of the *T. turgidum* parent are usually emasculated (removal of anthers) and pollinated using anthers from a selected *Ae. tauschii* accession. Developing embryos are rescued by excision from the developing seeds and further developed on culture media. Root and shoot growth is initiated from differentiated embryos forming plantlets, which are examined for chromosomal composition (genome ABD). Colchicine treatment is used to induce chromosome doubling in the crown of plantlets so that hexaploid seeds set upon self fertilisation (McFadden and Sears 1946; Lange and Jochemsen 1992; Mujeeb-Kazi *et al.* 1996; Fernandes *et al.* 2000; Mujeeb-Kazi *et al.* 2008). Before a primary synthetic wheat is crossed to bread wheat cultivar it is screened for the characters initially observed in the parental *T. turgidum* and *Ae. tauschii* accessions (Figure 4) (Reynolds *et al.* 2005).

The development and use of primary synthetic wheat for use as a genetic bridge provide several advantages over direct crossing between bread wheat and *T. turgidum* or *Ae. tauschii*. Embryo rescue and colchicine treatment are not always required for the

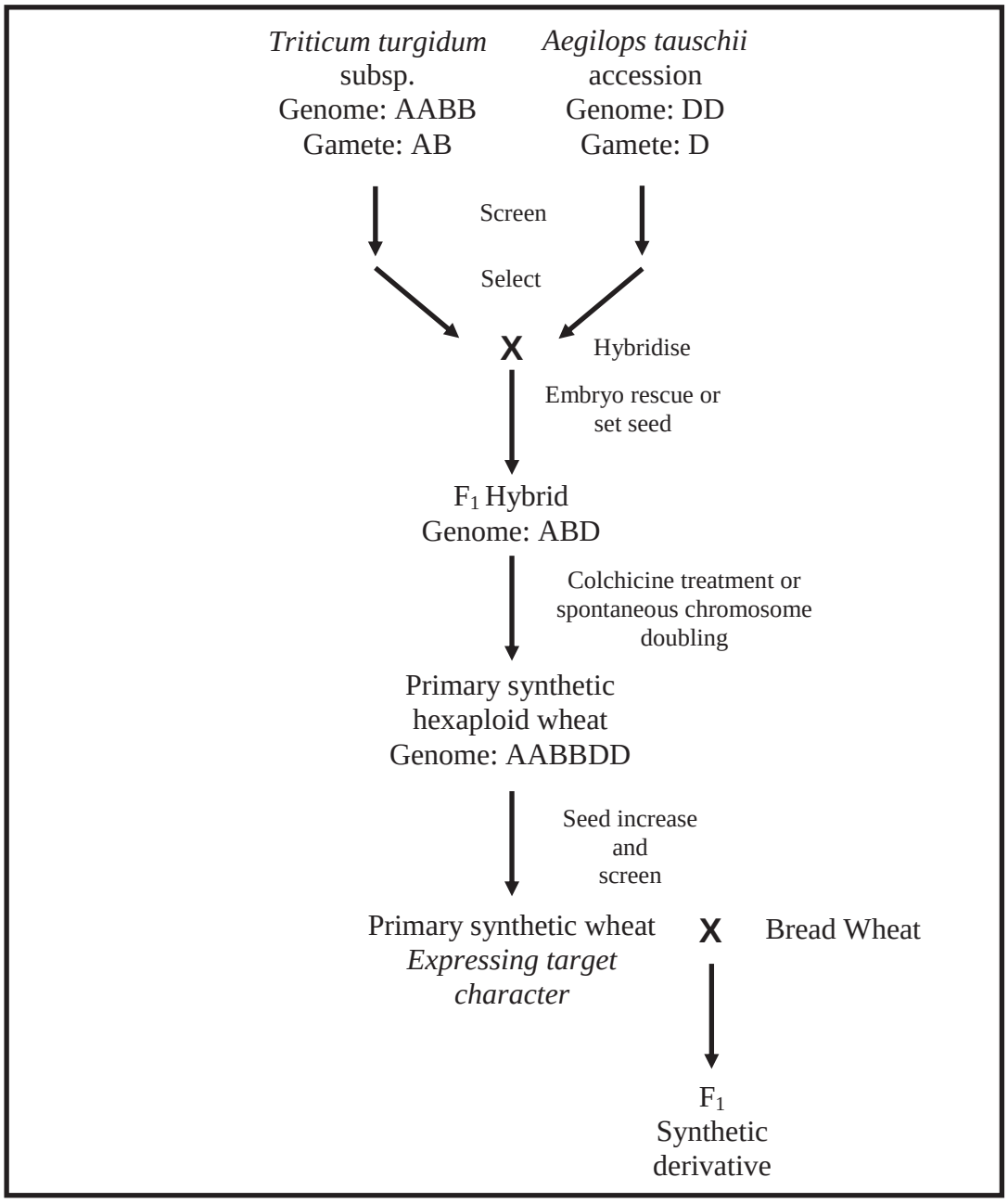

Figure 4. Creation of primary synthetic hexaploid wheat. Primary synthetic wheats are crossed with bread wheat cultivars forming synthetic derivatives, which are typically used in backcrossing strategies within breeding programs and research studies (Reynolds *et al.* 1999; Reynolds *et al.* 2005; van Ginkel and Ogbonnaya 2007; Mujeeb-Kazi *et al.* 2008; Trethowan 2008).

production of primary synthetic wheat. Some combinations of *T. turgidum and Ae. tauschii* can produce F_1 hybrids without human assistance, and some hybrids can spontaneously set hexaploid seed at relatively high frequencies (Liu *et al.* 2002; Matsuoka and Nasuda 2004). Once made, primary synthetic wheat can be readily crossed to bread wheat cultivars, leading to genetically stable F_1 synthetic derivatives without the need of backcrossing (Lange and Jochemsen 1992; Mujeeb-Kazi *et al.* 2008). The genomes of both *T. turgidum* and *Ae. tauschii* can be simultaneously exploited using primary synthetic wheat, which can provide a platform to observe trait expression from these progenitor genomes at the hexaploid level before crossing to a bread wheat cultivar.

The first attempts to make primary synthetic wheat were performed in the early 1900's, with 'synthetic spelta' being created during an investigation to determine the progenitors of *Triticum aestivum* subsp. *spelta* (L.) Thell., a primitive form of bread wheat (McFadden and Sears 1946). The term 'synthetic hexaploid wheat' was used by McFadden and Sears (1946) to describe the synthesis of this allopolyploid hybrid. Successful re-synthesis attempts by McFadden and Sears (1946) were only achieved using *T. turgidum* subsp. *dicoccoides* with *Ae. tauschii*, and not with other subspecies of *T. turgidum*. More recent efforts to create primary synthetic wheat have successfully hybridised different accessions of *Ae. tauschii* with *T. turgidum* subspecies *durum* (Desf.) Husn. (durum wheat) (Dreccer *et al.* 2007; Ogbonnaya *et al.* 2007), *dicoccoides* (Korn. Ex Ash. & Graebn.) Thell. ('wild emmer' wheat) (Lange and Jochemsen 1992), *dicoccon* (Schrank) Thell. ('emmer' wheat) (Lage *et al.* 2003) and *carthlicum* (Nevski) A. Love & D. Love (Persian black wheat) (Liu *et al.* 2006). In most primary synthetic wheats *Ae. tauschii* is the source of novel genetic material, as a durum wheat cultivar or breeding line is commonly used as the tetraploid parent (Trethowan and Mujeeb-Kazi 2008).

2.3.3.3 Production and use of synthetics by CIMMYT and the world

CIMMYT began investigating the use of synthetic wheat in the late 1980's, as a breeding resource to obtain genetic diversity and specific traits, such as karnal bunt resistance (Mujeeb-Kazi and Hettel 1995). CIMMYT had produced approximately 650 primary synthetic wheats from its wide crossing program by 1999, using its large bank of *Ae. tauschii* accessions and elite durum cultivars. Accessions of *T. turgidum* subsp. *dicoccoides* and *dicoccon* were used less frequently (Reeves *et al.* 1999; van Ginkel and Ogbonnaya 2007). Some of these synthetic wheats expressed desirable high molecular weight glutenins and many biotic and abiotic stress resistances and tolerances (Villareal

1994; Diaz-de-Leon and Mujeeb-Kazi 1997; Reeves *et al.* 1999). CIMMYT has used primary synthetic wheat to introgress resistance to *Septoria tritici* blotch into its breeding lines, with some CIMMYT breeding programs using primary synthetic wheat to improve drought tolerance and yield responsiveness (Reeves *et al.* 1999). Over 1014 spring habit and 186 winter habit primary synthetic wheat have been created by CIMMYT since 1991, with an estimated one third of the advanced lines distributed by CIMMYT to global breeding programs being synthetic derivatives (van Ginkel and Ogbonnaya 2007). A synthetic derivative, 'Chuanmai 42', has been released in China as a commercial variety due to its high yielding ability, high grain weight and stripe rust resistance (Yang *et al.* 2009). A second synthetic derivative evaluated in Spain, named 'Carmona', has been identified as having high commercial potential (Li *et al.* 2006; van Ginkel and Ogbonnaya 2007).

2.3.3.4 Production and use in Australia

Australian wheat breeding programs began to import CIMMYT developed primary synthetic wheat and derived lines in small quantities in the 1990's, with larger germplasm sets being imported annually from 2001 (Trethowan 2004; Moody and Emebiri 2008b). Derived synthetics in the earlier shipments had CIMMYT bread wheat as the recurrent and/or top cross parents, with more recent shipments of derived synthetics having Australian bread wheats as their recurrent parents (Dreccer *et al.* 2007). The Synthetic Enriched Resources for Genetic Enhancement (SynERGE) program in Australia produced many primary and derived synthetics for phenotypic and genotypic evaluation in local conditions. This program used tetraploid, diploid and hexaploid wheats from both CIMMYT and the Australian Winter Cereals Collection (AWCC). The program identified new sources of biotic stress resistance (cereal cyst nematode and yellow leaf spot resistance) and abiotic stress tolerances (drought, salinity and pre-harvest tolerance), which are desirable for Australian bread wheat cultivars. Breeding entities were encouraged to adopt and use this material for the genetic enrichment of bread wheat breeding programs (van Ginkel and Ogbonnaya 2007).

2.4 Increasing grain yield of bread wheat using primary synthetic wheat

2.4.1 Grain yield components

Grain yield in bread wheat can be broken down into two major numerical components, mean individual grain weight and grains per m^2 (as reviewed by Slafer *et al.* 1996). Increases in grain yield over the past 40 years, due to breeding practices, have come from

the production of more grains per m^2 and not heavier grains. The *Rht* dwarfing genes have played a significant role in this phenomenon, with modern semi-dwarf bread wheats setting more grains in distal floret positions compared to standard height wheats (Miralles *et al.* 1998; Calderini and Reynolds 2000). As the number of grains per m^2 has increased over time, mean individual grain weight has remained unchanged or has decreased. This negative relationship has been identified in many germplasm pools (Slafer *et al.* 1996). As grain weight is also a quality component of bread wheat, affecting milling yield, there is a commercial incentive to increase the grain weight of bread wheat whilst improving grain yield (Marshall *et al.* 1986).

Primary synthetic wheats have been reported to show genetic variation for the major grain yield components, especially grain weight (Villareal *et al.* 1994a; Villareal *et al.* 1994b; Mujeeb-Kazi 1995; Calderini and Reynolds 2000; Calderini and Ortiz-Monasterio 2003). Mean individual grain weights of up to 67 mg have been reported for CIMMYT primary synthetic wheat grown under field conditions in Mexico (Calderini and Reynolds 2000). Under hydroponic conditions, primary synthetic wheat has produced grains up to 58% heavier compared to bread wheat cultivars (Dreccer *et al.* 2004).

2.4.2 Breeding strategies to increase grain weight and grain yield

Primary synthetic wheat could be used in 'pre-breeding' strategies to increase the grain weight of bread wheat whilst maintaining or increasing grain yield. Calderini and Ortiz-Monasterio (2003) suggested a strategy based on selecting 'for grain yield by increasing average grain weight' when using primary synthetic wheat as a donor genetic source. A similar strategy has already been performed in bread wheat. Ten cultivars and breeding lines were intercrossed, with progeny recurrently selected for high grain weight over eight cycles (Wiersma *et al.* 2001). Grain weight was found to increase (4.5% per cycle), however no significant increase in grain yield was identified. A compensation effect was observed where grains per spikelet, spikelets per spike and spikes (tillers) per m^2 all decreased as a consequence of improved grain weight (Wiersma *et al.* 2001).

Backcross (BC) strategies using primary synthetic wheat have produced derivatives with higher grain weights and grain yields, compared to recurrent bread wheat parents. Del Blanco *et al.* (2001) reported on six populations of BC_2 synthetic-derived lines, developed from four CIMMYT primary synthetic wheats and four CIMMYT recurrent bread wheat cultivars. When grown in environments in Mexico, more than 80% of the synthetic

backcross lines across these populations had superior grain weights. Across the six populations, the grain yield component grains per m^2 was more strongly associated with grain yield than mean individual grain weight, however, eight derivatives showed improved grain yields (up to 111% of the recurrent bread wheat parent) that were due to an increase in grain weight (up to 142.2% of the recurrent bread wheat parent) (del Blanco *et al.* 2001). As such lines were not identified in every population, the use of different primary synthetic and bread wheat combinations may have influenced the production of these desirable lines.

2.4.3 Synthetic backcross lines with Australian bread wheat parentage

CIMMYT created some of the first synthetic backcross lines with Australian bread wheats as recurrent parents. These lines were evaluated in drought nurseries at Ciudad Obregón, north-western Mexico, together with other lines that had CIMMYT bread wheat parentage. Under terminal moisture stress conditions (drying soil profile) grain yields up to 79.2% higher than Australian recurrent or top cross parents were identified (Trethowan 2004). The lack of adaptation of the Australian bread wheats under Mexican environments may have contributed to the comparatively high grain yield performance by the synthetic derivatives (Dreccer *et al.* 2007). Nevertheless, based on this success, lines that produced grain yields at least 10% greater than their bread wheat parents were sent to Australia for further evaluation, and since 2001 Australia has received shipments (~100 lines p.a.) of synthetic backcross lines (Trethowan 2004; Dreccer *et al.* 2007).

The grain yield performance of synthetic backcross lines from CIMMYT has been evaluated in northern and southern regions of the Australian wheat belt (Dreccer *et al.* 2007; Ogbonnaya *et al.* 2007; Rattey and Shorter 2010). These studies have found grain yield performance to be commonly greater in northern Australia than in the south, with synthetic backcross lines achieving up to 30% higher grain yields compared to the best local check cultivar. Reasons why synthetic derivatives and other material from CIMMYT perform better under northern Australian environments compared to southern Australian environments have been well documented (Cooper *et al.* 1993; Brennan and Quade 2004; Dreccer *et al.* 2007; Ogbonnaya *et al.* 2007; Dreccer *et al.* 2008). The use of Australian bread wheats as recurrent and/or top cross parents, rather than CIMMYT bread wheats was suggested to provide a grain yield advantage in both northern and southern Australia. Synthetic backcross lines with Croc 1 (CIMMYT durum wheat) or *Ae. tauschii* accessions WX205 or WX224 in their primary synthetic wheat parents' pedigree were also found to

perform well across both northern and southern sites. Although the evaluations of synthetic backcross lines reported by Ogbonnaya *et al.* (2007) and Dreccer *et al.* (2007) did show that certain primary synthetic wheat and Australian bread wheat combinations were particularly beneficial for improving grain yield, they did not identify which grain yield component(s) (grains per m^2 or mean individual grain weight) were responsible for the improvements.

Primary synthetic wheats may have the potential to increase yield in the breeding of bread wheat for moisture-limited environments in Australia. In northern Australia, Rattey and Shorter (2010) assessed a similar set of synthetic-derived material to Dreccer *et al.* (2007) and found that the best grain yield advantage (up to 8%) over Australian bread wheats was achieved in the lowest yielding environments. In southern Australia Gororo *et al.* (2002) assessed BC_1 synthetic derivatives created from one CIMMYT primary synthetic wheat and one recurrent southern Australian bread wheat parent. In a low yielding environment, up to 49% higher grain yields were identified compared to the Australian recurrent bread wheat parent (Gororo *et al.* 2002). The production of more grains per m^2 was commonly found to be responsible for grain yield improvements in such conditions. Improved grain yields observed in relatively high grain yielding environments were achieved by small non-significant increases in both grain weight and grains per m^2 or by significant increases in grain weight (Gororo *et al.* 2002).

2.5 Introgressing novel genetic material into bread wheat

2.5.1 History and issues

The introgression of genetic material into bread wheat from related species began in the late 19^{th} century. These activities were chiefly performed to acquire levels of biotic stress resistance not readily observed in bread wheat gene pools (Appels and Lagudah 1990). The successful development of triticale, a hybrid of bread wheat and rye (*Secale cereale* L.) (for review, Briggle 1969), was the starting point for the introgression of exotic chromosome segments into bread wheat. The 1RS.1BL translocation event, involving the short arm of rye chromosome 1R and the long arm of bread wheat chromosome 1B, has been important in global bread wheat breeding for disease resistance and grain yield improvement in some genetic backgrounds (Zeller 1973; Berzonsky and Francki 1999). The introgression of other large chromosomal segments into bread wheat have involved species from the *Aegilops*, *Agropyron*, *Elymus*, *Haynaldia*, *Hordeum*, *Secale* and *Triticum* genera, which have chromosomes with varying levels of homology with those of bread

wheat (for review, Gupta *et al.* 2005). Large chromosome segments from novel sources can carry undesirable genes, expressing undesirable characters such as the poor end use quality (sticky dough) associated with the 1RS.1BL translocation (Barbeau *et al.* 2003). Primary synthetic wheats can also express deleterious characters, such as late maturity α-amylase activity, tough rachis, lodging and excessive height, which has prevented their direct use as commercial cultivars (Pritchard *et al.* 2002; Tyagi *et al.* 2004; Ogbonnaya 2005; van Ginkel and Ogbonnaya 2007; Mrva *et al.* 2009). The introgression of smaller chromosome segments from novel germplasm sources like primary synthetic wheat is therefore desirable, reducing 'linkage drag' between genes conferring desirable and undesirable characters (Paterson *et al.* 1990).

2.5.2 Backcross strategies using synthetic wheat

Backcross and selection strategies can reduce the size of introgression segments from donor sources and promote recombination between desirable and undesirable genes to limit linkage drag (Frisch and Melchinger 2001; Hospital 2001). Such strategies have been employed to introgress germplasm from wild relative species into cultivars of barley (von Korff *et al.* 2004), tomato (Tanksley and Nelson 1996) and peanut (Burow *et al.* 2001), and from primary synthetic wheat into modern bread wheat cultivars (del Blanco *et al.* 2001; Gororo *et al.* 2002; Trethowan 2004; Dreccer *et al.* 2007). As described by Allard (1960), backcrossing typically involves crossing an elite parent deficient in a target character to a donor source, and then performing a backcross between the resulting F_1 or selfed F_2 with the elite (recurrent) parent (Figure 5). BC_1F_1 individuals would be expected to be homozygous at more loci for the elite parent allele than found in the F_1 from a single cross. Combined with suitable selection protocols, backcrossing can produce superior synthetic derivatives, which have vital genetic contributions from the primary synthetic donor in an elite genetic background.

The number of backcrosses required when breeding with primary synthetic wheat depends on the breeding objective. Trethowan and Mujeeb-Kazi (2008) reported that at least one backcross is required to the adapted recurrent bread wheat parent to achieve high stable yield in synthetic derivatives. BC_1 strategies (Figure 5) have been successful in introgressing quality and yield/adaptation characters from primary synthetic wheat into bread wheat backgrounds (Gororo *et al.* 2002; Dreccer *et al.* 2007; Mares and Mrva 2008). Single and advanced backcrossing (BC_2, BC_3, BC_4 for example) combined with selection

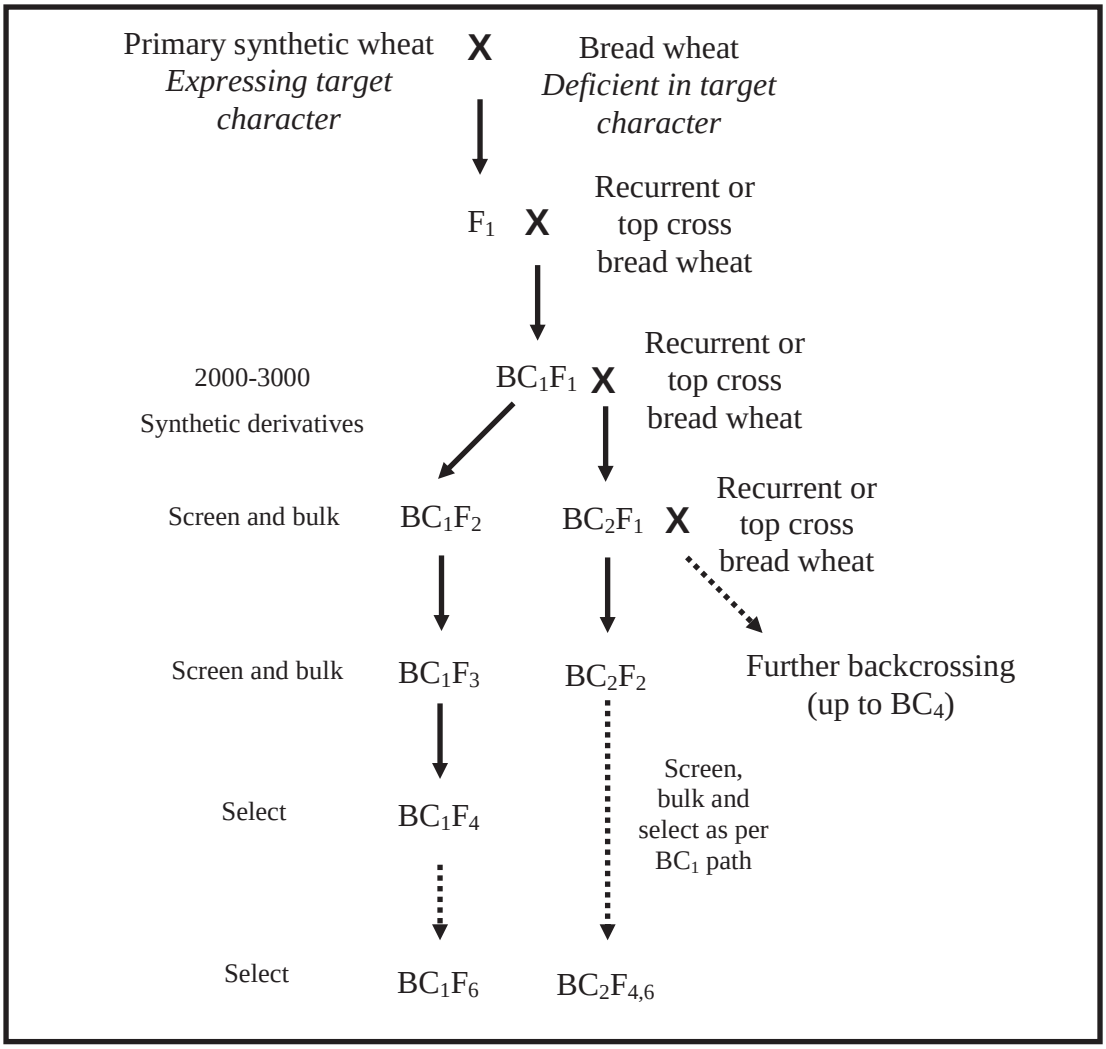

Figure 5. Backcross breeding strategies for the introgression of genetic material from primary synthetic wheats into a bread wheat background (van Ginkel and Ogbonnaya 2007; Trethowan 2008).

can minimise the drag of undesirable genes whilst maximising the chances of introgressing genes associated with a target character (Tanksley and Nelson 1996). However, for single backcrossing strategies the development of large populations (>2000 BC_1F_2 lines) has been recommended to increase the opportunity to select high performing lines without deleterious characters (van Ginkel and Ogbonnaya 2007). Advanced backcrossing with molecular marker selection can be used to develop introgression lines, each with a single small defined chromosome segment from the donor source, however this strategy may require over six generations of backcrossing and selection (for review, Zamir 2001). Backcrossing strategies, such as BC_2 (del Blanco *et al.* 2001; Narasimhamoorthy *et al.* 2006; Kunert *et al.* 2007), BC_3 (Röder *et al.* 2008) and BC_4 (Liu *et al.* 2006) have been successful in identifying and introgressing quality characters and superior yield components from primary synthetic wheat into bread wheat. After the desired number of backcrosses has been made, F_2 and F_3 populations are typically established and allowed to self to reach a homozygous state (Figure 5). Further selection of families/individuals may take place, after which character evaluations can be performed more thoroughly.

2.5.2 Primary synthetic wheat alleles at quantitative trait loci

Quantitative trait loci (QTL) are chromosome regions that contain one or more genes that affect a quantitative trait, like grain yield in bread wheat (for review, Liu 1998). Statistical analysis of molecular marker and phenotypic data, typically from a population of lines, is used to map the chromosomal location of QTL. Backcross populations are suitable for QTL mapping, with this structure also being used to simultaneous identify QTL and introgress genetic material from wild relatives into modern cultivars, a technique established in tomato (Eshed and Zamir 1994; Tanksley *et al.* 1996). Similar strategies have been applied to simultaneously introgress genetic material from primary synthetic wheat into modern bread wheat cultivars, and to identify QTL alleles from *T. turgidum* and *Ae. tauschii* that have positive effects on preharvest sprouting tolerance (Imtiaz *et al.* 2008), baking volume (Kunert *et al.* 2007) and length of grain filling period (Börner *et al.* 2002).

QTL for grain yield and its major components (grain weight and grains per m^2) have been identified on many of the 21 chromosomes of bread wheat. Grain yield is a complex quantitative trait, subject to genotype by environment interactions and is readily associated with QTL for flowering time, maturity, plant height, grain weight and grain per m^2 for example (Cuthbert *et al.* 2008). Across populations and environments several regions on

chromosomes 1B, 2A, 2B, 3B 6A 7B and 7D have been recurrently associated with grain weight (Campbell *et al.* 1999; Börner *et al.* 2002; Groos *et al.* 2003; Kumar *et al.* 2006; Li *et al.* 2007; Hai *et al.* 2008; Sun *et al.* 2009; Wang *et al.* 2009). Beneficial alleles from primary synthetic wheat have been identified at QTL for grain weight on chromosomes 3A, 3B, 5A, 6A, 7A and 7D (Börner *et al.* 2002; Huang *et al.* 2004; Röder *et al.* 2008).

2.6 Research questions

Primary synthetic wheats have shown potential value for use in Australian bread wheat breeding. This study aimed to investigate primary and derived synthetic wheats in three research areas to further evaluate this value, which included genetic diversity, grain weight and grain yield in drought stressed environments, and synthetic allele introgression.

The genetic diversity of Australian bread wheat has not significantly reduced over time due to breeding activities; however, primary synthetic wheats may offer additional genetic diversity for Australian bread wheat breeders. Genetic diversity between primary synthetic wheats and modern Australian bread wheat cultivars has not been investigated before. This study aimed to identify the genetic similarity within and among 44 primary synthetic wheats and nine modern Australian bread wheat cultivars using Diversity Array Technology™ (DArT) markers.

Maintaining a viable level of grain yield production from bread wheat in drought stressed environments is a high priority in Australia. Past studies have indicated that primary synthetics can improve the grain yield of Australian bread wheat through the development of synthetic backcross lines. However, grain yield and its major components (grain weight and grains per m^2) of synthetic backcross lines have not been measured before in moisture-limited environments in southern Australia. This study aimed to evaluate grain yield and its major components in 27 families of synthetic backcross lines, created from 27 primary synthetic wheats and a common recurrent Australian bread wheat parent, in drought stressed environments of southern Australia.

Primary synthetic alleles with positive effects on grain weight and grains per m^2, under Australian environments, have not been identified before within an Australian bread wheat genetic background. This study aimed to identify the presence of such alleles introgressed at QTL in two families of synthetic backcross lines. The retention of these beneficial alleles in high yielding and high grain weight lines was also investigated.

Chapter 3
Plant Materials

This chapter provides detailed information on the plant materials used for the research that will be reported in subsequent chapters of this thesis.

3.1 Summary of plant material

- 44 Primary synthetic hexaploid wheats
- 5 Durum wheat that are the recorded parents of one or more of the 44 primary synthetic wheat
- 27 Families of BC_1F_4-derived synthetic lines, derived from backcrosses between 27 primary synthetic wheat (a sub-set of the 44 referred to above) and recurrent parent Yitpi, an Australian semi-dwarf bread wheat cultivar
- Yitpi and 19 other Australian bread wheat cultivars
- 2 Durum wheat cultivars that are grown in Australian

3.2 Primary synthetic wheat and parental durum wheat

The forty-four primary synthetic wheat used in this study were created by the Wide Crossing Unit of the International Maize and Wheat Improvement Center (CIMMYT). These primary synthetic wheats were formed by hybridising 23 CIMMYT durum wheats (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) with one or more of 37 *Aegilops tauschii* Coss. accessions from CIMMYT's working germplasm bank (Table 2). Further information regarding the primary synthetic wheat used and their *Ae. tauschii* accessions is detailed in Appendix 1 and Appendix 2. Of the 44 primary synthetic wheats, 17 corresponded in pedigree with synthetics that were short listed by CIMMYT as having desirable agronomic characteristics (including "morphological, growth, biotic, and abiotic attributes" (Mujeeb-Kazi *et al.* 2000)) in environments across Mexico out of approximately 800 created between 1995 and 2001 (Mujeeb-Kazi and Delgado 2001). In 2001, Dr Richard Trethowan (formerly at CIMMYT, now at the University of Sydney) sent seed of a set of primary synthetic wheat to Australia (Quarantine importation number: ZSE01) (Moody and Emebiri 2008b). In 2005, Dr Francis Ogbonnaya (then at the Department of Primary Industries, Victoria, now at the International Centre for Agricultural Research in the Dry Areas) provided seed of 44 of these synthetics to this project.

Table 2. Primary synthetic wheats used in this study, their pedigrees, Australian Winter Cereals Collection accession numbers and abbreviations used.

Primary synthetic	Australian Winter Cereals Collection accession number	Recorded pedigree (durum parent & *Ae. tauschii* parent)	Abbreviated pedigree
S1	AUS 29642	ACO89/*Ae. tauschii* (WX309) [1]	D1T309
S2	AUS 29643	ACO89/*Ae. tauschii* (WX309) [1]	D1T309
S3	AUS 29637	ALTAR_84/*Ae. tauschii* (WX211) [1]	D2T211
S4	AUS 29641	AOS/*Ae. tauschii* (WX269)	D3T269
S5	AUS 29638	ARLIN_1/*Ae. tauschii* (WX218) [2]	D4T218
S6	AUS 29668	CETA/*Ae. tauschii* (WX661)	D5T661
S7	AUS 29655	CETA/*Ae. tauschii* (WX819)	D5T819
S8	AUS 29663	CETA/*Ae. tauschii* (WX895) [1]	D5T895
S9	AUS 29664	CETA/*Ae. tauschii* (WX895) [1]	D5T895
S10	AUS 29636	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (WX208) [1]	D6T208
S11	AUS 29680	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (WX390)	D6T390
S12	AUS 29653	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (WX629)	D6T629
S13	AUS 29676	CROC_1/*Ae. tauschii* (WX256)	D7T256
S14	AUS 29681	CROC_1/*Ae. tauschii* (WX436)	D7T463
S15	AUS 29677	DOY1/*Ae. tauschii* (WX258)	D8T258
S16	AUS 29678	DOY1/*Ae. tauschii* (WX264)	D8T264
S17	AUS 29652	DOY1/*Ae. tauschii* (WX488)	D8T488
S18	AUS 29682	DOY1/*Ae. tauschii* (WX517)	D8T517
S19	AUS 29684	DOY1/*Ae. tauschii* (WX1030) [1]	D8T1030
S20	AUS 29640	DVERD_2/*Ae. tauschii* (WX247)	D9T247
S21	AUS 29649	GAN/*Ae. tauschii* (WX437)	D10T437
S22	AUS 29650	GAN/*Ae. tauschii* (WX446)	D10T446

Table 2. *Continued.*

Primary synthetic	Australian Winter Cereals Collection accession number	Recorded pedigree (durum parent & *Ae. tauschii* parent)	Abbreviated pedigree
S23	AUS 29669	GAN/*Ae. tauschii* (WX890)	D10T890
S24	AUS 29673	GARZA/BOY//*Ae. tauschii* (WX484)	D11T484
S25	AUS 29672	KAPUDE/*Ae. tauschii* (WX385)	D12T385
S26	AUS 29685	LOCAL RED/*Ae. tauschii* (WX220)	D13T220
S27	AUS 29657	RABI//GS/CRA/3/*Ae. tauschii* (WX895)	D14T895
S28	AUS 29674	ROK/KML//*Ae. tauschii* (WX507)	D15T507
S28	AUS 29648	SCOOP_1/*Ae. tauschii* (WX434)	D16T434
S30	AUS 29667	SCOOP_1/*Ae. tauschii* (WX634)	D16T634
S31	AUS 29666	YAR/*Ae. tauschii* (WX518) [1]	D17T518
S32	AUS 29675	YAV_2/TEZ//*Ae. tauschii* (WX170)	D18T170
S33	AUS 29670	YAV_2/TEZ//*Ae. tauschii* (WX895) [1]	D18T895
S34	AUS 29651	YAV79//DACK/RABI/3/SNIPE/4/*Ae. tauschii* (WX477)	D19T477
S35	AUS 29644	68.111/RGB-U//WARD/3/*Ae. tauschii* (WX326) [1]	D20T326
S36	AUS 29659	68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (WX809)	D21T809
S37	AUS 29660	68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (WX878) [1]	D21T878
S38	AUS 29661	68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (WX878) [1]	D21T878
S39	AUS 29662	68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (WX878) [1]	D21T878
S40	AUS 29656	68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (WX882) [1]	D21T882
S41	AUS 29645	68.111/RGB-U//WARD RESEL/3/STIL/4/*Ae. tauschii* (WX332)	D22T332
S42	AUS 29654	68.111/RGB-U//WARD RESEL/3/STIL/4/*Ae. tauschii* (WX783)	D22T783
S43	AUS 29646	68112/WARD//*Ae. tauschii* (WX369) [1]	D23T369
S44	AUS 29647	68112/WARD//*Ae. tauschii* (WX369) [1]	D23T369

[1] Synthetic hexaploids short listed in elite set 1 (Mujeeb-Kazi *et al.* 2000).
[2] Synthetic hexaploids short listed in elite set 2 (Mujeeb-Kazi and Delgado 2001).

CIMMYT durum wheats Altar 84 (D2), Croc 1 (D7), Gan (D10), Kapude (D12) and Scoop 1 (D16), which were the recorded tetraploid parents of one, three, one and two primary synthetic wheat respectively (Table 2), were also used in the current study. Seed of these cultivars was made available by Dr Francis Ogbonnaya.
(Contact details for Dr Francis Ogbonnaya: International Center for Agricultural Research in the Dry Areas (ICARDA), PO Box 5466, Aleppo, Syria).

3.3 Bulk synthetic seed

An Australian bread wheat cultivar Yitpi (AUS 30462) and 27 primary synthetic wheats (Table 3), were used by Dr Francis Ogbonnaya to generate 27 BC_1F_4 bulk seed sets. Undertaken at the Department of Primary Industries (Horsham, Victoria, Australia), the development of these bulk seed sets involved Yitpi being individually crossed with each of the 27 primary synthetic wheat as the female parent, and five F_1 progeny from each cross were backcrossed to Yitpi. Two hundred BC_1F_1 seeds from each backcross were used to develop BC_1F_3 synthetic derivatives via single seed decent. A single spike was taken from each derivative. These spikes were bulked together with other derivatives from the same cross, forming 27 BC_1F_4 bulk seed sets. Dr Francis Ogbonnaya kindly provided 10 g of seed from each BC_1F_4 bulk set for use in this investigation.

3.4 Bread and durum wheat cultivars

Yitpi is the recurrent parent of the synthetic-derived families of lines under investigation here. It is a hard white bread wheat, semi-dwarf in stature with an intermediate growth habit, early to mid-season spike emergence, medium glaucoused flag leaf, non-pubesent glumes and has a high yield potential when grown in the medium to low rainfall districts of South Australia, north-western Victoria and south-western New South Wales (Figure 6) (AWB Limited 2003; Hollamby 2003). Yitpi was bred at the University of Adelaide in South Australia. Yitpi was used in every experiment undertaken in this study. Nineteen other Australian bread wheats were also used in this study. In winter field experiments bread wheat cultivars Annuello, Carinya, Stylet (not commercially released, Australian Grain Technologies (AGT) Pty Ltd), and Wyalkatchem were used as high yielding checks. These cultivars are all well adapted to the same south-eastern Australian environments as Yitpi (Figure 6). Genetic diversity investigations used bread wheat from different Australian breeding organisations and gene pools, including the cultivars AGT Scythe, Annuello, Carinya, GBA Ruby, Janz, Kukri, Stylet and Wyalkatchem (Table 4).

Table 3. Families of BC_1F_4-derived lines developed using Yitpi as the recurrent parent and 27 primary synthetics as the donor parent.

Primary synthetic parent	Synthetic-derived family	Number of BC_1F_4 derivatives selected
S1	Y1	34
S3	Y3	49
S5	Y5	35
S6	Y6	27
S7	Y7	24
S9	Y9	28
S10	Y10	32
S11	Y11	35
S12	Y12	30
S13	Y13	22
S14	Y14	49
S15	Y15	30
S16	Y16	26
S18	Y18	53
S19	Y19	46
S20	Y20	41
S26	Y26	25
S27	Y27	22
S31	Y31	27
S33	Y33	29
S35	Y35	24
S36	Y36	35
S37	Y37	27
S40	Y40	25
S41	Y41	16
S42	Y42	28
S43	Y43	20
	Total	839

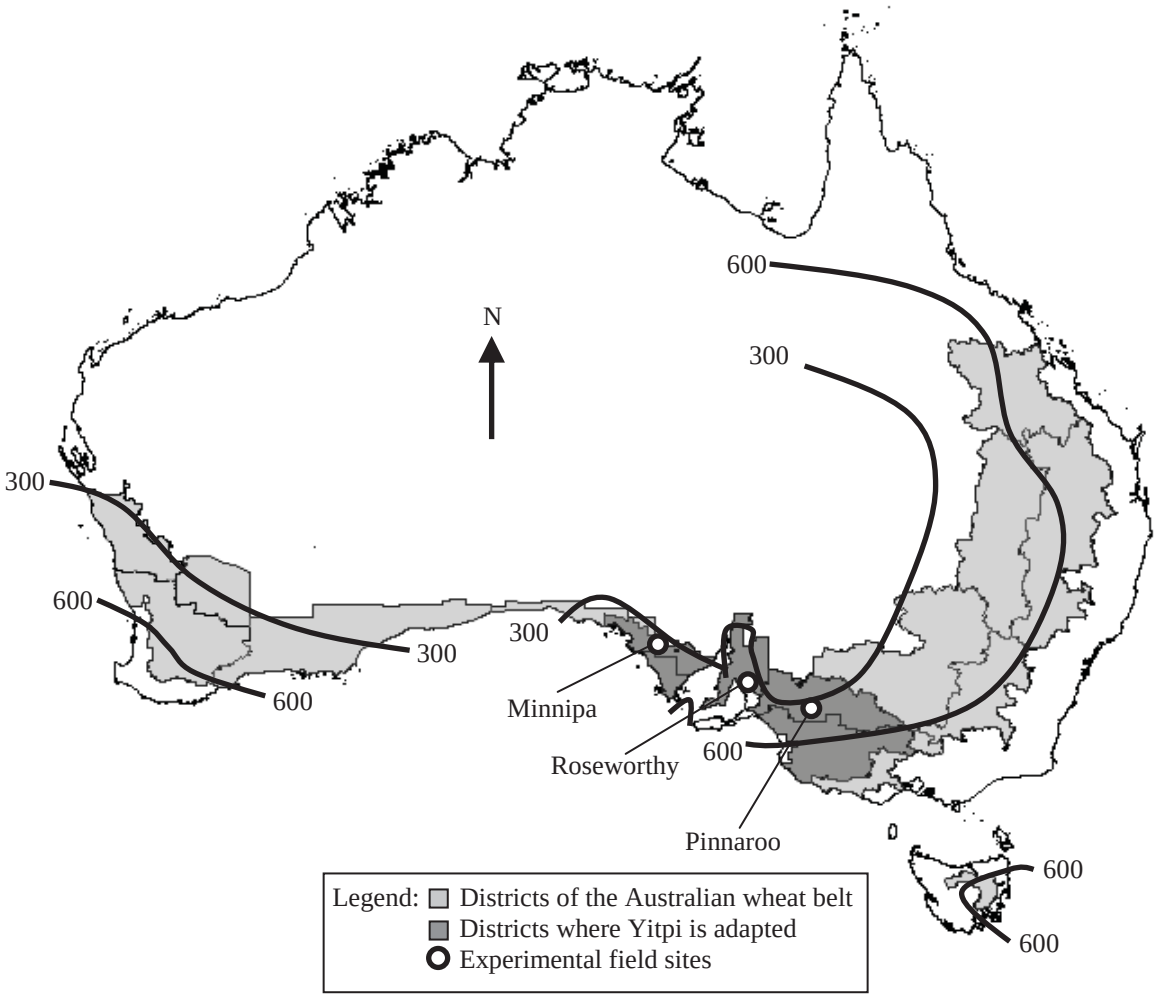

Figure 6. Districts of the Australian wheat belt where bread wheat cultivar Yitpi is adapted. Field experiments for this study were conducted near Minnipa, Roseworthy and Pinnaroo. Isohyets represent zones receiving an annual rainfall of 300mm and 600mm (AWB Limited 2003; Bureau of Meteorology 2008c; Bureau of Rural Sciences 2008).

Table 4. Australian-grown bread and durum wheat cultivars used in this research, their cultivation environments within the Australian wheat belt and breeding entities from which they were bred.

Cultivar	Cultivated environment of Australian wheat belt	Breeding Institution[1]
Bread wheat		
Annuello	South-eastern	Department of Primary Industries Victoria
AGT Scythe	South-eastern	Australian Grain Technologies
Carinya	South-eastern	Australian Grain Technologies
Kukri	South-eastern	University of Adelaide-RAC
Stylet	South-eastern	Australian Grain Technologies
Yitpi	South-eastern	University of Adelaide-WI
GBA Ruby	Western	Grain Biotech Australia
Wyalkatchem	Western and south-eastern	Western Australian Department of Agriculture
Janz	Broadly adapted	Queensland Department of Primary Industries
Durum wheat		
Arrivato	South-eastern	New Zealand Crop and Food Research
EGA Bellaroi	Eastern	Enterprise Grains Australia

[1] RAC = Roseworthy Agricultural College, WI = Waite Institute

Two durum wheat cultivars that are grown in Australia, Arrivato and EGA Bellaroi, were also included within genetic diversity investigations performed in this thesis. These genotypes were included for genetic contrast reasons as they come from different breeding pools (Table 4) to that of the parental CIMMYT durum wheat of the primary synthetic wheat investigated in this thesis.

3.5 Families of synthetic derived lines

The 27 BC_1F_4 bulk synthetic seed sets donated to this study (as described previously) were grown at Roseworthy (South Australia, Australia) in 2005 as full plots per the methods described by Kuchel *et al.* (2007) (6 row, 1.3 x 3.2m; 4.16m^2). These were sown with 17 other experimental plots in a randomised block (12 x 4 plots), with four Yitpi plots sown throughout in regular intervals. At maturity, 16 to 53 single plant spikes (lines) were selected per plot (Table 3) based on similarity of their height, growth habit and lodging status to those of Yitpi. Selected spikes were threshed and seeds were sown in 1.3 m rows in an irrigated summer nursery at Roseworthy for seed increase. Sowing occurred in mid-December (2005) and plants remained under irrigation until early April (2006). For the promotion of healthy plant growth general fertiliser, herbicide, fungicide and pest control measures were used. BC_1F_4-derived F_5 lines from this nursery were selected against if they did not mature before harvest, had poor threshability or produced an inadequate amount of seed (less than 35g for the sowing of one field plot). Selected BC_1F_4-derived F_6 lines (in 2006) and F_7 lines (in 2007) were evaluated in this thesis. Lines that failed to mature or failed to generate at least 160g of seed (sufficient to sow at least 4 field plots in 2007) were selected against from the 2006 field experiments. Further removal of lines also took place at this time due to contamination during harvest and seed cleaning. This contamination was partly attributable to poor threshability.

Chapter 4

Molecular characterisation of primary synthetic wheat

4.1 Introduction

The genomes of *Triticum turgidum* L. (genome AABB) and *Aegilops tauschii* Coss. (genome DD) are sources of genetic variation for bread wheat (*Triticum aestivum* L., genome AABBDD) breeding, as bread wheat has evolved from multiple but limited hybridisations between these progenitor species (McFadden and Sears 1946; Lubbers *et al.* 1991; Dvorak *et al.* 1998; Talbert *et al.* 1998; Reif *et al.* 2005; Chabane *et al.* 2007). Genetic variation from *T. turgidum* and *Ae. tauschii* can be introgressed into modern bread wheat cultivars using primary synthetic wheat as a genetic bridge (Lange and Jochemsen 1992). Primary synthetic wheats (genome AABBDD) are created by hybridising cultivars and landraces of *T. turgidum* with accessions of *Ae. tauschii* (Mujeeb-Kazi *et al.* 1996; Mujeeb-Kazi *et al.* 2008). Identifying genetic diversity among modern bread wheat cultivars and primary synthetic wheats may aid bread wheat breeders in choosing combinations suitable for developing populations for selection (Franco *et al.* 2001; Saffdar *et al.* 2009).

Molecular markers are powerful tools for identifying genetic similarity and variation between genotypes of bread wheat and germplasm sources for breeding. These tasks have employed some of the major marker systems, such as amplified fragment length polymorphisms (AFLP) (Hirano *et al.* 2008), simple sequence repeats (SSR) (Naghavi *et al.* 2009) and Diversity Arrays Technology (DArT™) (Akbari *et al.* 2006). Each marker system varies for implementation time, cost and ability to detect polymorphism across diverse germplasm. AFLP and DArT markers can be developed without prior sequence information, unlike SSR based markers (Mueller and Wolfenbarger 1999; Jaccoud *et al.* 2001). AFLP and DArT markers are therefore desirable for assessing the genomes of primary synthetic wheats, as these markers can be easily developed for non-model species like *Ae. tauschii*. The microarray platform of DArT permits a large number of loci to be assayed efficiently and simultaneously, unlike AFLP markers (Jaccoud *et al.* 2001; Akbari *et al.* 2006). DArT markers also provide the chromosome coverage to assess the large genome and allohexaploid nature of bread and primary synthetic wheats (Francki *et al.* 2009). As DArT markers have been successfully used to evaluate the genetic similarity among bread wheats (Stodart *et al.* 2007; White *et al.* 2008), this marker system could be

an effective tool to identify genetic diversity within and between modern bread wheat cultivars and primary synthetic wheats.

Molecular markers have shown Australian bread wheat cultivars to be more genetically diverse than those from western Europe, the UK and the USA (Akbari *et al.* 2006; White *et al.* 2008). Despite this diversity, *T. turgidum* and *Ae. tauschii* still have the potential to genetically improve Australian bread wheat pools. Primary synthetic wheats have been imported into Australia from CIMMYT for agronomic assessment and use in pre-breeding activities (Moody and Emebiri 2008a). Most of these primary synthetic wheats had A and B genomes derived from durum wheat cultivars or lines (*T. turgidum* subsp. *durum* (Desf.) Husn.) from CIMMYT's own breeding programs, with their D genomes being the novel source of genetic variation (Reeves *et al.* 1999; van Ginkel and Ogbonnaya 2007; Moody and Emebiri 2008a). The comparison of genetic diversity within and among a group of primary synthetic wheats imported into Australia and modern Australian bread wheat cultivars has not been performed before.

The aim of this study was to assess the genetic diversity within and among a group of primary synthetic wheats and modern Australian bread wheat cultivars using DArT markers. Genetic similarity matrices, dendrograms and principal co-ordinate analyses will form the basis of these investigations.

4.2 Materials and methods

4.2.1 Plant material

The plant materials used in this investigation have all been described in Chapter 3. They consisted of:

- 44 primary synthetic wheat genotypes (S1 through S44),
- nine Australian bread wheat cultivars (AGT Scythe, Annuello, Carinya, GBA Ruby, Janz, Kukri, Stylet (not commercially released), Wyalkatchem and Yitpi),
- five CIMMYT durum wheat cultivars (Altar84, Croc1, Gan, Kapude and Scoop1), parents of one, two, three, one and two primary synthetic wheats respectively, and
- two durum wheat cultivars that are grown in Australia (Arrivato and EGA Bellaroi)

According to the recorded pedigree information for the primary synthetic wheats, some of them had common durum and/or *Ae. tauschii* parents, as shown in Table 5.

Table 5. Recorded parentage of 32 primary synthetic wheats from the set of 44 used here that had pedigree relationships.

	Primary synthetic wheats											
Ae. tauschii	Durum wheat parent [2]											
parent [1]	D1	D5	D6	D7	D8	D10	D14	D16	D18	D21	D22	D23
T170									S32			
T208			S10									
T256				S13								
T258					S15							
T264					S16							
T269												
T309	S1											
	S2											
T332											S41	
T369												S43
												S44
T385												
T390			S11									
T434								S29				
T437						S21						
T446						S22						
T463				S14								
T484												
T488					S17							
T507												

Table 5. *Continued.*

	Primary synthetic wheats											
Ae. tauschii	Durum wheat parent[2]											
parent[1]	**D1**	**D5**	**D6**	**D7**	**D8**	**D10**	**D14**	**D16**	**D18**	**D21**	**D22**	**D23**
T517					S18							
T629			S12									
T634								S30				
T661		S6										
T783											S42	
T809										S36		
T819		S7										
T878										S37		
										S38		
										S39		
T882										S40		
T890						S23						
T895		S8					S27		S33			
		S9										
T1030					S19							

[1] CIMMYT's *Ae. tauschii* accession numbers are prefixed by WX in recorded pedigrees.
[2] Durum wheat pedigrees, D1 = ACO89, D5 = Ceta, D6 = CPI/GEDIZ/3/GOO//JO69/CRA, D7 = Croc1, D8 = Doy1, D10 = Gan, D14 = RABI//GS/CRA, D16 = Scoop1, D18=YAV2/TEZ, D21 = 68.111/RGB-U//WARD/3/FGO/4/RABI, D22 = 68.111/RGB-U//WARD RESEL/3/STIL, D23 = 68112/WARD

4.2.2 DNA extraction

The extraction of DNA was based on a phenol-chlorophorm protocol described by Rogowsky *et al.* (1991). Leaf tissue (2g) was collected from a single plant per genotype into polypropylene screw cap culture tubes. Stainless steel ball bearings were then placed into each tube. Tubes were immersed in liquid nitrogen for 5 minutes, then placed on a vortex mixer (VOR-MIX, S.E.M. (SA) Pty Ltd, AUS) until frozen leaf tissue was ground to a fine powder. After removing the ball bearings using a magnet, 4.5mL of DNA extraction buffer (1% sarkosyl, 100mM Tris-HCl, 100mM NaCl, 10mM EDTA, 2% polyvinyl-polypyrrolidone (insoluble), pH 8.5 adjusted using concentrated HCl) was added and slowly mixed with the leaf tissue powder using a suspension mixer (Ratek Instruments Pty. Ltd., AUS) for 1 minute. Next, 4.5mL of phenol:chloroform:isoamyl alcohol (25:24:1) was added, mixed vigorously for 1 minute using a vortex mixer and mixed for a further 5 minutes using a suspension mixer. Separated aqueous phase was decanted into a silica matrix tube and re-extracted as above using 4mL phenol:chloroform:isoamyl alcohol then centrifuged for 10 minutes. In a fresh tube, DNA was precipitated by adding 400µL 3M sodium acetate (pH 4.8) and 4mL isopropanol and mixed for 5 minutes on an orbital mixer (Ratek Instruments Pty. Ltd., AUS). DNA was spooled using a Pasteur pipette, transferred to a 2mL tube, washed with 1mL 70% ethanol, pelleted and dried. DNA was resuspended in 350µL of sterile water and stored overnight at 4˚C. Long term storage was at -20˚C. DNA quality and quantity was checked using a ND-1000 spectrophotometer (NanoDrop Technologies, USA).

4.2.3 DArT marker application and output

DNA from each test sample was diluted and prepared for DArT analysis as per instructions provided by Triticarte Pty Ltd (Australian Capital Territory, Australia) (http://www.triticarte.com.au/content/services-quality.html). DArT marker application was performed by Triticarte Pty Ltd, using a prototype of the version 2.5 high-density DArT array that had about 75% more clones than the standard array version 2.3. The hybridisation of each marker to a test sample was scored as either present (1), absent (0) or missing (X, for unreliable scores). Markers that obtained a Q-value (estimate of marker quality) below 77 were not used as errors in scoring become significant below this value (E. Huttner, personal communication, 2008).

4.2.4 DArT marker data analysis

DArT markers were considered to be polymorphic amongst the genotypes assessed here when both the '1' and '0' alleles were observed at a frequency where the rarer allele was at least 0.05. Polymorphic information content values (Botstein *et al.* 1980) were calculated for each marker as per the method of De Riek *et al.* (2001) for dominant markers. Polymorphic markers were assigned to chromosomes of the A, B and D genomes using 17 reference genetic maps by comparison. The reference genetic maps referred to here were as follows:

- Triticarte version 1.2 set of nine integrated genetic maps (Triticarte Pty Ltd 2008),
- Gladius x Drysdale and Kukri x Excalibur genetic map (provided by Dr Ken Chalmers, University of Adelaide).
- Ajana x WAWHT2074, P92201D5-2 x P91193D1-10, Cadoux x Reeves and EGA Blanco x Millewa genetic maps (Francki *et al.* 2009),
- Colosseo x Lloyd genetic map (tetraploid wheat) (Mantovani *et al.* 2008), and
- Langdon x Wild emmer wheat (G18-16) (tetraploid wheat) (Peleg *et al.* 2008).

Unmapped DArT markers assigned to chromosome regions by Francki *et al.* (2009), through the use of nullisomic-tetrasomic and deletion lines, were also used.

The software package NTSYSpc (version 2.20N) (Rohlf 2005) was used to perform the following computations. Genetic similarity matrices between pairs of genotypes were estimated using the Nei and Li coefficient (Nei and Li 1979) in subprogram SIMQUAL. Correlation between similarity matrices were estimated using a 2-way Mantel test in subprogram MXCOMP (Mantel 1967). Unweighted pair group method of arithmetic averaging (UPGMA) dendrograms, which demonstrate genetic similarity through hierarchical clustering were generated using subprogram SAHN (Sokal and Michener 1958; Sneath and Sokal 1973). Durum wheat and *Ae. tauschii* parentage of primary synthetics were shown in the dendrograms by the use of pedigree abbreviations in parentheses. (For corresponding pedigree abbreviation and explanation refer to Chapter 3). Principal coordinate analysis, which displays spatial genetic structure between genotypes was performed using subprograms DCENTER and EIGEN (Gower 1966). The first two axes were extracted to represent the total diversity detected. These operations were applied to analyse the A, B and D genomes of the primary synthetic wheats and bread wheat cultivars in four ways: (1) using data from D genome assigned polymorphic markers, (2) using data from A and B genome assigned polymorphic markers, (3) using data from A, B

and D genome assigned markers together, and (4) using data from all polymorphic markers, with and without chromosome assignment.

4.3 Results

4.3.1 Detection of polymorphism

A total of 1808 DArT markers detected polymorphism across the 44 primary synthetic wheats and nine bread wheat cultivars. These markers had a mean call rate of 95.5% and polymorphic information content values distributed from 0.1 to 0.5 (Figure 7). The mean polymorphic information content value was 0.39. Of the marker loci that were given chromosomal assignment from 17 reference genetic maps by comparison, most represented the B genome (472), followed by the A genome (317) and the D genome (179). Although DArT markers wPt-2592, wPt-2689, wPt-2910, wPt-5765, wPt-6292, wPt-6661, wPt-6736, wPt-8321 and wPt-9488 were assigned to the D genome, using the reference genetic maps, they were found to hybridise to at least one reference durum wheat cultivar, and hence these markers were removed from further analyses. Of the polymorphic markers that were not given chromosomal assignment, 168 markers were found to hybridise to all primary synthetic wheats but not to any of the bread wheat cultivars. Six unassigned markers hybridised in the opposite type of polymorphism (‘0’ in all synthetics, ‘1’ in all bread wheats).

4.3.2 Genetic similarities

The mean similarity coefficient between primary synthetic wheat pairs based on A and B genome and D genome DArT marker data sets was 0.67. The ranges of similarity coefficients between primary synthetic wheat pairs using A and B genome and D genome DArT marker sets were 0.51 to 1.00 and 0.38 to 1.00 respectively (Table 6). Primary synthetic wheats S43 and S44, both of which had the same recorded durum parent (D23) had a similarity coefficient of 1.00 between their A and B genomes. A similarity coefficient of 1.00 was identified between the D genomes of thirteen primary synthetic wheat pairs, which included genotypes S3(T221), S5(T218), S8(T895), S12(T629), S42(T783), S43(T369) and S44(T369). The mean pair-wise similarity coefficient for the bread wheat cultivars was 0.68 for their A and B genomes, with this value being 0.83 between their D genomes (Table 6). Similarity coefficients between primary synthetic and bread wheat pairs ranged from 0.40 to 0.72, with a mean of 0.54 for their A and B genomes. The D genomes of primary synthetic and bread wheat pairs had similarity coefficients ranging from 0.41 to 0.86, with a mean of 0.66 (Table 6).

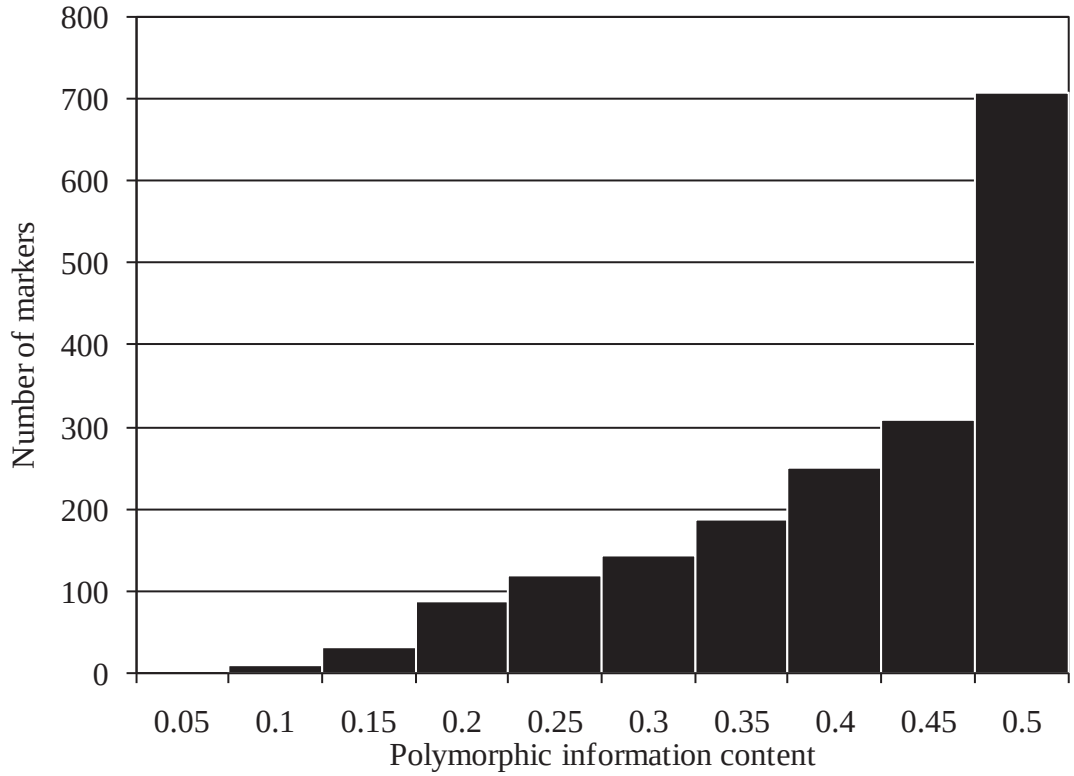

Figure 7. Distribution of polymorphic information content values of 1808 DArT marker loci displaying polymorphism between 44 primary synthetic wheat and nine Australian bread wheat cultivars.

Table 6. Range and mean genetic similarity coefficients between and within 44 primary synthetic wheat and nine Australian bread wheat cultivars using A, B and D genome DArT data.

Genotypes in matrix	Genome marker sets [1]	Genetic similarity coefficients	
		Range	Mean
Primary synthetic and bread wheats	D	0.41 to 0.86	0.66
	A and B	0.40 to 0.72	0.54
	A, B and D + unassigned	0.45 to 0.78	0.58
Primary synthetics	D	0.38 to 1.00	0.67
	A and B	0.51 to 1.00	0.67
	A, B and D + unassigned	0.54 to 1.00	0.68
Bread wheat	D	0.71 to 0.94	0.83
	A and B	0.58 to 0.92	0.68
	A, B and D + unassigned	0.70 to 0.94	0.77

[1] D genome marker set = 179 D genome assigned markers; A and B genome marker set = 317 A genome, and 472 B genome assigned markers; A, B and D genome plus unassigned markers = 317 A genome, 472 B genome, 179 D genome assigned markers plus 840 markers without chromosome assignment.

A correlation coefficient ‘r’ of 0.97 (Mantel test, $p = 0.01$) was identified between two genetic similarity matrices involving the primary synthetic and bread wheats, where one matrix was based on 968 markers assigned to the A, B and D genome, and the other based on 1808 markers with and without chromosomal assignment. Similarity coefficient values from this point forth refer to the use of all A, B and D genome polymorphic markers, with and without chromosomal assignment. Among primary synthetic wheat pairs and bread wheat pairs mean similarity coefficients were 0.68 and 0.77 respectively, which were both higher than the mean similarity coefficient (0.58) identified between pairs of genotypes from these two germplasm groups (Table 6). Out of the primary synthetic wheat pairs that shared the same recorded pedigree, S43(D23T369) and S44(D23T369) was the only one to record a similarity coefficient of 1.00.

4.3.3 Cluster and principle coordinate analyses

A UPGMA dendrogram involving the primary synthetic and bread wheats, generated using data from D genome assigned DArT markers, identified three major subclusters (G1, G2, and G3), which separated at the 0.57 similarity level (Figure 8a). Subcluster G1 mainly included primary synthetic wheats derived from *Ae. tauschii* accessions from Afghanistan. Primary synthetic wheats within G2 and G3 were derived from *Ae. tauschii* accessions from varying origins. All bread wheat cultivars clustered within G2, and were also found to associate with the primary synthetic wheats of G2 in the principal coordinate analysis (Figure 9a), where axis 1 and axis 2 accounted for 21.6% and 12.1% of the total variance. Of the 11 primary synthetic wheats that had the same *Ae. tauschii* accession in their pedigrees, four clustered together (Figure 8a). Primary synthetic wheats of minor subcluster G4 separated at similarity levels above 0.99, and were closely associated in diversity space (Figure 9a). Most of these primary synthetic wheats had parental *Ae. tauschii* accessions from the Mazandaran province of Iran.

Cluster analysis of data from markers assigned to the A and B genomes separated most primary synthetic wheats (H1) from the bread wheat cultivars (H2) at the 0.59 similarity level (Figure 8b). The primary synthetic wheats formed two lesser defined sub-groups, ‘durum wheat-like’ (H3) and ‘intermediate’ (H4). These two sub-groups were more clearly separate in the principal coordinate analysis, where the first two principal coordinates accounted for 18.5% (axis 1) and 5.9% (axis 2) of the total variance (Figure 9b). The H3 primary synthetic wheats represented 27 of the primary synthetic genotypes assessed, which included those with

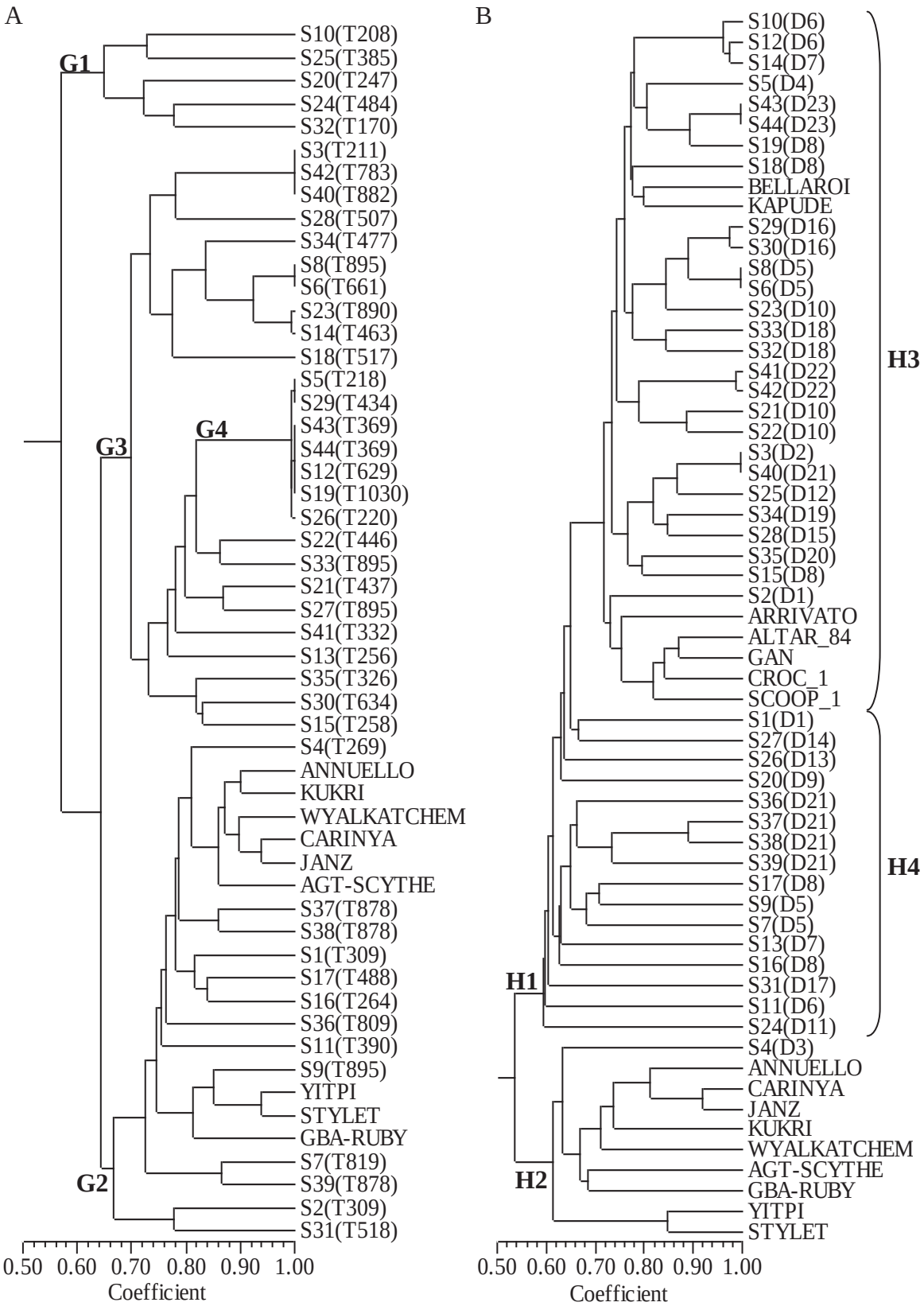

Figure 8. UPGMA dendrograms for 44 primary synthetic wheats, nine Australian bread wheats and seven reference durum wheats, based on (A) 179 D genome DArT markers, and (B) 287 A genome and 451 B genome DArT marker loci. Abbreviation of *Ae. tauschii* and durum wheat parentage of the primary synthetic wheats is denoted within parentheses.

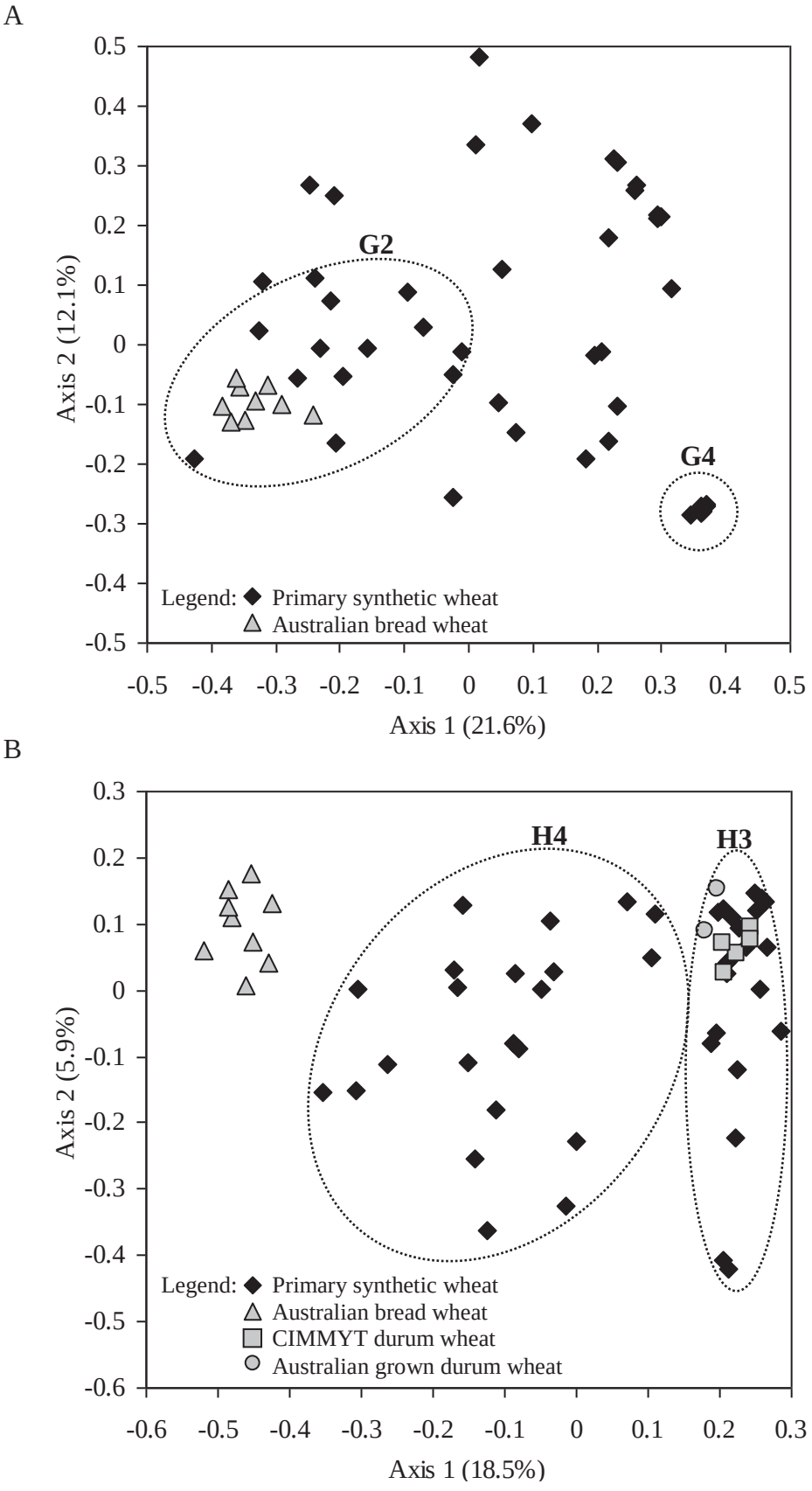

Figure 9. Principal coordinate analysis (2-dimensional) of 44 primary synthetic wheat genotypes and nine Australian bread wheat, five CIMMYT durum wheat and two Australian grown durum wheat cultivars using data from (A) 179 D genome DArT markers, and (B) 287 A genome and 451 B genome DArT markers.

Altar_84 (D2), Gan (D10), Kapude (D12) or Scoop_1 (D16) as the A and B genome donor in their pedigrees (Figure 8b). None of the reference durum wheats clustered within a primary synthetic wheat which had them as their recorded A and B genome parent. Among primary synthetic wheats with common durum wheat parentage, 68.8% were found to cluster together.

Two major clusters of genotypes (J1 and J2) were identified in the A, B and D genome UPGMA analysis of the primary synthetic and bread wheat cultivars, which used markers with and without chromosomal assignment to the A, B and D genomes (Figure 10). Clusters J1 and J2 separated at approximately the 60% similarity level, however the genotypes within each cluster were influenced by the A and B genome marker data. This could be seen by 96.2% of primary synthetic wheats that clustered in H3 of the A and B genome dendrogram (Figure 8b) clustering similarly in J3 (Figure 10). Of the primary synthetic wheats with 'intermediate' A and B genomes (H4), 43.8% of these clustered with the bread wheats in J4 (Figure 10). Although 8 primary synthetic wheats cluster with the bread wheats, their association within diversity space was intermediate where 18.5% (axis 1) and 5.9% (axis 2) of the total variance was represented (Figure 11).

4.3.4 Correlation between genetic distance matrices

Of the DArT markers that were polymorphic among the primary synthetic and bread wheat genotypes, 786 were not given chromosomal assignment from 17 reference maps and studies assigning DArTs to nullisomic-tetrasomic and deletion lines. A correlation coefficient 'r' of 0.83 (Mantel test, $p = 0.01$) was identified for the comparison of similarity matrices calculated from 786 unassigned markers and those that represent the A and B genomes in the genotypes assessed here. This relationship was stronger than the 0.69 correlation coefficient (Mantel test, $p = 0.01$) identified between similarity matrices calculated from the unassigned and D genome assigned markers.

4.4 Discussion

This study demonstrates that the DArT marker system can successfully identify genetic similarity between the genomes of primary synthetic wheat and bread wheat cultivars. Between the 44 primary synthetic wheats and the nine modern Australian bread wheat cultivars investigated here greater dissimilarity was observed than that identified within these germplasm groups, suggesting that these primary synthetic wheats are a source of genetic variation for Australian bread wheat breeders.

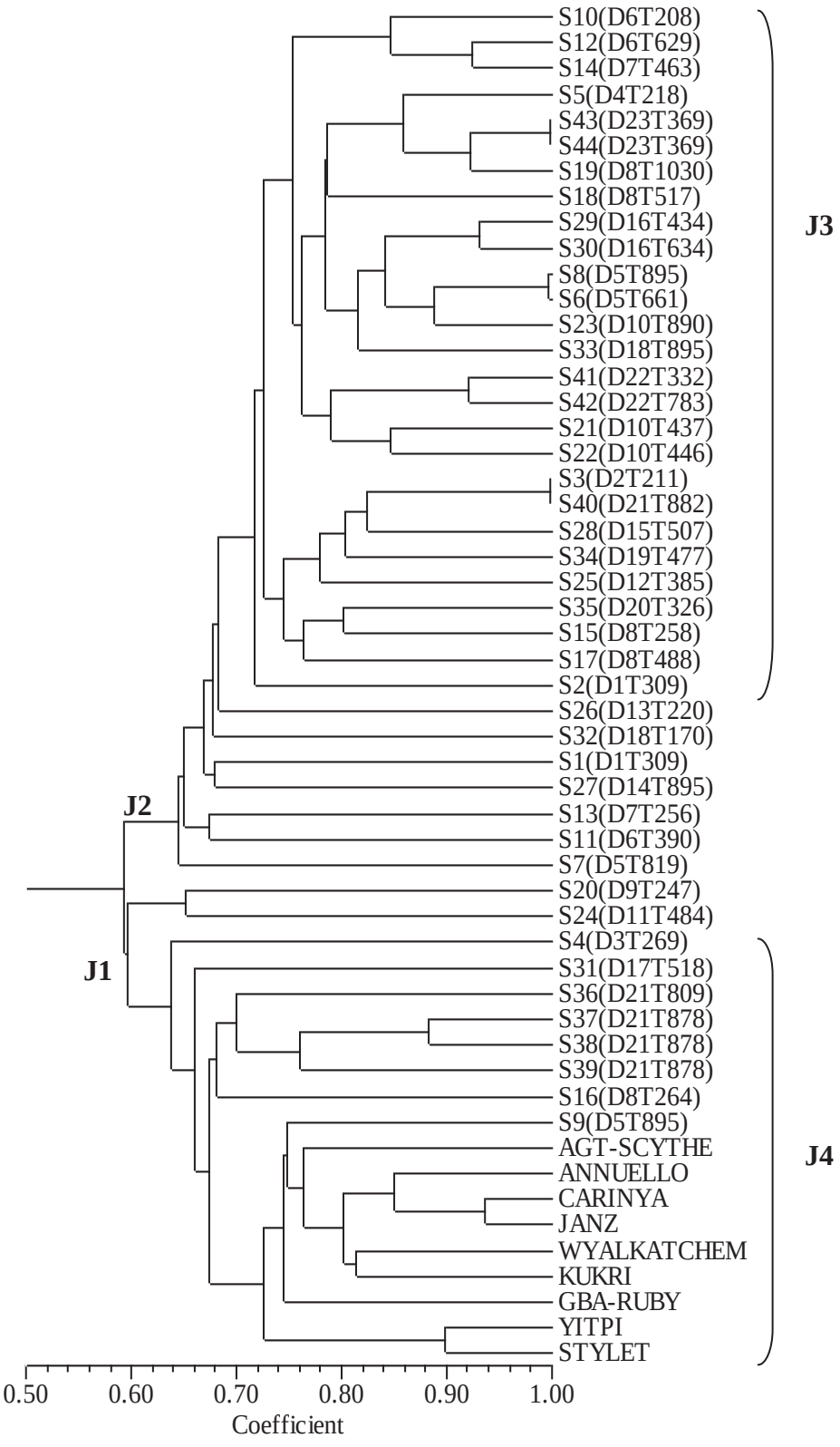

Figure 10. UPGMA dendrograms for 44 primary synthetic wheats and nine Australian bread wheat cultivars, based on 1808 polymorphic DArT markers with and without chromosomal assignment to A, B and D genome chromosomes. Abbreviation of *Ae. tauschii* and durum wheat parentage of the primary synthetics is denoted within parentheses.

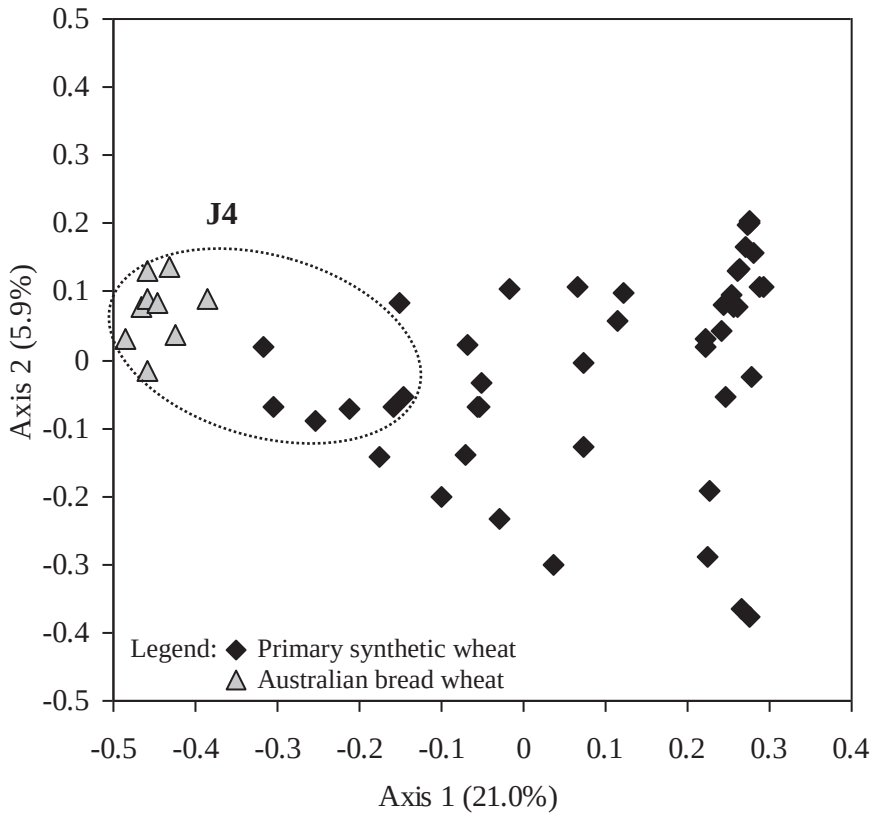

Figure 11. Principal coordinate analysis (2-dimensional) of 44 primary synthetic wheat genotypes and nine Australian bread wheats, created using data from all polymorphic DArT markers with and without chromosomal assignment to the A, B and D genomes.

The mean level of genetic similarity identified between the Australian bread wheat cultivars used in this study was between the upper limits of similarity reported by Paull *et al.* (1998) and White *et al.* (2008) for pairs of Australian bread wheat cultivars. The use of nine bread wheat cultivars from varying Australian breeding programs therefore provided a reasonable representation of genetic similarity among modern Australian bread wheats. However, to investigate the genetic similarity between primary synthetic wheats and cultivars that represent Australian bread wheat breeding history, an extensive range of modern and older bread wheats would be required. Such a collection would reveal the classic pedigree groups among Australian bread wheat cultivars, which were not observed in the current study (Paull *et al.* 1998; Akbari *et al.* 2006).

The high similarity between the bread wheat cultivars, based on their D genome DArT marker data reflected the common observation of low polymorphism in modern bread wheat cultivars (Röder *et al.* 1998; Chalmers *et al.* 2001). This supports part of the rationale for using primary synthetics in bread wheat breeding, to increase the genetic diversity of bread wheat. Many primary synthetic wheat investigated here had genetically divergent D genomes compared to those of the bread wheat cultivars examined. Interestingly the A and B-genomes of the synthetic wheats were more divergent from those of the bread wheats, compared to divergence observed between the D genomes of the synthetic wheats and the bread wheats. Even though durum wheat, the A and B-genome component of these primary synthetic wheats can be quite genetically divergent from the A and B genome of bread wheat (Chabane *et al.* 2007), these observations could be explained by the following. The D genome of *Ae. tauschii* could be less divergent from that of bread wheats (Naghavi *et al.* 2009), which is contrary to previous studies, or that too few D genome DArT markers are available to sufficiently account for the polymorphism found in *Ae. tauschii*. Approximately half of the markers on the prototype version 2.5 high-density DArT array used here were developed from bread and durum wheats, with the other half representing *Triticum* species of various ploidy (Triticarte Pty Ltd 2009). *Aegilops tauchii* was included in the latter germplasm group, however relatively few markers on the array were polymorphic for the D genome of bread wheat. Triticarte Pty Ltd has sought to address this issue by adding more D genome markers to the current DArT arrays from D genome enriched sources (Triticarte Pty Ltd 2009). It is also important to note that estimates of similarity may have been inflated. This is due to an assumption that the absence of hybridisation (‘0’) for a particular DArT clone always represented the same

allele, whereas in some cases there may have been multiple mutations or deletions leading to absence of hybridisation.

The high correlation of the A and B genome DArT marker data with the unassigned DArT marker data suggests that many of the markers without chromosomal assignment could be A and B genome derived. The significant and moderate correlation between the unassigned markers and the D genome assigned markers however suggested that some new D genome markers are present, which require mapping. Nearly five times more DArT markers were found to hybridise to the primary synthetic wheats and not the bread wheat cultivars, than in the opposite type of polymorphism. Due to the germplasm used to develop the DArT array used here it is surprising to have a high level of hybridisation with the synthetics. The use of primary synthetic wheats as parents of mapping populations could aid in assigning additional DArT markers to chromosomes.

The low genetic similarity coefficients and low incidence of clustering between primary synthetic wheat with the same recorded pedigree components (durum and/or *Ae. tauschii* accession) raises concerns about the accuracy of their recorded pedigrees. Discrepancies in the pedigrees of some primary synthetics created by CIMMYT in the late 1980's and 1990's have been discovered through research investigations. As reported by Singh *et al.* (2000), the primary synthetic wheat used to develop the recombinant inbred population 'Opata 85 x Synthetic' for the International Triticeae Mapping Initiative (ITMI) (Van Deynze *et al.* 1995) repeatedly failed to exhibit expected rust-resistance infection types along with progeny from the population . Some of the synthetics imported into Australia have been found to differ in their glutenin composition from what would be expected based on pedigree information (Cornish 2008). A high degree of out-crossing has been observed in primary synthetic wheat imported into Australia, and has been thought to be the cause of approximately 6% of these primary synthetic wheats not being true to type (F. Ogbonnaya, personal communication, 2008). Therefore the use of CIMMYT durum wheat as accurate parental references in the current study had its limitations. The methods used to analyse the synthetic wheats had limitations. With only a single plant sampled from each accession, it was not possible to detect heterogeneity within accessions. Therefore the results observed may have not been fully representative of each accession.

4.5 Conclusion

The present DArT marker analysis, characterising the genetic diversity of primary synthetic wheat and Australian bread wheat shows that primary synthetic wheat is a source of genetic diversity that could be suitable for breeding. The primary synthetic and bread wheat groups used here showed a lack of genetic divergence between their D genomes, emphasising the need to develop more polymorphic D genome markers, possibly from *Ae. tauschii* accessions. This action is likely to occur in the near future. Based on the current study it seems that the pedigree information for some CIMMYT developed primary synthetic wheats must be viewed with caution. The practice of thorough pedigree record keeping and the collecting and retention of seed from the exact parental plants used for developing primary synthetic wheats is encouraged for later molecular studies.

Chapter 5

Grain yield and its component traits in synthetic backcross wheat lines grown under drought stress

5.1 Introduction

Increases in global grain yields of bread wheat (*Triticum aestivum* L.) over the last 40 years partly come from the use of gibberellic acid-sensitive dwarfing genes (*Rht1* and *Rht2*), which were globally distributed during the 'green revolution' (Smale *et al.* 2002). In addition to reducing plant height to a semi-dwarf form, the expression of these genes increased grain yield through the production of more grains per m^2, compared to normal stature wheats. Grains per m^2 is one of two major grain yield components of bread wheat, and increases in this component have commonly been accompanied by reductions in the other component, grain weight (Miralles and Slafer 1995; Miralles *et al.* 1998; Araus *et al.* 2008). Mean individual grain weight is a major grain yield component and a quality character of bread wheat. High grain weight has a favourable effect on milling yield due to higher endosperm volume to surface area ratios, reducing the percent of by-products (bran and pollard) from the milling process (Marshall *et al.* 1986; Wiersma *et al.* 2001). Although semi-dwarf cultivars have shown a negative relationship between grain weight and grains per m^2, further increases of grain yield may be possible through exploring genetic variation to simultaneously increase grain weight and grains per m^2 (Gaju *et al.* 2009).

Primary synthetic wheats are a potential genetic resource for increasing grain weight and grains per m^2 in semi-dwarf bread wheats. Genetic variation for both grain yield components has been reported in primary synthetic wheats (Villareal *et al.* 1994a; Villareal *et al.* 1994b; Mujeeb-Kazi 1995; Calderini and Reynolds 2000; Calderini and Ortiz-Monasterio 2003). As primary synthetic and bread wheats have the same genomic constitution (genome AABBDD) and can be readily hybridised, genetic material from

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In October 2010, a modified version of this chapter was submitted as a manuscript to the journal Crop and Pasture Science for consideration for publication. The title and authors of this manuscript are as follows:

Grain yield and its component traits in synthetic backcross wheat lines grown under drought stress
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primary synthetic wheats can be exploited for the breeding of improved bread wheat cultivars (Reynolds *et al.* 2005).

In northern and southern Australian environments, synthetic derivatives with improved grain yield performance have been identified (Dreccer *et al.* 2007; Ogbonnaya *et al.* 2007; Rattey and Shorter 2010). In these studies, synthetic backcross lines achieved grain yields up to 30% higher than the best check bread wheat in the north, while in the south, grain yields up to 11% higher than the best check bread wheat were reported. Gororo *et al.* (2002) investigated a set of synthetic backcross lines at low and high yielding environments in southern Australia. In the high yielding environments, grain yield of synthetic backcross lines were similar to that of their Australian recurrent bread wheat parent. In the lower yielding environments, synthetic derivatives produced significantly higher grain yields, up to 149% of their Australian recurrent bread wheat parent (Gororo *et al.* 2002). However, the ability of synthetic backcross lines to achieve such superior grain yields, in diverse drought stressed environments in southern Australia, is unknown.

There is high priority to develop bread wheat cultivars in Australia that maintain a viable level of grain yield in drought stressed environments. The occurrence of drought can severely reduce the grain yield of bread wheat in Australia, where it is grown in rainfed environments (275mm to 700mm p.a.) (Fischer 1999). In the growing seasons of 2006 and 2007, drought in the southern Australian wheat belt resulted in severe yield losses (Bureau of Meteorology 2008a; Bureau of Meteorology 2008b). Reductions in the Australian national production of wheat were in the order of 49.7% in 2006/07 and 39.4% in 2007/08, compared with 2001/02-2005/06 average production (ABARE 2003; ABARE 2007; ABARE 2008). The conditions of 2006 and 2007 provided an opportunity to investigate the grain yield, grain weight and grains produced per m^2 of synthetic backcross lines grown in southern Australian environments under natural drought conditions.

The potential of primary synthetic wheats to increase grain weight and grains per m^2 of a bread wheat cultivar, in drought stressed environments, may be different because primary synthetic wheat can be created from different *Triticum turgidum* L. and *Aegilops tauschii* Coss. accessions (Mujeeb-Kazi *et al.* 1996). Variation in the grain yield components of synthetic derivatives assessed in Australia has not been extensively reported, and hence the effect that different primary synthetic wheat parents may have on mean individual grain weight and grains per m^2 is unknown (Dreccer *et al.* 2007; Ogbonnaya *et al.* 2007). Gororo

et al. (2002) has shown that one primary synthetic wheat significantly improved the grain yield of an Australian bread wheat, in a low yielding environment, through increases to the number of grain produced per m^2. To understand the potential of primary synthetic wheats to simultaneously increase grain weight and grains per m^2 of an Australian bread wheat, in Australian drought stressed environments, synthetic backcross lines with different primary synthetic parentage need to be assessed in such conditions.

The aim of the current study was to assess 27 families of BC_1 synthetic-derived lines, each with a different primary synthetic parent but a common bread wheat parent for grain yield and its component (grains per m^2 and grain weight) performance, in selected rainfed environments in southern Australia. The families of synthetic-derived lines were evaluated under serious to severe drought conditions in 2006 and 2007, which provided ideal conditions for the analysis of their performance for grain yield and its component in low-yielding and moisture limiting environments. To identify genotypes suitable for further breeding activities, the mechanism of superior grain yield production by individual synthetic backcross lines is also discussed.

5.2 Materials and methods

5.2.1 Plant material

An Australian bread wheat cultivar, Yitpi (recurrent parent), and 27 primary synthetic wheats (donor parents, Table 7) were used to generate 27 families of BC_1F_4-derived synthetic lines, as described in Chapter 3. These families comprised of 10-48 lines per family (Table 7), and were evaluated as BC_1F_4-derived F_6 plants in 2006 and as BC_1F_4-derived F_7 plants in 2007. Australian bread wheat cultivars, Annuello, Carinya, Stylet (not commercially released), Yitpi and Wyalkatchem (Table 8) were used as reference check cultivars for grain yield as they are highly adapted to the southern Australian wheat belt (Figure 12).

5.2.2 Field experimentation

Synthetic backcross lines were assessed in five environments in South Australia wheat belt. Experiments were conducted near Roseworthy (34°53’S, 138°74’E) and Minnipa (32°85’S, 135°15’E) in 2006 and 2007, and near Pinnaroo (35°26’S, 140°9’E) in 2007 only (Figure 12). These experience Mediterranean-like climate. Annual rainfall (25-year average) at Roseworthy, Minnipa and Pinnaroo is 446 mm, 320 mm and 301 mm respectively, about 65% of which falls within the growing season (1 June to 30

Table 7. Primary synthetic wheats, their associated derived families and the number of synthetic-derived lines evaluated per family in field experiments at Minnipa, Pinnaroo and Roseworthy in 2006 and 2007.

Primary synthetic wheat	Synthetic-derived family	Number of lines evaluated		
		2006		2007
		Minnipa	Roseworthy	Minnipa, Pinnaroo and Roseworthy
S1	Y1	15	23	22
S3	Y3	27	37	37
S5	Y5	11	23	22
S6	Y6	16	24	23
S7	Y7	21	19	18
S9	Y9	17	25	25
S10	Y10	8	17	16
S11	Y11	23	29	28
S12	Y12	24	27	27
S13	Y13	17	20	19
S14	Y14	39	48	47
S15	Y15	22	26	25
S16	Y16	13	20	19
S18	Y18	41	48	47
S19	Y19	25	34	34
S20	Y20	16	29	29
S26	Y26	7	19	18
S27	Y27	13	20	19
S31	Y31	16	23	23
S33	Y33	22	28	28
S35	Y35	11	18	18
S36	Y36	19	25	25
S37	Y37	18	23	23
S40	Y40	9	16	14
S41	Y41	5	10	9
S42	Y42	20	27	25
S43	Y43	13	15	13
	Total	488	673	653

Table 8. Date of sowing for each field experiment, replication of synthetic derivatives per experiment and number of reference check cultivar replicates sown per experiment.

	Field experiments				
	2006		**2007**		
	Minnipa	**Roseworthy**	**Minnipa**	**Pinnaroo**	**Roseworthy**
Sowing date	9 June	7 June	2 June	6 June	22 May
Replication of synthetic derivatives	38%	25%	20%	20%	20%
Replicates of check cultivars					
Annuello	8	6	12	12	10
Carinya	5	5	8	11	10
Stylet	5	5	10	10	10
Yitpi	8	6	15	14	14
Wyalkatchem	6	5	12	10	10

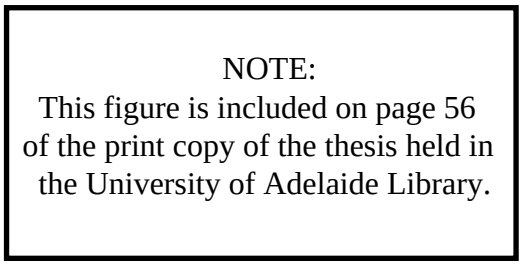


Figure 12. Location of field experiments (Minnipa, Pinnaroo and Roseworthy) within the in South Australian wheat belt. Isohyets represent zones receiving an annual rainfall of 300mm and 600mm (Bureau of Meteorology 2008c).

November). DiGGer design search software (Coombes 2002) was used to design the field experiments, using random, partially replicated designs (Cullis *et al.* 2006). In each experiment there was at least 20% replication of the synthetic backcross lines, with up to 15 replicates of each reference check cultivar (Table 8). Plots within experiments used 34 g of seed, sown as full plots (6 row, 1.3 x 3.2m; 4.16m^2), and were mechanically harvested using methods similar to those described by Kuchel (2007). Chemical pest control management and fertiliser application followed local practice at each experimental location.

5.2.3 Phenotypic evaluation

Mean individual grain weight (mg) was derived from counting a random sample of 500 threshed grains from each field plot and dividing by 500. As the area harvested from each plot was 4.16 m^2, grain yield in kg/ha was calculated by dividing the number of kg of grain harvested by 4.16 x 10^{-4}. The number of grains produced per m^2 per plot was determined by dividing plot grain yield (g) by grain weight (g), then by plot area (m^2).

5.2.4 Statistical analysis

Multi-environment trial (MET) analysis was performed on field and postharvest measurements. Mixed linear models were fitted using residual maximum likelihood (REML) (Patterson and Thompson 1971; Gilmour *et al.* 1995) to manage genotype by environment interactions. This was performed in the 'R' statistical environment (R Development Core Team 2008) (version 2.5.0) using the ASReml package (Butler *et al.* 2007). Best linear unbiased predictions (BLUPs) (Henderson 1975; Piepho *et al.* 2008) were generated for each measurement, for each genotype, at each environment, incorporating adjustments for spatial fixed and random effects. A summary of these effects is found in Appendix 3. BLUPs for each family of synthetic backcross lines were calculated as the mean of BLUPs of lines within each family at each environment. Across-year MET BLUPs (Minnipa 2006 and 2007 BLUPs, and Roseworthy 2006 and 2007 BLUPs) for reference bread wheat checks, families and synthetic backcross lines within were calculated as the mean of corresponding BLUPs from experiments conducted in 2006 and 2007 at a trial location. Estimates of heritability for grain yield and grain weight at individual environments were calculated using methods described by Cullis (2006). Associations among grain weight, grains per m^2 and grain yield were investigated across all synthetic backcross lines and within families by applying simple linear correlation

analysis to the MET BLUP means for Roseworthy (2006 and 2007), Minnipa (2006 and 2007), and Pinnaroo (2007).

5.3 Results

5.3.1 Climatic and growing conditions

Drought was experienced in southern Australia during the growing seasons of this study (1 June to 30 November in 2006 and 2007). At Minnipa and Roseworthy in 2006, growing season rainfall was 41.6% and 40.5% of their respective 25-year means (Table 9). In 2007, Minnipa, Pinnaroo and Roseworthy received 36.6%, 62.4% and 63.4% of their respective 25-year mean rainfall for the same five month period. Unseasonal rainfall occurred at these localities in several summer and autumn months prior to the growing seasons of 2006 and 2007, resulting in near mean annual rainfall (Table 9).

Experiments at Roseworthy exhibited symptoms of crown rot. The severity of crown rot symptoms was fitted as a fixed covariate in the MET analysis (Appendix 3).

5.3.2 Heritability, genetic variance and genetic correlations between environments

Estimates of heritability of grain yield and grain weight ranged from 0.37 to 0.89 across environments (Table 10). Genetic variance for grain yield was higher for the environments with higher grain yields. Estimates of genetic correlation for grain yield ranged from 0.51 to 0.85 between environments, and from 0.59 to 0.82 for grain weight (Table 11).

5.3.3 BLUP analysis of grain yield and its components

Grain yield and its components reported here for genotypes grown under the Roseworthy and Minnipa environments are for 2006 and 2007 across year MET BLUPs. Data from the Pinnaroo environment are for 2007 MET BLUPs.

5.3.3.1 Grain yield performance

Grain yields were highest at Roseworthy, followed by Pinnaroo and Minnipa, with Yitpi yielding 2547.9 kg/ha, 1212.1 kg/ha and 405.9 kg/ha, respectively (Figure 13). Across environments, estimated grain yield of synthetic backcross families ranged from 73.8% to 103.3% of that of Yitpi. Y18 produced the highest estimated family grain yield in every environment, and was superior (p<0.05) to 18 other families at Roseworthy, 14 at Minnipa and three at Pinnaroo. Synthetic backcross lines within families produced grain yields up to 12.0% higher at Roseworthy, 4.7% higher at Pinnaroo and 43.8% higher at Minnipa

Table 9. Rainfall data from Minnipa, Pinnaroo and Roseworthy during the years 2006 and 2007.

		Rainfall (mm) [1]	
Year	**Location**	**Growing season (% of 25-year mean) [2]**	**Annual mean (% of 25-year mean) [3]**
2006	Roseworthy	115.8 (40.5%)	340.6 (76.3%)
2006	Minnipa	90.7 (41.6%)	246.6 (77.1%)
2007	Roseworthy	181.4 (63.4%)	440.4 (98.7%)
2007	Minnipa	79.6 (36.6%)	NA[4]
2007	Pinnaroo	119.8 (62.4%)	NA[4]

[1] Bureau of Meteorology (2008d).
[2] 25-year mean = mean of growing season rainfall period (1 June to 30 November) from 1982 to 2007.
[3] 25-year mean = mean of annual rainfalls (1 January to 31 December) from 1982 to 2007.
[4] Complete 12 monthly rainfall data sets were unavailable for this site.

Table 10. Estimation of heritability and genetic variance for grain yield and grain weight in experiments conducted under environments at Minnipa, Pinnaroo and Roseworthy in 2006 and 2007.

Environments		Heritability	Genetic variance
Grain yield			
2006	**Minnipa**	0.78	4846.1
	Roseworthy	0.87	188869.1
2007	**Minnipa**	0.81	27373.7
	Pinnaroo	0.48	15306.0
	Roseworthy	0.78	187058.0
Grain weight			
2006	**Minnipa**	0.37	8.5
	Roseworthy	0.78	7.7
2007	**Minnipa**	0.63	7.8
	Pinnaroo	0.87	12.9
	Roseworthy	0.89	19.8

Table 11. Estimated genetic correlation matrices between environments for grain yield and grain weight.

		Environment				
		2006		2007		
Grain yield		**Minnipa**	**Roseworthy**	**Minnipa**	**Pinnaroo**	**Roseworthy**
2006	**Minnipa**	1.00				
	Roseworthy	0.79	1.00			
2007	**Minnipa**	0.82	0.85	1.00		
	Pinnaroo	0.51	0.67	0.63	1.00	
	Roseworthy	0.71	0.83	0.80	0.65	1.00
Grain weight						
2006	**Minnipa**	1.00				
	Roseworthy	0.64	1.00			
2007	**Minnipa**	0.75	0.63	1.00		
	Pinnaroo	0.72	0.75	0.82	1.00	
	Roseworthy	0.59	0.67	0.64	0.69	1.00

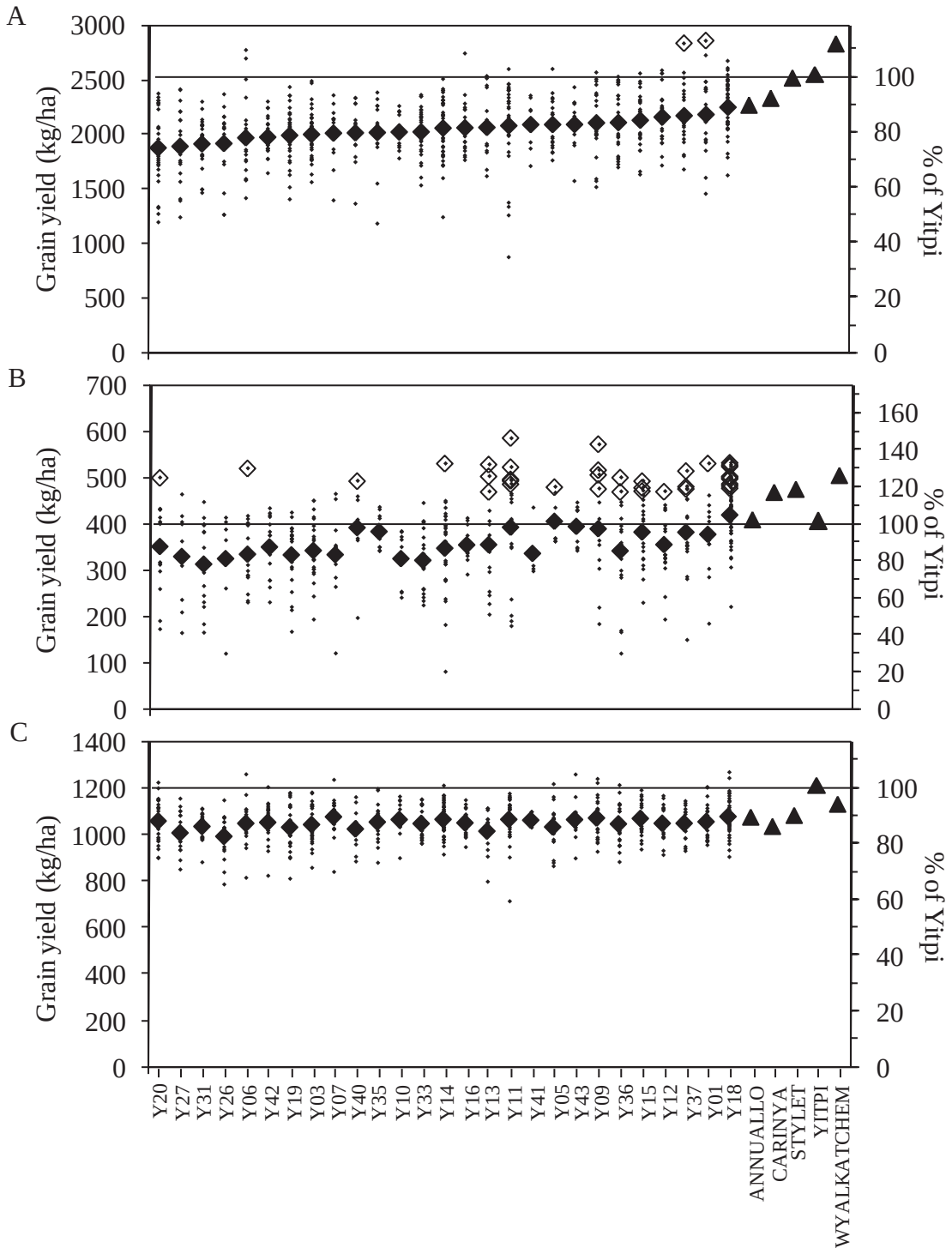


Figure 13. BLUPs for grain yield of 27 families of synthetic-derived lines (◆), for individual lines within those families (⬩ and ◈, where ◈ designates lines with significantly (p<0.05) higher grain yield than their recurrent parent Yitpi) and for five bread wheat cultivars (▲), grown at (A) Roseworthy in 2006 and 2007, (B) Minnipa in 2006 and 2007 and (C) Pinnaroo in 2007.

compared with that of Yitpi (Figure 13). Thirty six synthetic backcross lines at Minnipa and two at Roseworthy produced significantly (p<0.05) higher grain yields than Yitpi. These lines were from 14 families (Y01, Y05, Y06, Y09, Y11, Y12, Y13, Y14, Y15, Y18, Y36, Y37 and Y40). At Minnipa these lines produced up to 54.3% more grains per m^2 (p<0.05) than Yitpi, and commonly showed no significant (p<0.05) penalty to grain weight compared with Yitpi. Derivative Y09-04 at Minnipa achieved a superior grain yield through a significant (p<0.05) 7.3% grain weight increase compared with that of Yitpi. At Roseworthy, higher yielding lines had significantly (p<0.05, up to 8.1%) heavier grains than Yitpi. These lines also showed no penalty to grains per m^2 compared with Yitpi. At Pinnaroo, 187 synthetic backcross lines produced grain yields not different to that of Yitpi. Synthetic backcross line Y01-08 was the most productive across all environments, with grain yields from 99.2% to 130.4% of that of Yitpi.

5.3.3.2 Grain weight and grain per m^2 performance

Mean individual grain weight of Yitpi was highest at Pinnaroo (38.7 mg/grain), followed by Roseworthy (32.2 mg/grain) and Minnipa (30.8 mg/grain). Family means for grain weight ranged from 82.9 to 107.6 % of that of Yitpi (Figure 14). Families Y12, Y14 and Y43 had higher estimated grain weights than Yitpi in every environment. Compared to Yitpi, synthetic backcross lines produced significantly (p<0.05) heavier grains, up to 133.2% at Roseworthy, 119.1% at Minnipa and 118.9% at Pinnaroo (Figure 14). These lines were identified across 26 families. Of the synthetic backcross lines evaluated in Roseworthy, Pinnaroo and Minnipa environments, 30.5%, 17.6% and 7.4% respectively produced significantly (p<0.05) heavier grains than Yitpi, with 4.3% of lines achieving this in every environment. Across all environments Y14-27 produced the heaviest grains compared with that of Yitpi, ranging from 10.9% to 25.6% heavier.

Yitpi produced 8041 grains per m^2 at Roseworthy, 3171 grains per m^2 at Pinnaroo and 1277 grains per m^2 at Minnipa (Figure 15).The 27 synthetic backcross families had estimated grain per m^2 ranging from 70.9% to 104.0% of that of Yitpi. The best three families for grains per m^2 were Y01, Y10 and Y37 at Roseworthy and Pinnaroo, and Y05, Y11 and Y18 at Minnipa. At Roseworthy, seven synthetic backcross lines produced significantly (p<0.05, up to 111.8%) more grain per m^2 than Yitpi (Figure 15). Across all lines at Minnipa, 10.3% produced significantly (p<0.05, up to 154.3%) more grains per m^2 than Yitpi (Figure 15). At Pinnaroo, derivative Y11-26 produced significantly (p<0.05, 111.7%) more grains per m^2 to that of Yitpi (Figure 15).

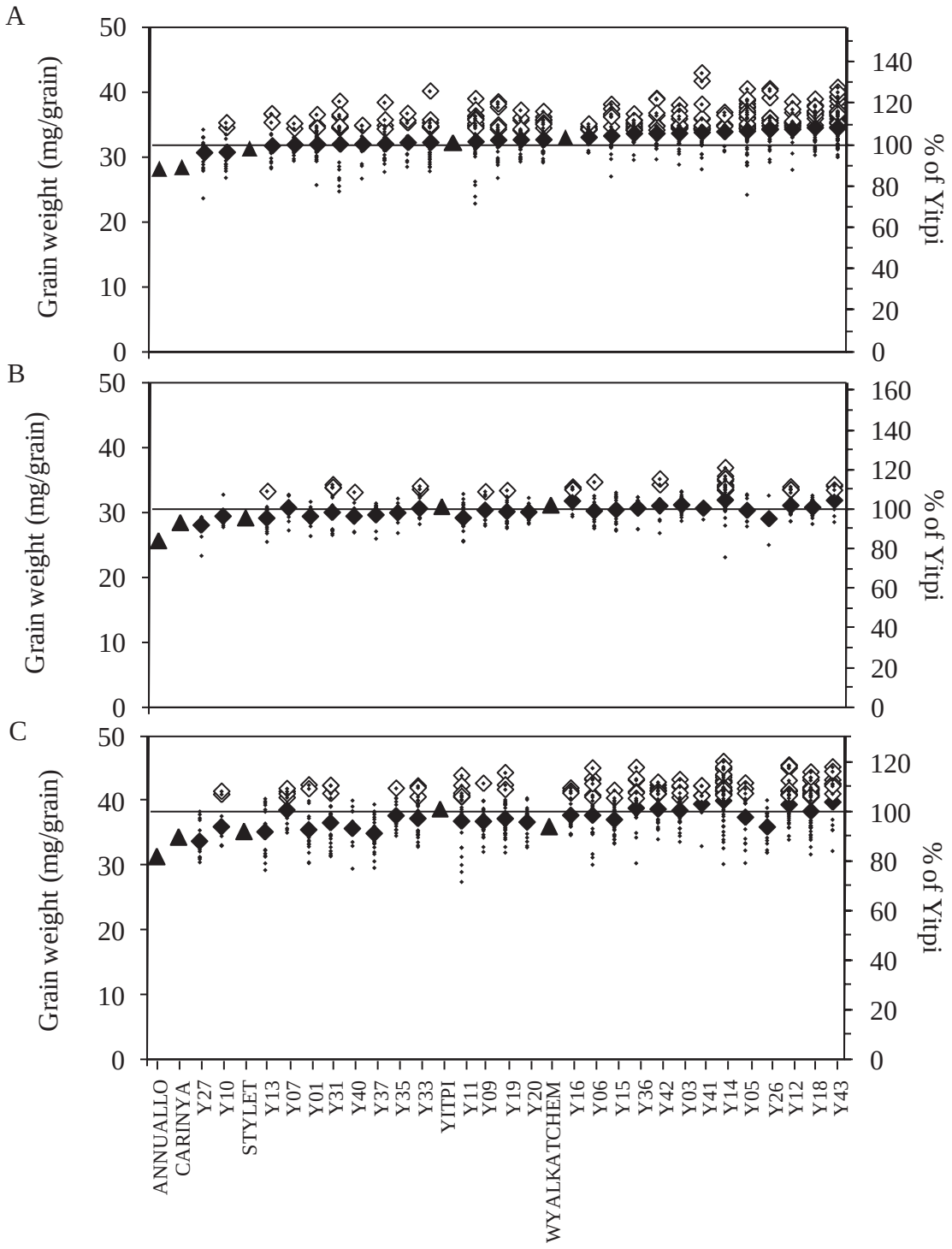


Figure 14. BLUPs for grain weight of 27 families of synthetic-derived lines (◆), for individual lines within those families (⬩ and ◈, where ◈ designates lines with significantly (p<0.05) heavier grain weight than their recurrent parent Yitpi) and for five bread wheat cultivars (▲), grown at (A) Roseworthy in 2006 and 2007, (B) Minnipa in 2006 and 2007 and (C) Pinnaroo in 2007.

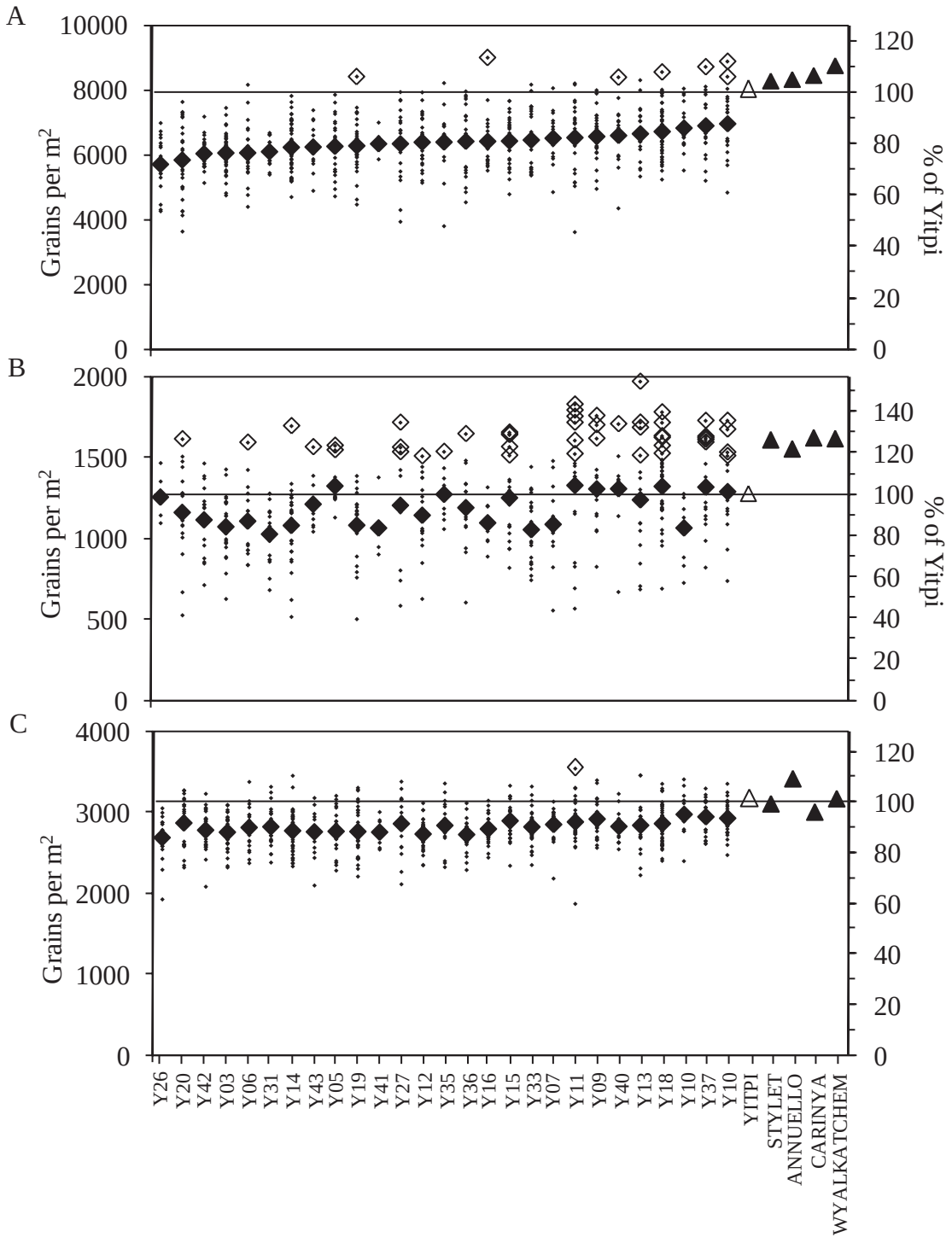


Figure 15. BLUPs for grains per m^2 of 27 families of synthetic-derived lines (◆), for individual lines within those families (⬩ and ◈, where ◈ designates lines with significantly (p<0.05) more grains per m^2 than their recurrent parent Yitpi) and for five bread wheat cultivars (▲), grown at (A) Roseworthy in 2006 and 2007, (B) Minnipa in 2006 and 2007 and (C) Pinnaroo in 2007.

5.3.6 Relationships between grain weight, grains per m^2 and grain yield

Across all synthetic backcross lines, grain weight exhibited statistically significant but very weak negative associations with grains per m^2 (Table 12). Negative associations between these traits were also observed within most families (Appendix 4), but within one family (Y31), a strong positive association was observed (r = 0.775) at Minnipa (Table 12). Grains per m^2 and grain yield were positively associated in all families in all environments, which was commonly significant ($p<0.01$). Across environments, 82.7% of families had a positive association between grain weight and grain yield (Appendix 4). The strongest association was observed within family Y31 at Roseworthy (r = 0.901, $p<0.01$).

5.4 Discussion

This study investigated the potential of 27 primary synthetic wheats to increase grain yield and its components (grain weight and grain per m^2) of a southern Australian bread wheat cultivar. The BC_1 synthetic-derived families of lines were evaluated in five environments in South Australia, all of which experienced serious to severe drought. Although grain yields were low, heritability was generally high and primary synthetic wheat and synthetic backcross lines suitable for use as a germplasm resource in further breeding programs for southern Australia were identified.

Of the 27 primary synthetic wheats evaluated, 14 (S01, S05, S06, S09, S11, S12, S13, S14, S15, S18, S36, S37 and S40) were donor parents to families with significantly ($p<0.05$) higher grain yielding lines compared to Yitpi (recurrent bread wheat parent) in at least one environment. This suggests that many, but not all primary synthetic wheats can improve the grain yield of a bread wheat cultivar in selected environments. However, the identification of superior grain yielding synthetic backcross lines here does support previous findings in that high grain yielding synthetic derivatives can be created from only one backcross to an elite bread wheat (Gororo *et al.* 2002; Dreccer *et al.* 2007; Ogbonnaya *et al.* 2007). Hence minimal backcrossing, as opposed to advanced backcrossing (BC_2 or more) may be sufficient to improve the grain yield of bread wheat when using primary synthetic wheat as a donor genetic source.

Synthetic backcross lines were commonly adapted to a particular environment rather than showing broad adaptation. Derivative Y01-08 showed the highest grain yields across the five environments (99.2% to 130.4% compared with Yitpi) and therefore could be suitable as a parent to develop bread wheat cultivars with improved grain yield in southern

Table 12. Simple linear correlation coefficients (*r*) for associations between grains per m^2, grain weight and grain yield across all synthetic backcross lines from 27 families and across lines within the family Y31, at Minnipa in 2006 and 2007, Pinnaroo in 2007 and Roseworthy in 2006 and 2007.

Synthetic-derived families and lines within	Grains per m^2 v grain weight		Grain yield v grains per m^2		Grain yield v grain weight	
	r	**df**	***r***	**df**	***r***	**df**
Minnipa 2006/07						
All lines	-0.157**	443	0.936**	454	0.146*	454
Y31	0.775**	14	0.960**	14	0.896**	14
Pinnaroo 2007						
All lines	-0.300**	649	0.776**	649	0.312**	649
Y31	-0.543**	21	0.699**	21	0.165	21
Roseworthy 2006/07						
All lines	-0.184**	645	0.817**	645	0.387**	645
Y31	0.156	21	0.554**	21	0.901**	21

df = degrees of freedom; *, ** significant at the 0.05 and 0.01 levels of probability, respectively.

Australian environments. S18 seemed to be the most suitable primary synthetic wheat for further crosses to other bread wheats as its associated family Y18 had the highest estimated family grain yield in every environment. However, some of the highest grain yielding lines in each environment were identified in lower grain yielding families. For example, family Y06 had the 20th highest estimated family grain yield at Minnipa, 18th at Pinnaroo and 23rd at Roseworthy, though one of its derivatives achieved a superior grain yield (127.8%) of Yitpi at Minnipa, with grain yields of other Y06 derivatives approaching significance (p<0.05) at both Pinnaroo (104.0%) and Roseworthy (108.6%). Therefore to identify genotypes that improve grain yield across a broad range of environments still remains a challenge.

Most synthetic backcross lines that displayed significantly (p<0.05) higher grain yields than Yitpi (up to 43.8% higher) were identified in the lowest yielding environments (Minnipa). At the highest yield environments (Roseworthy) few derivatives produced superior (p<0.05) grain yields, up to 12.0% higher than Yitpi. The magnitude of these increases in grain yield with respect to the recurrent bread wheat parent were similar to the 49% (Gororo *et al.* 2002) and 11% (Ogbonnaya *et al.* 2007) high grain yields achieved by synthetic backcross lines at similar rain-fed environments in southern Australia. Further evaluations of superior synthetic backcross lines from the current study, conducted by Australian Grain Technologies Pty Ltd at Roseworthy in 2008 also showed grain yields up to 111% compared with Yitpi (H. Kuchel, personal communication, 2008). These results suggest that genetic gain for grain yield can be readily and consistently achieved by synthetic backcross lines in southern Australian environments. However one negative trait, poor threshability, was observed in each environment. In several instances during the development and evaluation of the material, synthetic derivatives were removed because of poor threshability. Poor threshability can be 'bred out' of a germplasm pool (H. Kuchel, personal communication, 2008), but this trait would inhibit the rapid development of commercial cultivars from this set of synthetic material.

Evaluation in the future of larger families of lines and the use of different bread wheat parents may produce further increases to grain yield. As discussed by Gororo *et al.* (2002), performing advanced selection within segregating lines, such as the superior BC_1F_4-derived lines of the current study, may also identify additional improvement to grain yield. These lines would have been a mixture of near homozygous genotypes at the time of evaluation (F_7), with their performance representing a mean of these genotypes (Allard

1960). Therefore, evaluating synthetic backcross lines selected from large bulks at more advanced generations could be a suitable strategy to identify the highest grain yielding synthetic backcross lines.

Primary synthetic wheats S12, S14, S42 and S43 were found to be the most valuable genetic resources for improving grain weight, with S01, S10 and S37 commonly improving grains per m^2. Interestingly, the grain yield component responsible for significant ($p<0.05$) grain yield improvements were similar between synthetic backcross lines from different families. Under the lowest yielding environments (Minnipa), improvements to grain per m^2 (up to 154.3% of that of Yitpi) were commonly responsible for superior grain yields, whereas under the highest yielding environments (Roseworthy), improvements to grain weight (up to 108.1% of that of Yitpi) were responsible. Gororo *et al.* (2002) also reported synthetic backcross lines that achieved superior grain yields in low yield conditions in southern Australia through significant ($p<0.05$) improvements to grain per m^2. Crop-simulation models for improving grain yield of bread wheat cultivars in Australian have suggested that in low yield environments selecting for enhanced grain number per unit area may achieve such improvements (Asseng *et al.* 2002). However, the magnitude of these improvements were predicted to be small due to limiting environmental factors.

Finally, this study assessed the potential of 27 primary synthetic wheats to improve the relationship between grain weight and grains per m^2 in bread wheat. This relationship was commonly found to be negative and very weak within the 27 synthetic-derived families of lines; hence there was little influence of the different primary synthetic parents on this relationship. As reviewed by Araus *et al.* (2008), the relationship between grain weight and grains per m^2 in bread wheat is commonly found to be negative. The results from within families in the present study were consistent with this. In contrast family Y31 did show a moderate positive association for this relationship under the lowest yielding conditions. This family was also the only one to have a strong association between grain weight and grain yield in the lowest and highest yielding environments. However, Y31 was one of the lowest yielding families across the environments and did not contain any lines with significantly ($p<0.05$) higher grain yields than Yitpi. Therefore, if lines within this family do represent an uncoupling of grain weight and grains per m^2, as reported by Gaju *et al.* (2009) to be a means of increasing grain yield potential, this situation does not provide a grain yield advantage under the environments evaluated. Del Blanco *et al.* (2001) suggested that indirect effects can affect the correlation strength between grain yield

components, which may be the case with family Y31. The different primary synthetic parents assessed here did affect the magnitude of these grain yield components in the form of grain yield differences between families, as discussed earlier.

5.5 Conclusion

This study identified synthetic backcross lines with superior grain yield compared with their recurrent bread wheat parent in drought conditions, particular in low yield moisture limiting environments. Here, improved grains per m^2 were commonly observed without penalty to grain weight. Therefore primary synthetic wheat can be a useful breeding resource to improve the grain yield of bread wheat in such conditions. In the highest yield environments evaluated, equal and superior grain yields were identified compared to the recurrent bread wheat parent. Improved grain weight was responsible for such achievements without penalty to grain per m^2. For high yielding environments primary synthetic wheats could be less useful to improve grain yield, but grain quality (grain weight) could be enhanced.

Chapter 6

Introgression of genetic material from primary synthetic wheat into an elite bread wheat background

6.1 Introduction

Primary synthetic wheats (genome AABBDD) can act as a biological bridge for the introgression of genetic material from *Triticum turgidum* L. (genome AABB) and *Aegilops tauschii* Coss. (genome DD) into bread wheat (T*riticum aestivum* L., genome AABBDD) (van Ginkel and Ogbonnaya 2007). Synthetic-derived populations, commonly created by backcrossing primary synthetic wheat (donor parent) to a bread wheat cultivar (recurrent parent), have been used to detect quantitative trait loci (QTL) for agronomic traits. QTL have been mapped at which synthetic-derived alleles had positive effects on preharvest sprouting tolerance (Imtiaz *et al.* 2008), baking volume (Kunert *et al.* 2007), length of grain filling period (Börner *et al.* 2002), and grain yield and its components (Börner *et al.* 2002; Huang *et al.* 2004; Narasimhamoorthy *et al.* 2006; Röder *et al.* 2008).

In synthetic-derived populations QTL for grain weight have been detected on chromosomes 1B, 1D, 2A, 2D, 3A, 3B, 3D, 4B, 5A, 6A, 7A and 7D, where the positive effect was due to the primary synthetic allele (Börner *et al.* 2002; Huang *et al.* 2004). Fine mapping on chromosome 7D has detected a locus where a primary synthetic allele has significant positive effects on grain weight, which could be selected for using a marker assisted selection strategy (Frisch and Melchinger 2001; Röder *et al.* 2008). However, synthetic-derived alleles having positive effects on grain weight often have negative effects on grain number (Börner *et al.* 2002; Huang *et al.* 2004). Therefore it would be beneficial to identify primary synthetic alleles that have a positive association for the major grain yield components, grain weight and grains per m^2.

In bread wheat, alleles that are positively associated with the major grain yield components can be enriched in selected lines with high grain yield performance (McIntyre *et al.* 2010). The retention of primary synthetic alleles, with or without positive association for the major grain yield components, has not been investigated in selected synthetic backcross lines with high grain weight and/or high grain yield performance. Understanding this would be important for improving the genetic diversity of bread wheat whilst performing breeding activities.

The work reported here investigated two synthetic-derived families of lines developed by independently backcrossing two primary synthetic wheats with a common bread wheat cultivar. Using DArT marker genotypes of these lines, this study aimed to identify:

1. introgressed synthetic alleles and compare patterns of introgression between the families,
2. genomic regions in which primary synthetic or bread wheat alleles have a positive effect on grain weight and/or grains per m^2, and

6.2 Materials and methods

6.2.1 Plant material

Two families of BC_1F_4-derived synthetic lines, Y14 and Y18 were used in this study, together with their recorded donor primary synthetic parents S14 (Croc1/*Ae. tauschii* WX436) and S18 (Doy1/*Ae. tauschii* WX517) respectively, and recurrent bread wheat parent Yitpi. These plant materials are fully described in Chapter 3. The 43 lines of Y14, and the 46 lines of Y18 were examined (at the F_6 and F_7 generations) in field experiments across five environments for grain yield and its components, grain weight and grains per m^2 (Chapter 5). These field experiments showed lines within Y14 and Y18 varied for their grain weight and grain yield performance, with Y14 having a high family grain weight BLUP (best linear unbiased predictor) and Y18 having a high family grain yield BLUP relative to 25 other synthetic-derived families of lines assessed in the same field experiments.

6.2.2 Marker application and detection of polymorphism

DNA was extracted from all genotypes and prepared for DArT marker application following the methods given in Chapter 4. DArT marker application was performed by Triticarte Pty Ltd (Australian Capital Territory, Australia). The hybridisation of DArT markers was scored as either present (1), absent (0) or unreliable (X) for each genotype. Markers with low quality scores (Q-value <77) were excluded from analyses (E. Huttner, personal communication, 2008). A DArT marker was considered polymorphic in a synthetic-derived family when the dominant ‘1’ allele was observed in only one of its parents, and when both the ‘1’ and ‘0’ alleles were observed across lines of the respective family at a frequency where the rarer allele was at least 0.05. The informativeness of each DArT marker locus screened was quantified using polymorphic information content values (Botstein *et al.* 1980), which were calculated as per the methods of De Riek *et al.* (2001) for dominant markers.

6.2.3 Estimation of marker loci positioning and linkage

Genomic positioning and linkage information of polymorphic DArT marker loci in the families Y14 and Y18 were estimated using 17 reference genetic maps. The steps below were followed.

1. The genetically diverse Gladius/Drysdale mapping population (provided by Dr Ken Chalmers, University of Adelaide) provided linkage information for DArT marker loci that were also found to be polymorphic in Y14 and Y18.
2. Groups of linked markers from step 1 were assigned to chromosomes using information from the genetic maps listed below.
   - Triticarte version 1.2 set of nine integrated genetic maps (Triticarte Pty Ltd 2008),
   - Kukri/Excalibur genetic map (provided by Dr Ken Chalmers, University of Adelaide),
   - Ajana/WAWHT2074, P92201D5-2/P91193D1-10, Cadoux/Reeves and EGA Blanco/Millewa genetic maps (Francki *et al.* 2009),
   - Colosseo/Lloyd genetic map (tetraploid wheat) (Mantovani *et al.* 2008), and
   - Langdon/Wild emmer wheat (G18-16) genetic map (tetraploid wheat) (Peleg *et al.* 2008).
3. Genetic distances between markers within linkage groups were inferred from the Gladius/Drysdale mapping population.
4. Orientation and order of linkage groups within chromosomes was determined using the reference genetic maps listed above.

Linkage between neighbouring markers separated by an interval greater than 20cM was broken. Additional linkage groups and single markers were incorporated into the genetic maps of Y14 and Y18 by following steps 1 to 4, using each reference genetic map in step 1, with genetic distance inferred from the reference maps in step 3.

MapChart (version 2.1) (http://www.biometris.wur.nl/UK/Software/MapChart.html) (Plant Research International 2002; Voorrips 2002) was used to diagrammatically display the positioning and linkage of polymorphic markers of Y14 and Y18. Some assayed DArT marker loci not given chromosomal assignment from the above positioning and linkage determination process were assigned to a chromosome through genotype matching. This involved comparing the genotypes of assigned markers to unassigned markers using the

'distribute' function in Map Manager QTX software (version 0.30) (Meer *et al.* 2004) and manual checking.

6.2.4 Marker data analysis

6.2.4.1 Assumptions and expectations of synthetic introgression

The lengths of chromosomal segments of synthetic derived introgression were estimated using the following assumptions, which were based on methods described by Hospital (2001):

1. that each chromosomal segment flanked by two markers displaying a non-Yitpi (or synthetic donor) genotype were of synthetic origin,
2. that recombination occurred half the distance between a marker displaying non-Yitpi genotype and a flanking marker displaying Yitpi genotype, and
3. that the segment from a marker displaying a non-Yitpi genotype to a recombination point is of synthetic origin.

Hence, a synthetic introgression segment is the region between two recombination events when marker(s) of a non-Yitpi genotype are located in that intervening region.

Chi-square tests were performed for each DArT marker that was polymorphic within Y14 and/or Y18 to assess the fit of observed synthetic introgression to the expected ratios for BC_1 germplasm. The expected ratio of Yitpi (recurrent parent) alleles to the primary synthetic (donor parent) alleles was 3:1. One degree of freedom (df) was used for each chi-square test (df = n - 1, where n =2 is the number of genotypic classes). Deviations of less than a 0.05 significance level from the expected were considered significant. MapChart software was used to highlight significant deviations from the expected at marker loci mapped in Y14 and Y18.

6.2.4.2 Association of parental alleles with grain weight and grains per m^2 data

Association of parental alleles at DArT marker loci assayed in Y14 and Y18, with grain weight and grains per m^2 data from five environments, was tested by single marker regression using Map Manager QTX software (Meer *et al.* 2004). Loci were identified where the Yitpi or primary synthetic allele was significantly (p<0.05) associated with increased grain weight and grains per m^2 in one or more environments. For each of these loci the percentage amount of trait (grain weight or grains per m^2) phenotypic variance explained was calculated using Map Manager QTX software, which was the difference between the total trait variance and the residual variance expressed as a percent of the total

variance. MapChart software was used to highlight mapped marker loci of Y14 and Y18 that identified such beneficial synthetic and Yitpi alleles.

Retention of beneficial synthetic and Yitpi alleles for increased grain weight and grains per m^2 was observed in three selected groups of lines within Y14 and Y18. The selection criteria for these groups were high grain weight (significantly (p<0.05) heavier grains than Yitpi), high grain yield (not significantly different (p>0.05) grain yield of that of Yitpi), and both high grain weight and high grain yield.

6.3 Results

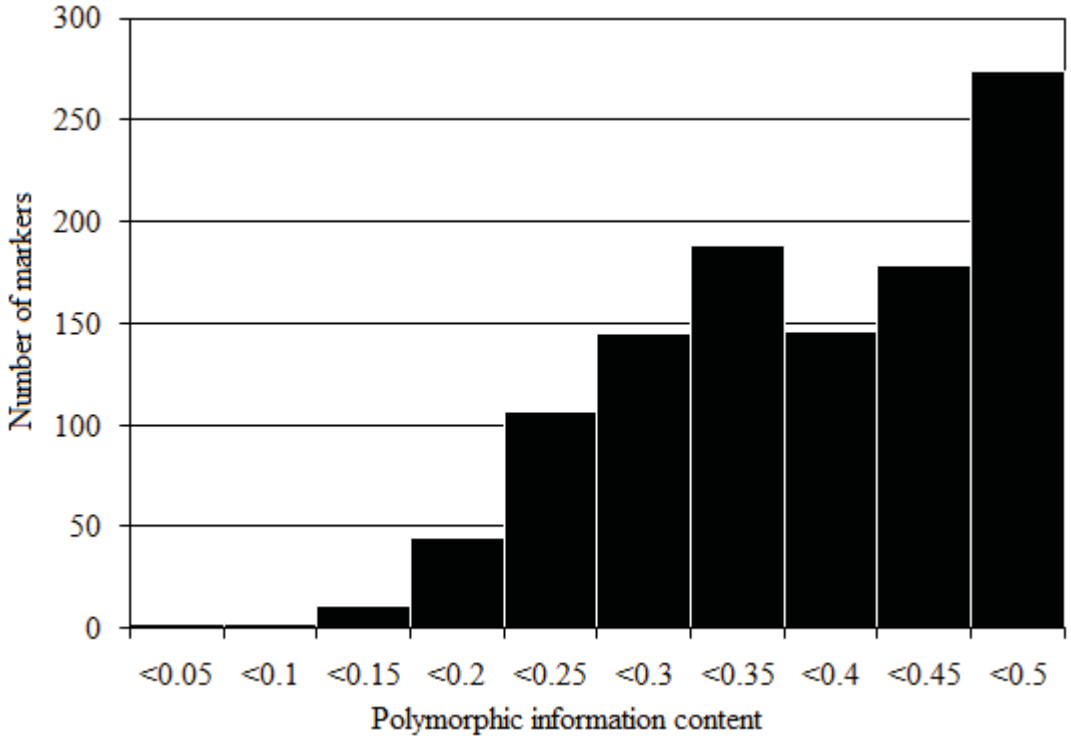
6.3.1 Marker polymorphism

Polymorphism between parents of Y14 (S14 and Yitpi) and Y18 (S18 and Yitpi) were identified by 1048 and 993 DArT markers respectively. Markers within these sets were of high quality (Q-value >77), with their mean call rate being 96.6 (Y14) and 96.5 (Y18). The polymorphism information content at these marker loci ranged from 0.1 to 0.5 (highest possible value for a biallelic marker) in both Y14 (Figure 16a) and Y18 (Figure 16b). Mean polymorphism information content values of the Y14 and Y18 marker sets were 0.36 and 0.37 respectively.

6.3.2 Genetic linkage of markers

The Gladius/Drysdale mapping population provided linkage information for 261 and 244 of the DArT marker loci found to be polymorphic in Y14 and Y18 respectively. Genetic linkage information for an additional 179 (Y14) and 165 (Y18) marker loci were sourced from the16 other reference genetic maps. A final 440 and 409 polymorphic DArT marker loci provided the chromosome information for the A, B and D genomes of Y14 and Y18 respectively (Table 13). In each family, fewer than 10 polymorphic marker loci were assigned to each of chromosomes 5A, 2D, 3D, 4D, 5D and 6D. A mean of 21 and 20 marker loci represented each chromosome of the Y14 (Figure 17) and Y18 (Figure 18) genetic maps respectively.

A



B

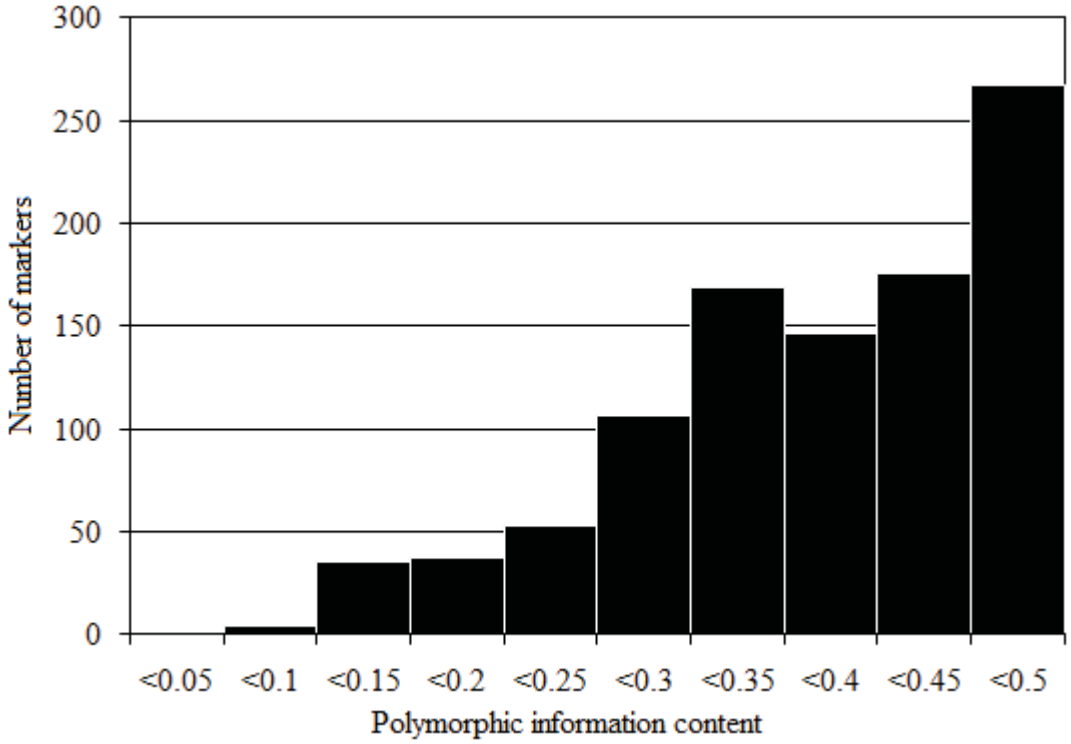


Figure 16. Distribution of polymorphic information content values of *A*. 1048 polymorphic DArT marker loci of synthetic-derived family of lines Y14, and *B*. 993 polymorphic DArT marker loci of synthetic-derived family of lines Y18.

Table 13. Number of DArT marker loci positioned per chromosome in the genetic maps of synthetic-derived families of lines Y14 and Y18.

	Synthetic-derived families of lines	
Chromosome	**Y14**	**Y18**
1A	31	34
2A	24	21
3A	18	20
4A	34	32
5A	4	3
6A	19	26
7A	26	26
1B	29	31
2B	42	37
3B	43	36
4B	17	13
5B	20	16
6B	35	29
7B	32	33
1D	30	20
2D	8	3
3D	8	4
4D	2	1
5D	0	7
6D	1	3
7D	17	14
Total	440	409

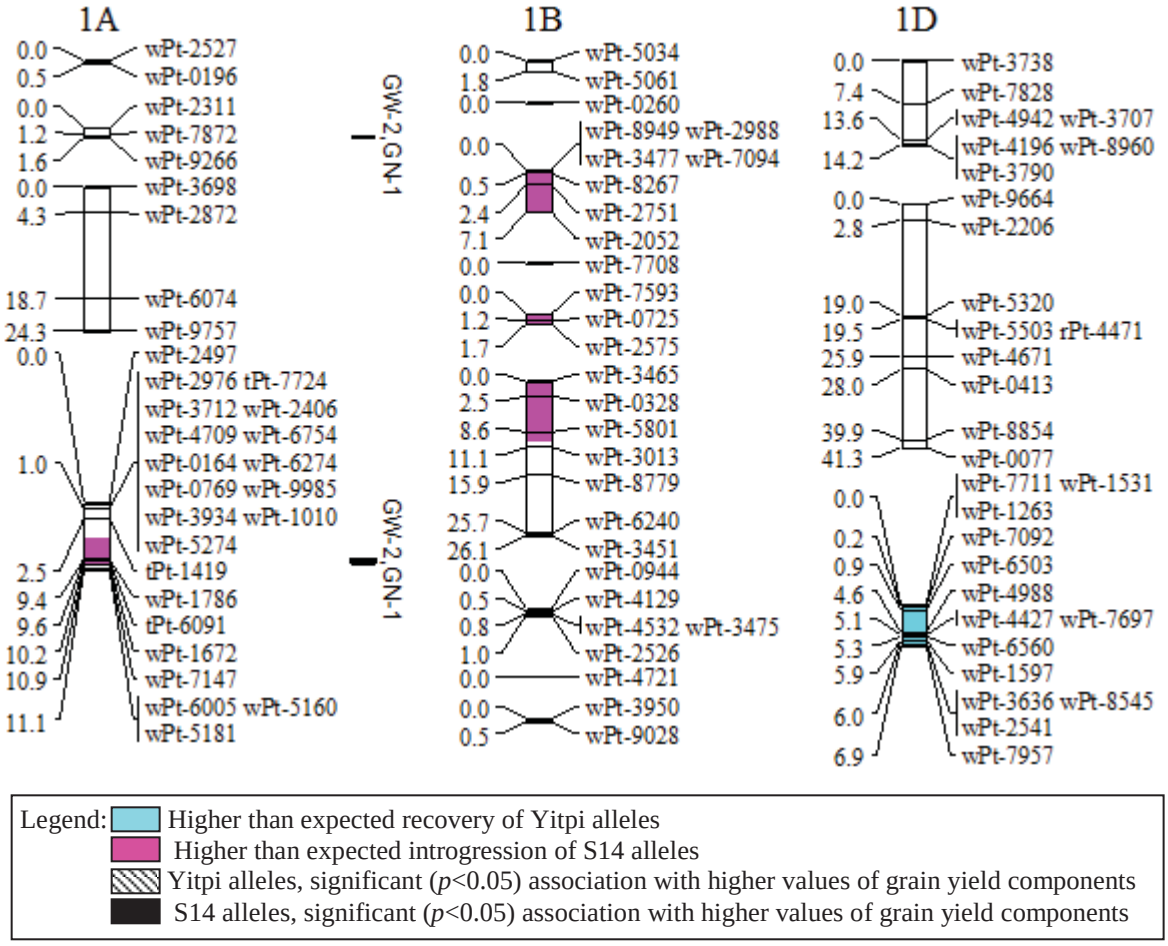


Figure 17. Estimated genomic positions of 440 DArT marker loci that exhibited polymorphism within the Y14 family of synthetic-derived lines. Previously constructed linkage maps were used to order markers within linkage groups, provide genetic distances between linked markers, assign markers to chromosomes and to orient and order markers within chromosomes. Shaded chromosome regions are those where Yitpi (recurrent parent) and S14 (donor primary synthetic parent) alleles were observed at ratios significantly deviating from the expected (3:1 for BC_1 material). Vertical bars to the right of chromosomes span loci that were significantly ($p<0.05$) associated with grain weight (GW) and grains per m^2 (GN), in one (GW-1,GN-1) or more (GW-2,GN-2 for example) environments using single marker regression. Shading of vertical bars indicates the parental allele (either synthetic or Yitpi) that was significantly associated with an increase in the trait.

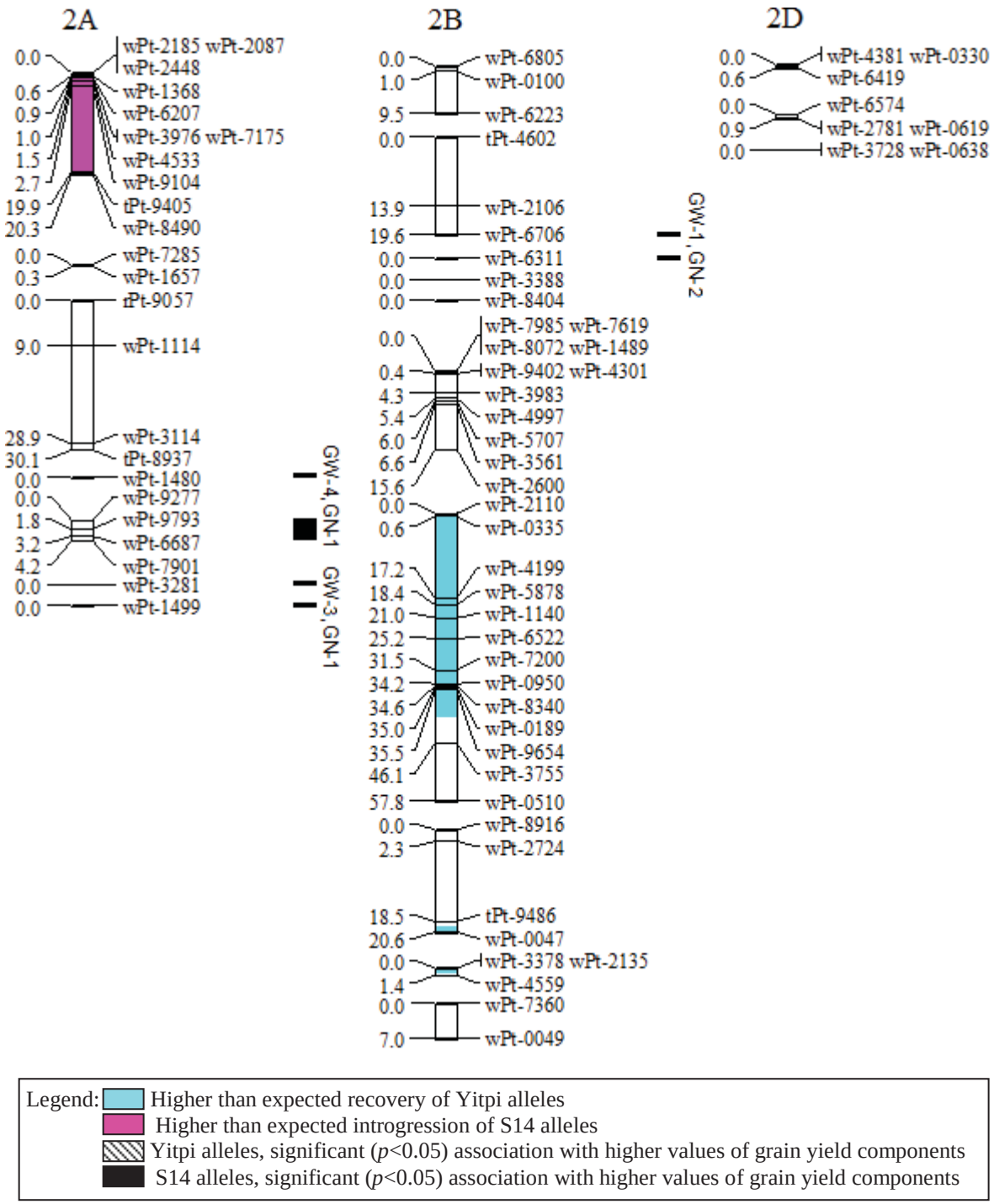


Figure 17. *Continued*.

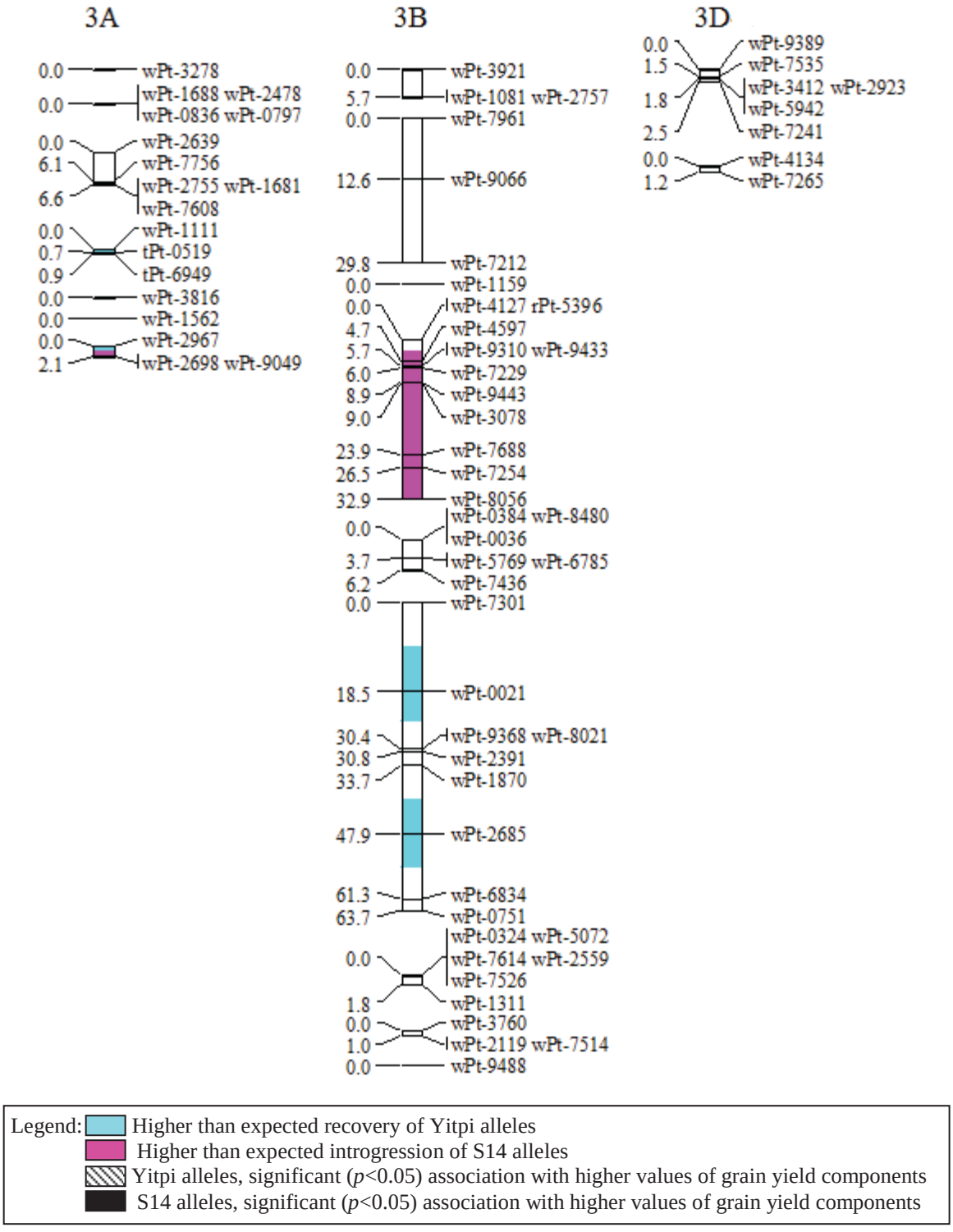


Figure 17. *Continued*.

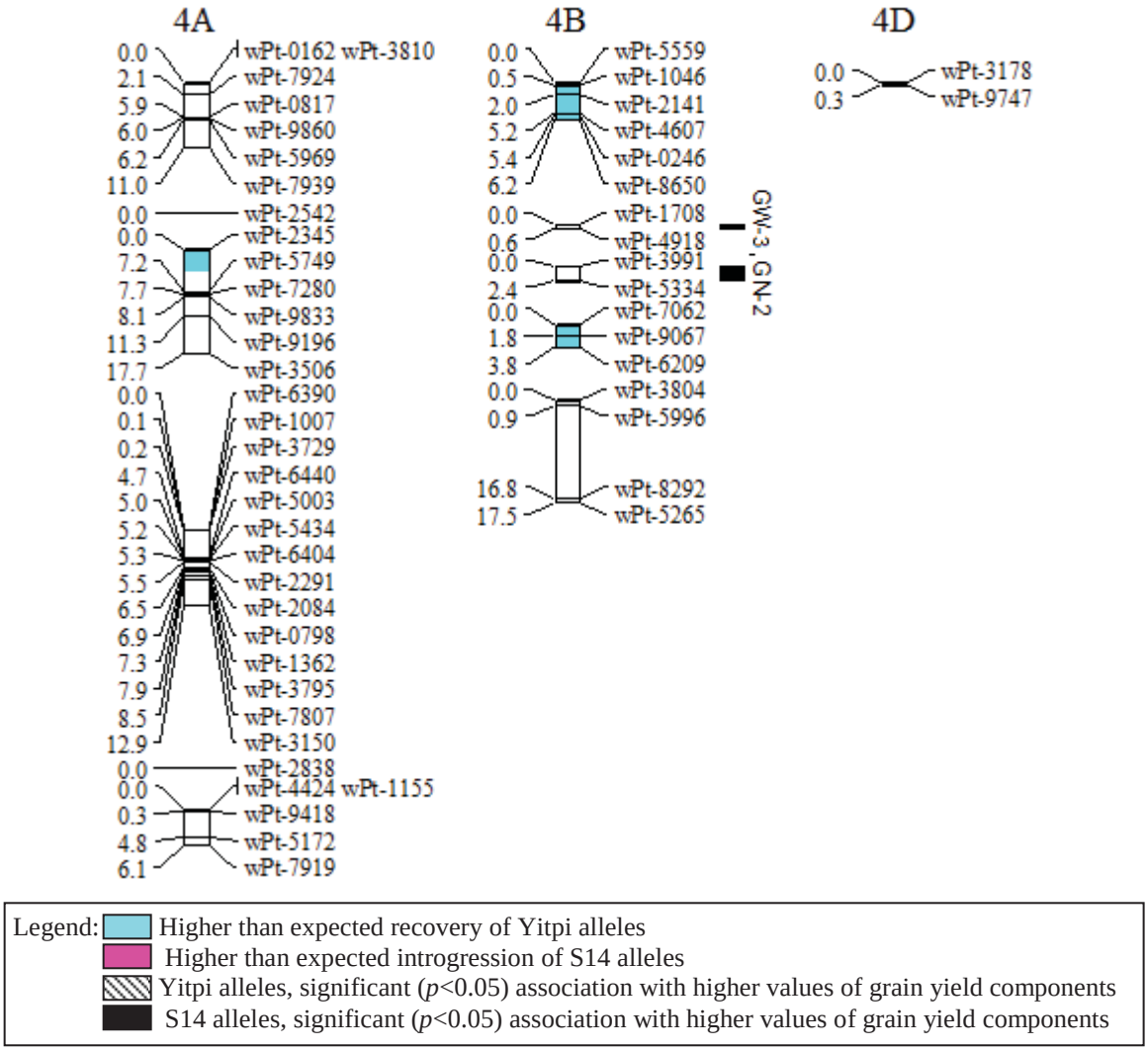


Figure 17. *Continued*.

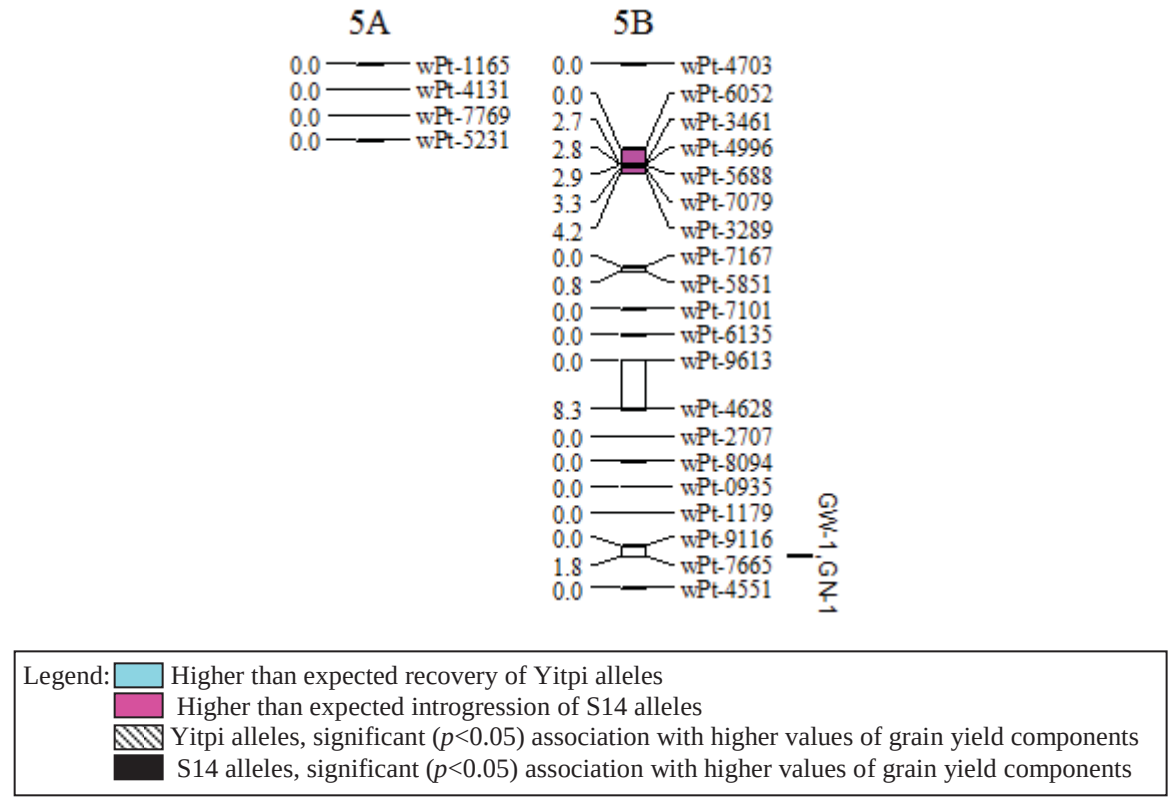


Figure 17. *Continued*.

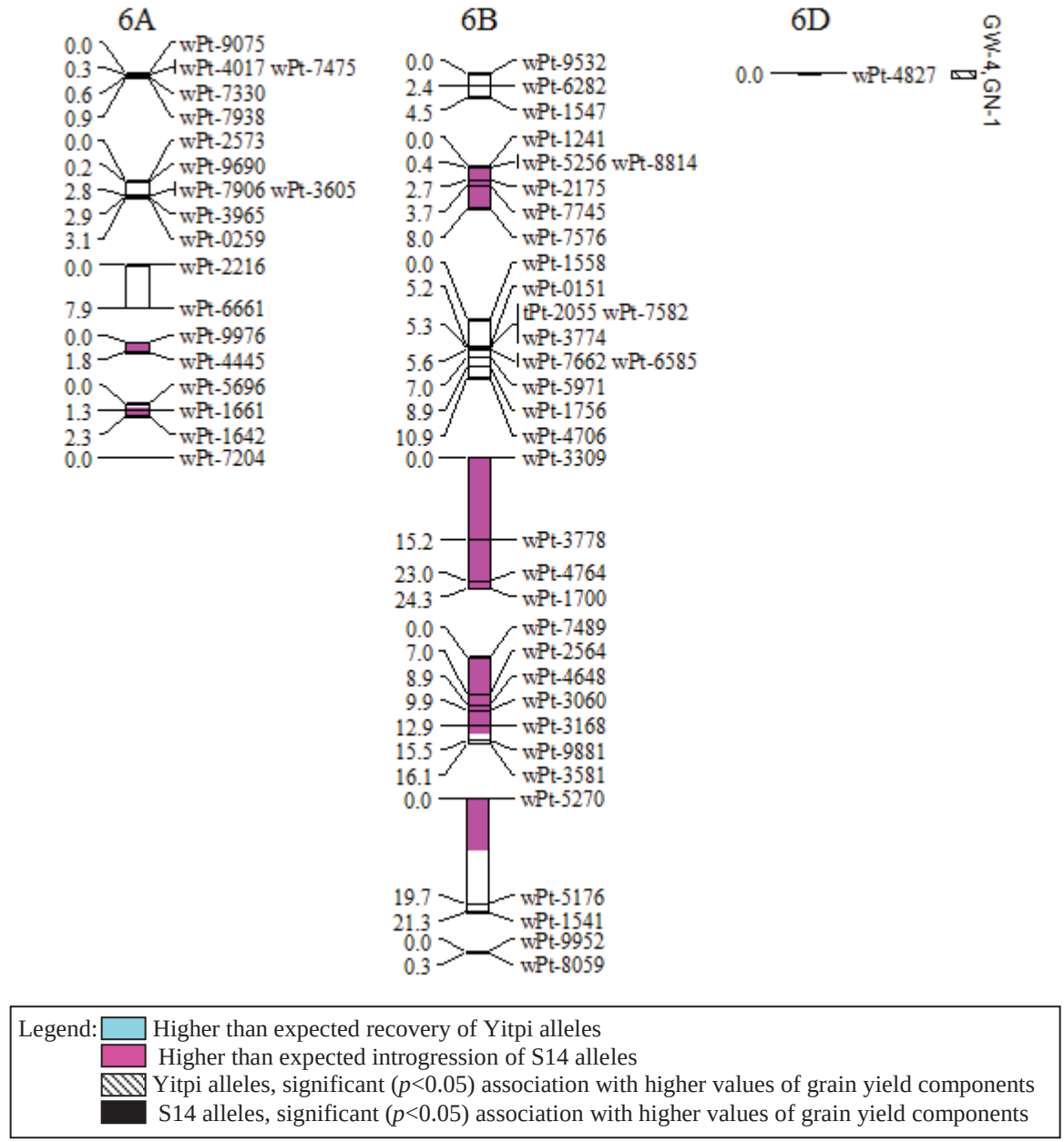


Figure 17. *Continued*.

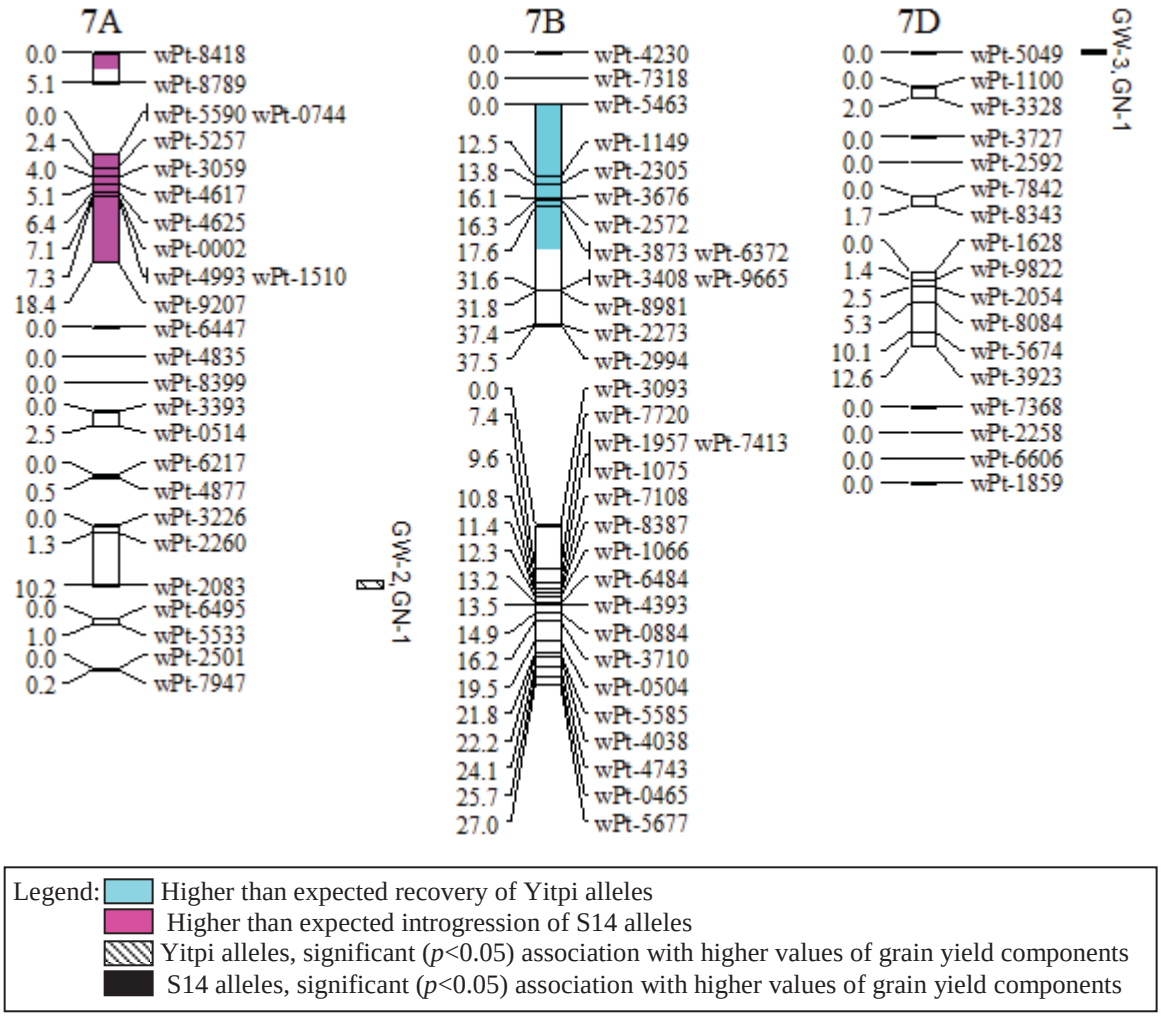


Figure 17. *Continued*.

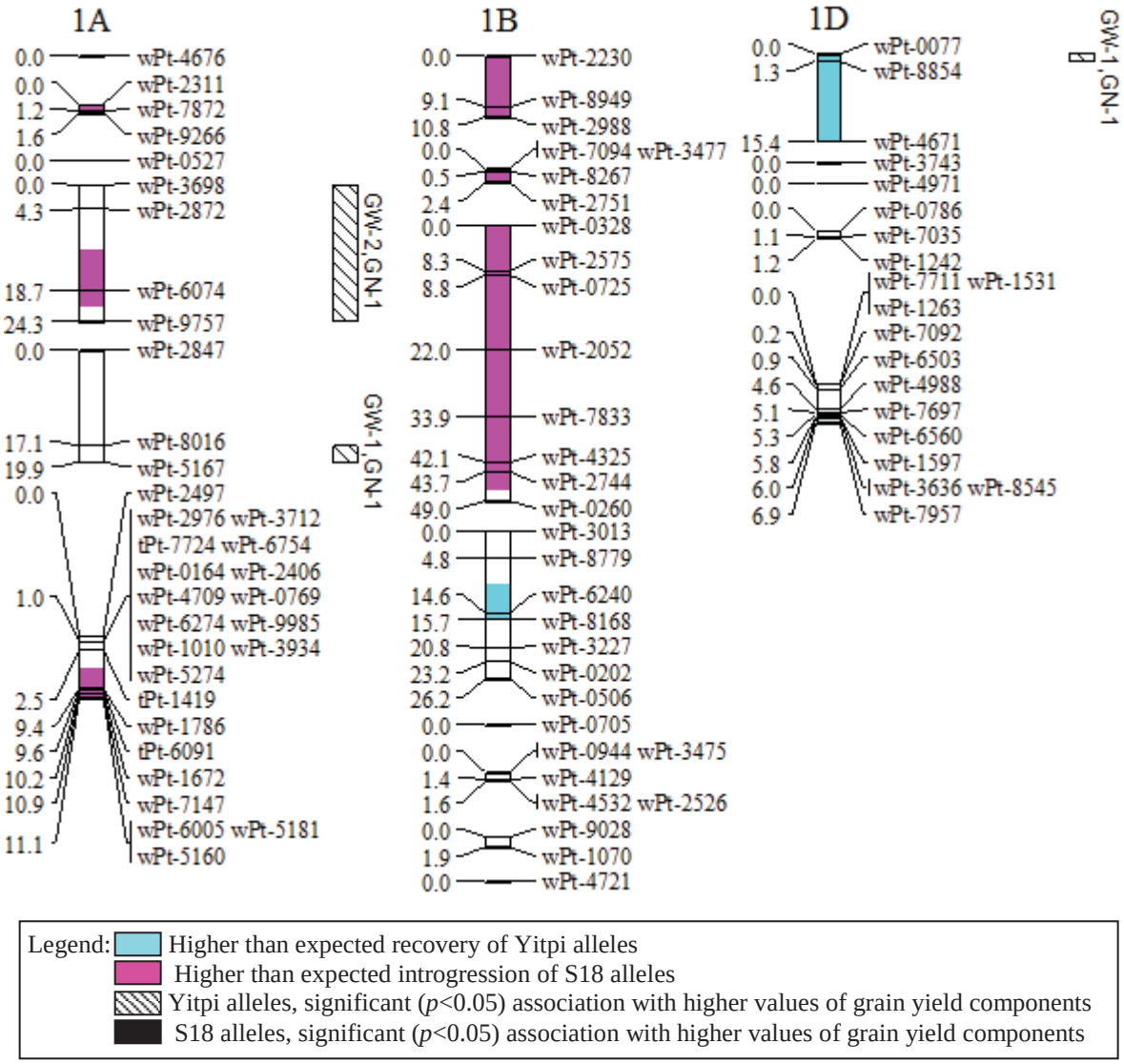


Figure 18. Estimated genomic positions of 409 DArT marker loci that exhibited polymorphism within the Y18 family of synthetic-derived lines. Previously constructed linkage maps were used to order markers within linkage groups, provide genetic distances between linked markers, assign markers to chromosomes and to orient and order markers within chromosomes. Shaded chromosome regions are those where Yitpi (recurrent parent) and S18 (donor primary synthetic parent) alleles were observed at ratios significantly deviating from the expected (3:1 for BC_1 material). Vertical bars to the right of chromosomes span loci that were significantly ($p<0.05$) associated with grain weight (GW) and grains per m^2 (GN), in one (GW-1,GN-1) or more (GW-2,GN-2 for example) environments using single marker regression. Shading of vertical bars indicates the parental allele (either synthetic or Yitpi) that was significantly associated with an increase in the trait.

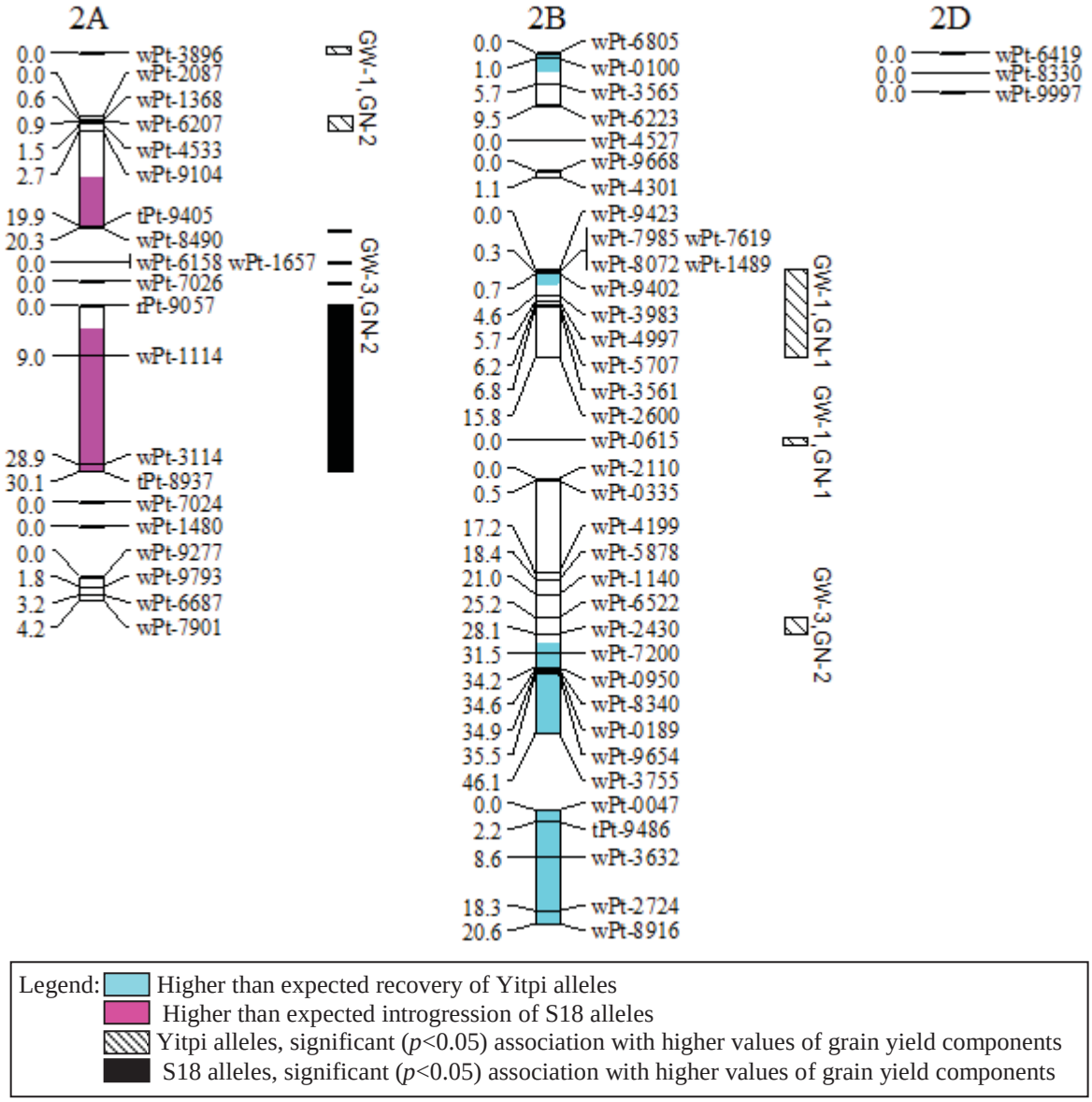


Figure 18. *Continued*.

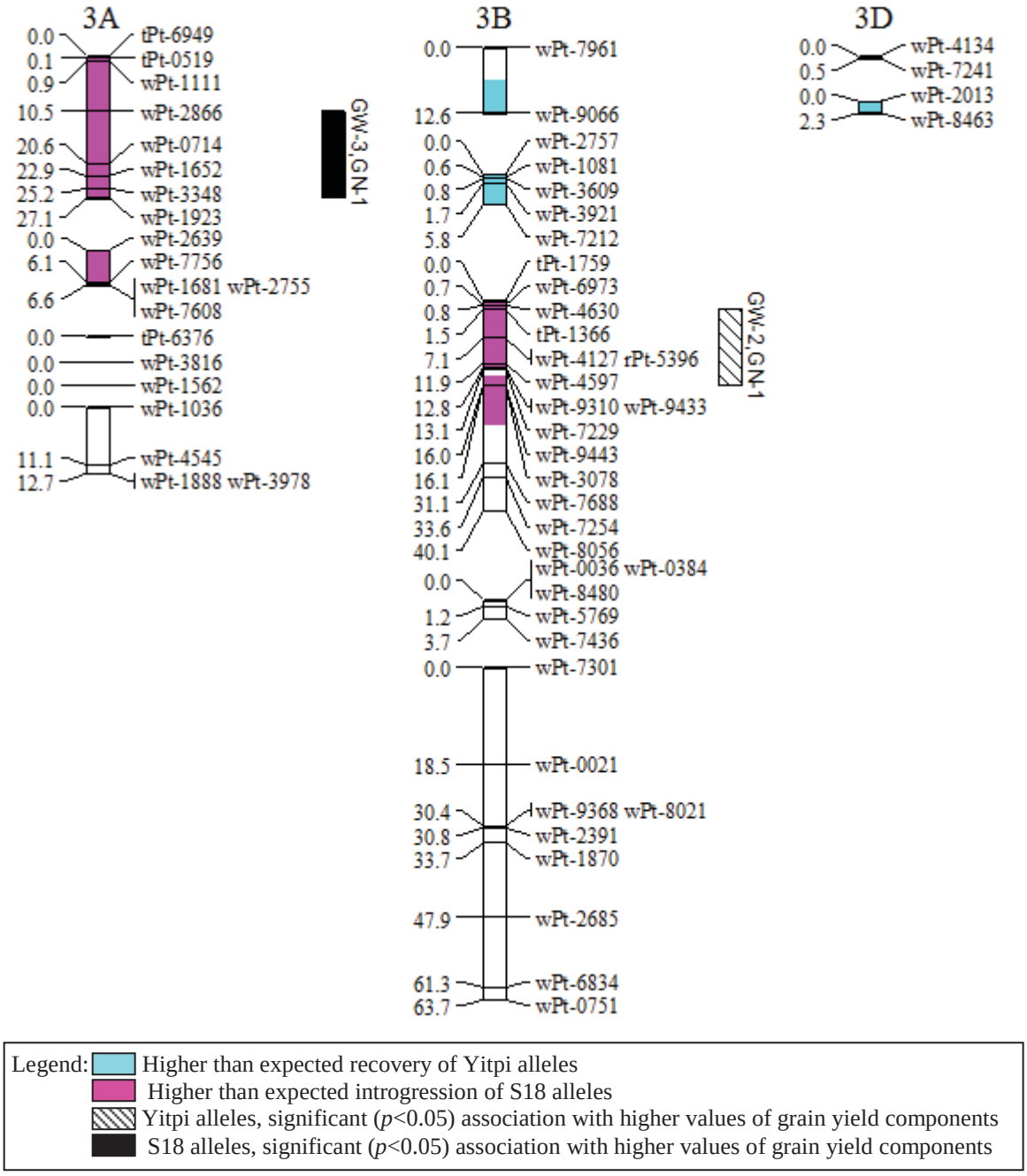


Figure 18. *Continued*.

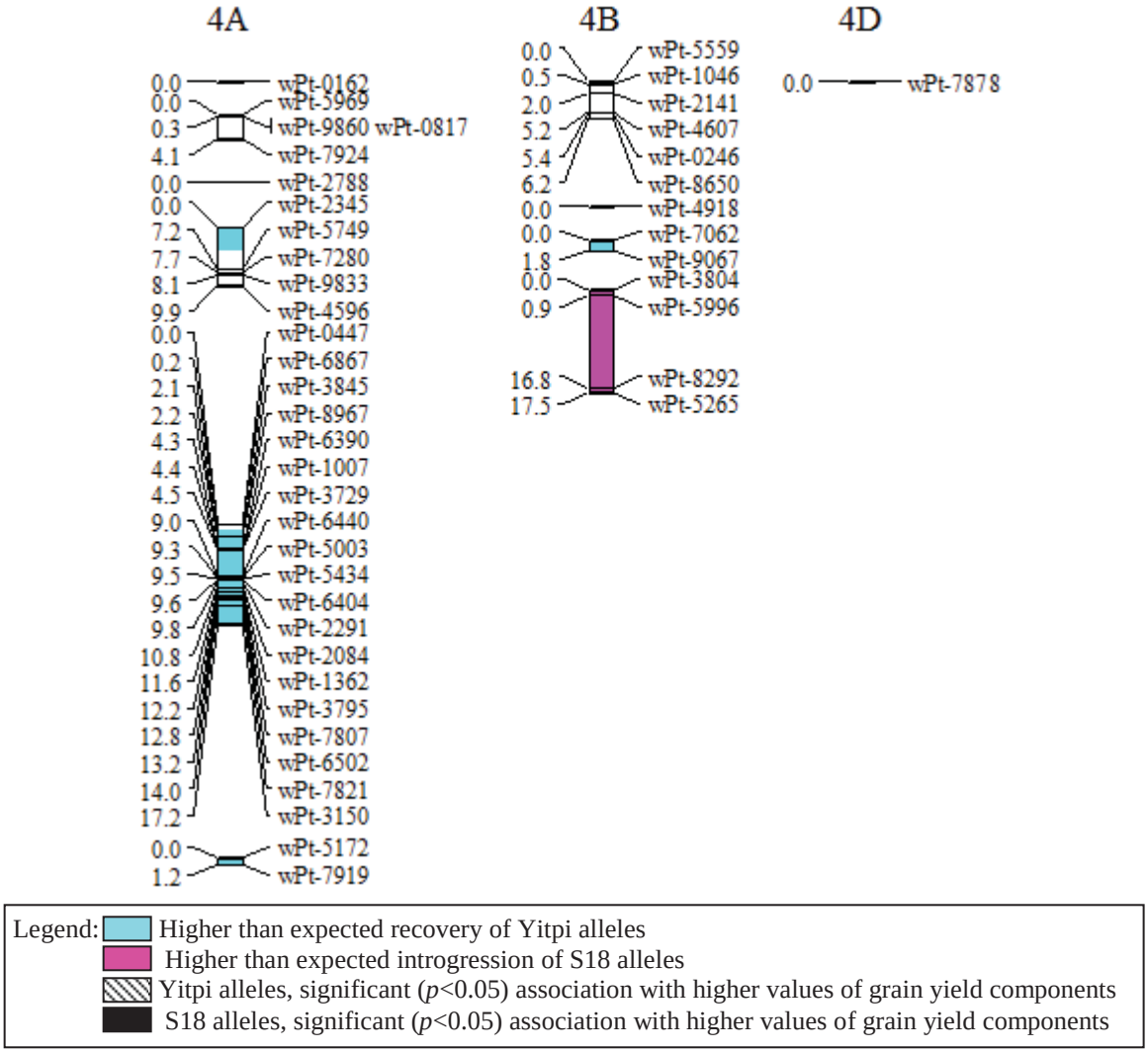


Figure 18. *Continued*.

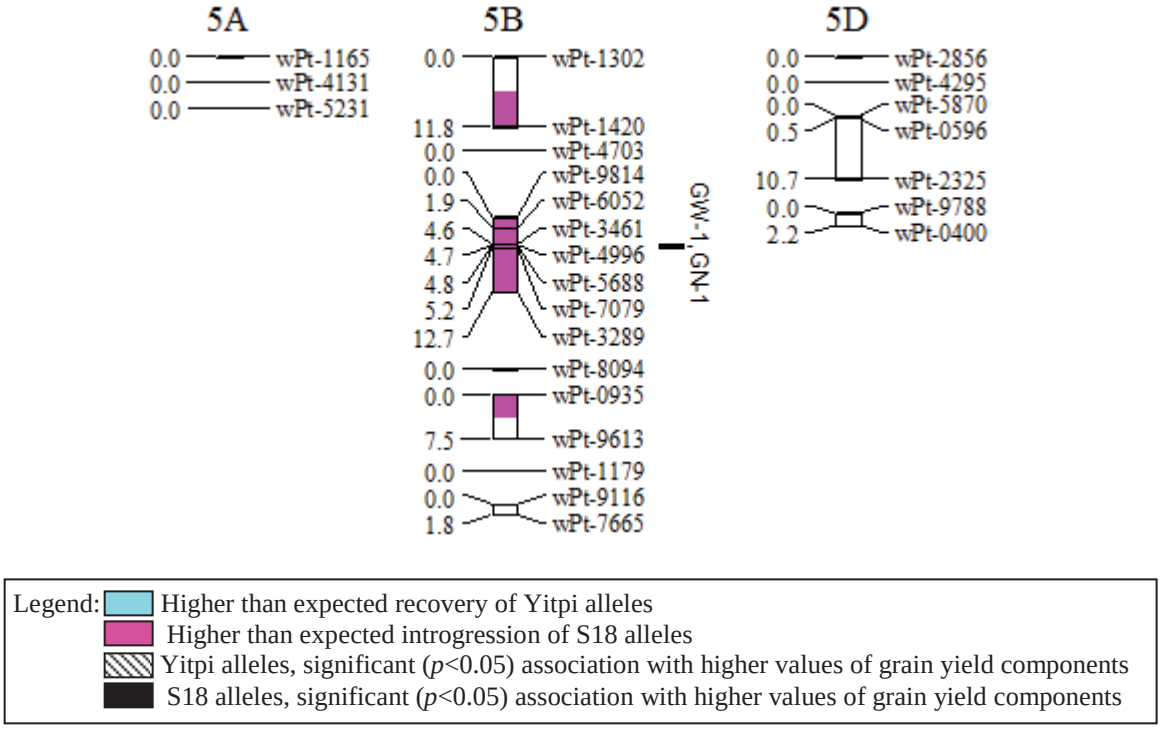


Figure 18. *Continued*.

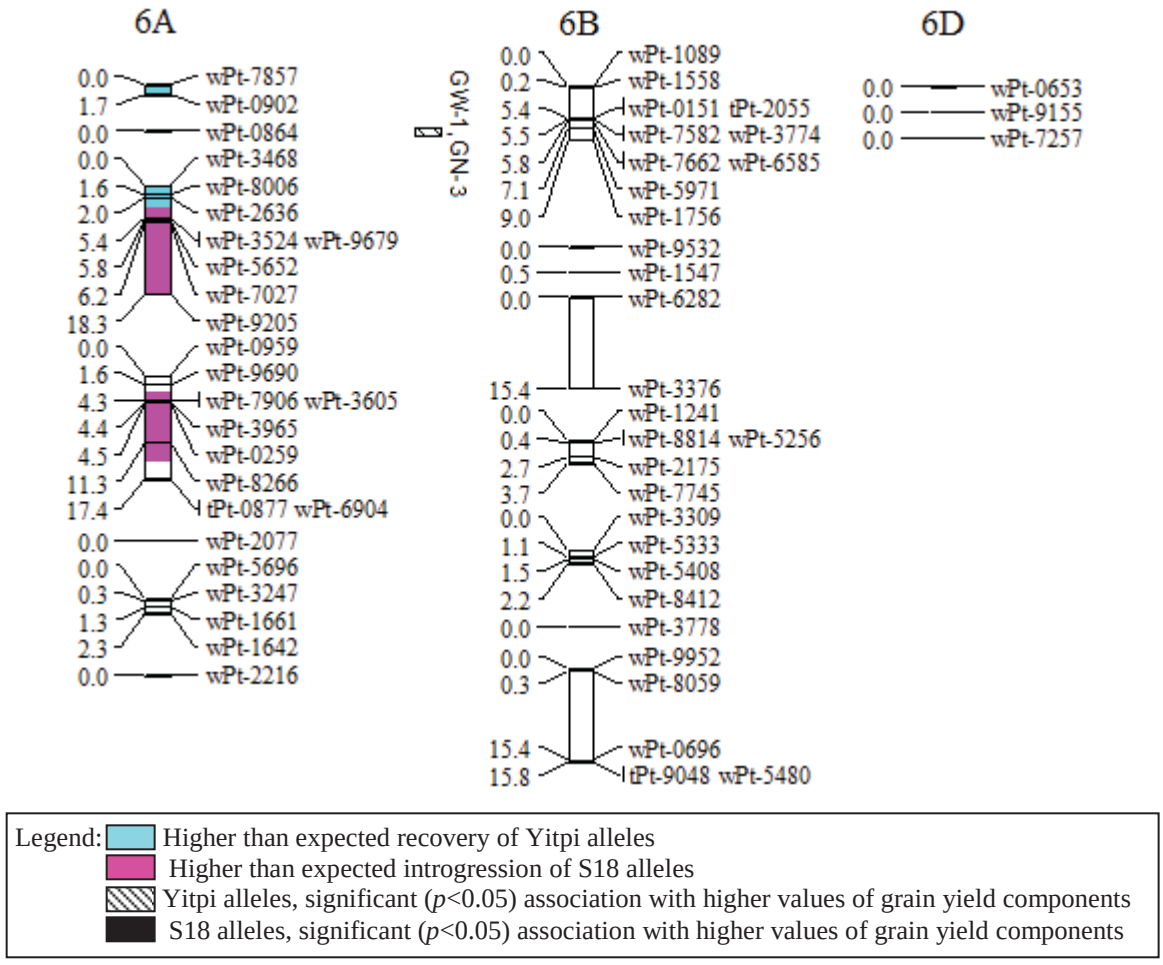


Figure 18. *Continued*.

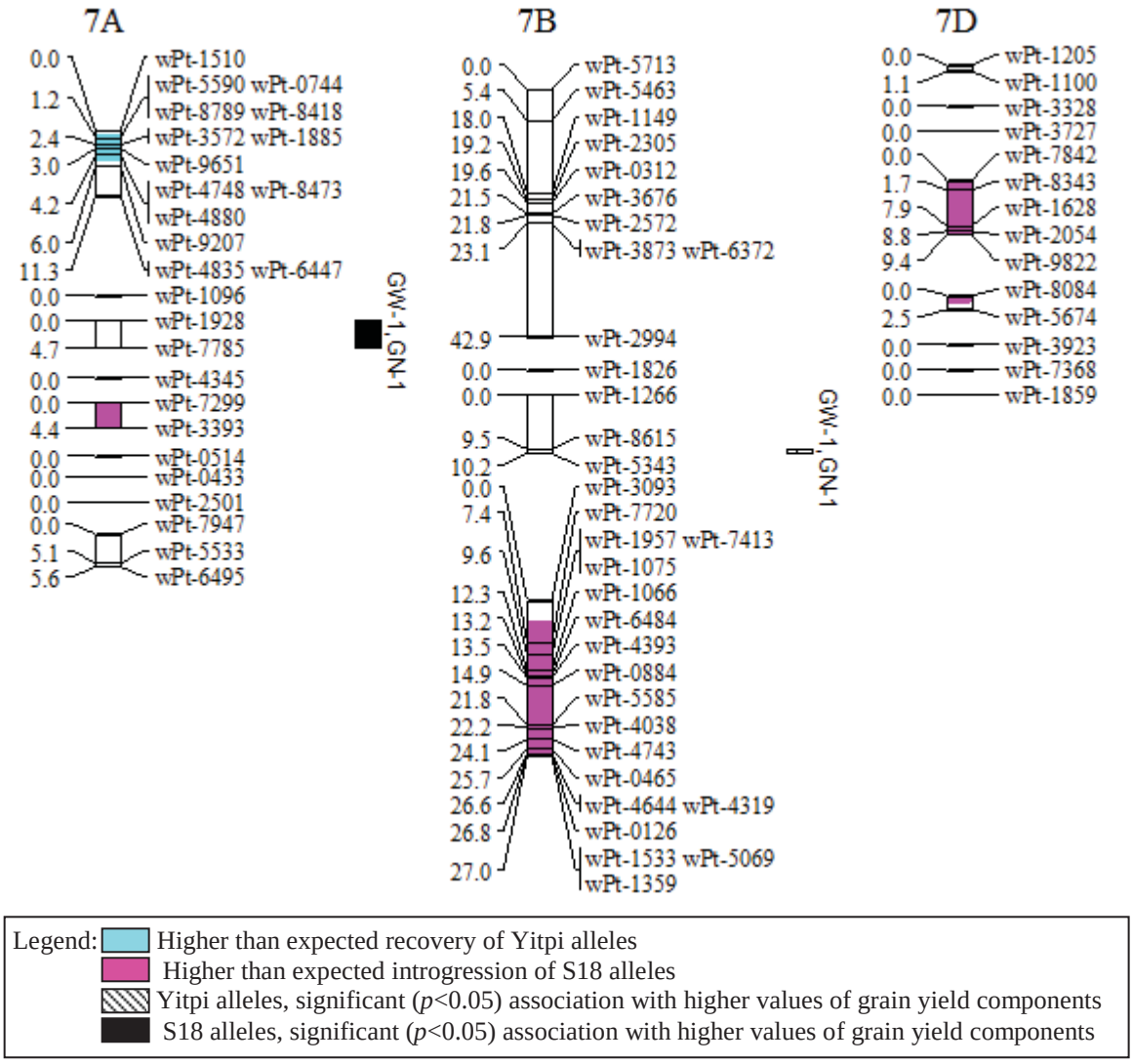


Figure 18. *Continued*.

6.3.3 Introgressed primary synthetic alleles at loci associated with grain weight and grains per m^2

Chi-squared tests identified significant (p<0.05) excess introgression of the primary synthetic allele at 79 (18.0%) and 103 (25.2%) mapped DArT marker loci of Y14 and Y18 respectively. These marker loci were located on chromosomes 1A, 1B, 2A, 3A, 3B, 5B, 6A and 7A of both families, and also on 6B of Y14 (Figure 17) and 4B, 7B and 7D of Y18 (Figure 18). Common regions of excess primary synthetic allele introgression were identified in both families at loci on chromosomes 1A, 1B, 2A, 3B and 5B.

At some loci with significant effects on grain weight and grains per m^2, the alleles introgressed from primary synthetics had positive effects on both traits. These loci were identified at regions on chromosomes 1A, 2A, 2B, 4B, 5B and 7D of Y14 (Figure 17), and 2A, 3A, 5B and 7A of Y18 (Figure 18). The loci in each chromosome region that explained the greatest percentage of variance for grain weight and grains per m^2 are listed in Table 14. In Y14, DArT marker loci wPt-6687 (2A) and wPt-3991 (4B) showed a significant (p<0.05) association for the major grain yield components at four and three environments respectively. Loci wPt-6687 (2A) and wPt-3991 (4B) explained up to 33% and 26% of grain weight variance, and up to 16% and 11% of grains per m^2 variance respectively (Table 14). DArT marker loci tPt-6091 (1A), wPt-9266 (1A) and wPt-6687 (2A) of Y14 were the only ones assayed in this family that showed a significant (p<0.05) association with both grain weight and grains per m^2 at a single environment. In Y18, DArT marker loci tPt-9405 (2A), wPt-1114 (2A) and wPt-1923 (3A) were all significantly (p<0.05) associated with grain weight and grains per m^2 at three test environments. Locus tPt-9405 (2A) explained the most variance for grain weight (up to 34%) and for grains per m^2 (up to 33%) in Y18 at any one environment (Table 14).

Of the marker loci that were not incorporated in the genetic maps of Y14 and Y18, five (Y14) and 15 (Y18) were significantly (p<0.05) associated with both grain weight and grains per m^2. These primary synthetic alleles were found to be significantly associated with an increase in these traits. Six of these unmapped loci in each family were assigned to a chromosome (4B and 5B in Y14, and 1B, 2B, 3A, 3D and 7A in Y18) using the reference genetic maps and genotype matching (Appendix 5).

Table 14. Mapped DArT marker loci of Y14 and Y18, which explain the highest percent of phenotypic variance for grain weight (mg/grain) and grains per m^2 at chromosome regions where loci that significant (p<0.05) association for these traits. Parental allele detected at these loci that were significantly associated with these higher values of grain yield components is displayed. Amount of trait variance explained by a QTL at each of these loci was the difference between total trait variance and the residual variance, expressed as a percent of the total variance.

				Grain weight (mg/grain)			Grains per m^2			
Family	**Chromosome**	**Marker**	**Environment**	**% variance**	**p-value**	**Regression coefficient**	**% variance**	**p-value**	**Regression coefficient**	**Allele**
Y14	1A	wPt-9266	Minnipa 2007	10	0.035	0.98	9	0.044	133.63	S14
			Roseworthy 2007	12	0.021	1.72				S14
		tPt-6091	Minnipa 2007	9	0.041	0.92	33	0.000	226.22	S14
			Roseworthy 2007	12	0.023	1.57				S14
	2A	wPt-6687	Minnipa 2007	9	0.040	0.86	16	0.005	164.8	S14
			Pinnaroo 2007	11	0.028	1.29				S14
			Roseworthy 2006	17	0.005	1.37				S14
			Roseworthy 2007	33	0.000	2.56				S14
	2B	wPt-6706	Minnipa 2006				11	0.043	49.45	S14
			Minnipa 2007	10	0.036	0.91				S14
			Roseworthy 2007	16	0.007	1.86				S14
	4B	wPt-3991	Minnipa 2006				11	0.043	57.14	S14
			Minnipa 2007				11	0.026	157.7	S14
			Pinnaroo 2007	15	0.009	1.75				S14
			Roseworthy 2006	19	0.003	1.67				S14
			Roseworthy 2007	26	0.000	2.72				S14
	5B	wPt-7665	Minnipa 2007				9	0.042	136.85	S14
			Roseworthy 2007	18	0.003	2.16				S14

Table 14. *Continued.*

				Grain weight (mg/grain)			Grains per m^2			
Family	**Chromosome**	**Marker**	**Environment**	**% variance**	**p-value**	**Regression coefficient**	**% variance**	**p-value**	**Regression coefficient**	**Allele**
Y14	6D	wPt-4827	Minnipa 2006				11	0.044	-48.94	Yitpi
			Minnipa 2007	12	0.021	-0.96				Yitpi
			Pinnaroo 2007	9	0.044	-1.19				Yitpi
			Roseworthy 2006	9	0.044	-1				Yitpi
			Roseworthy 2007	17	0.006	-1.77				Yitpi
	7A	wPt-2083	Roseworthy 2006				14	0.011	-510.41	Yitpi
			Roseworthy 2007	24	0.000	-2.51				Yitpi
Y18	1A	wPt-6074	Minnipa 2007				32	0.000	-239.41	Yitpi
			Pinnaroo 2007	17	0.003	-1.27				Yitpi
			Roseworthy 2007	9	0.038	-1.12				Yitpi
		wPt-5167	Minnipa 2007				11	0.019	-165.22	Yitpi
			Pinnaroo 2007	10	0.031	-1.13				Yitpi
	1D	wPt-8854	Minnipa 2007				8	0.048	-190.65	Yitpi
			Pinnaroo 2007	12	0.017	-1.71				Yitpi
	2A	tPt-9405	Minnipa 2007				33	0.000	259.47	S18
			Roseworthy 2006	20	0.001	1.18				S18
			Pinnaroo 2007	36	0.000	1.88				S18
			Roseworthy 2007	34	0.000	2.26				S18
		wPt-3896	Minnipa 2006	15	0.015	-1.02				Yitpi
			Roseworthy 2007				10	0.031	-83.43	Yitpi
		wPt-6207	Minnipa 2006	17	0.009	-0.99				Yitpi
			Pinnaroo 2007				10	0.031	-83.43	Yitpi
		wPt-1114	Minnipa 2006				14	0.016	56.38	S18
			Minnipa 2007				24	0.000	207.28	S18

Table 14. *Continued.*

				Grain weight (mg/grain)			Grains per m^2			
Family	**Chromosome**	**Marker**	**Environment**	**% variance**	**p-value**	**Regression coefficient**	**% variance**	**p-value**	**Regression coefficient**	**Allele**
Y18	2A	wPt-1114	Pinnaroo 2007	16	0.005	1.2				S18
			Roseworthy 2006	12	0.015	0.87				S18
			Roseworthy 2007	20	0.001	1.68				S18
	2B	wPt-2430	Minnipa 2007	20	0.001	-0.8	15	0.006	-190.83	Yitpi
			Pinnaroo 2007	29	0.000	-1.95				Yitpi
			Roseworthy 2007	43	0.000	-2.87				Yitpi
		wPt-9402	Minnipa 2007				20	0.002	-269.88	Yitpi
			Roseworthy 2007	14	0.008	-2.23				Yitpi
	3A	wPt-1923	Minnipa 2007				10	0.026	141.51	S18
			Pinnaroo 2007	12	0.014	1.09				S18
			Roseworthy 2006	11	0.019	0.87				S18
			Roseworthy 2007	14	0.009	1.43				S18
	3B	wPt-4127	Minnipa 2007				24	0.000	-203.94	Yitpi
			Pinnaroo 2007	17	0.003	-1.22				Yitpi
			Roseworthy 2007	10	0.024	-1.17				Yitpi
	5B	wPt-4996	Minnipa 2006	13	0.021	0.73				S18
			Pinnaroo 2007				10	0.027	73.21	S18
	6A	wPt-0864	Minnipa 2007				12	0.013	-248.78	Yitpi
			Pinnaroo 2007				10	0.029	-133.31	Yitpi
			Roseworthy 2006				9	0.040	-562.31	Yitpi
			Roseworthy 2007	9	0.038	-2.05				Yitpi
	7A	wPt-1928	Minnipa 2007				16	0.005	191.13	S18
			Pinnaroo 2007	18	0.002	1.42				S18
	7B	wPt-8615	Minnipa 2006	17	0.008	-0.89				Yitpi
			Roseworthy 2007				15	0.006	-291.65	Yitpi

6.3.4 Recovered bread wheat alleles at loci associated with grain weight and grains per m^2

Recovered Yitpi alleles were found to be significantly ($p<0.05$) in excess at 50 (11.5%) and 67 (16.4%) mapped DArT marker loci of Y14 and Y18 respectively. These loci were positioned on 1D, 2B, 3B, 4A and 4B in both families, and also on 3A and 7B in Y14 (Figure 17), and 1B, 3D, 6A and 7A in Y18 (Figure 18). In both families a similar pattern of excess Yitpi allele recovery was found on 2B, 3B, 4A and 4B.

Some alleles introgressed from Yitpi were significantly associated with increases in both grain weight and grains per m^2. These alleles were identified at loci on chromosomes 6D and 7A of Y14 (Figure 17), and on 1A, 1D, 2A, 2B, 3B, 6A and 7B of Y18 (Figure 18). The loci in each chromosome region that explained the highest significant ($p<0.05$) percentage of phenotypic variance for grain weight and grains per m^2 are listed in Table 14. In Y14 DArT marker locus wPt-4827 (6D) showed significant ($p<0.05$) associations with the grain yield components at four environments, explaining up to 17% of grain weight variance, and up to 11% of grain per m^2 variance (Table 14). In Y18 DArT marker locus wPt-2430 (2B) showed a significant ($p<0.05$) association with both grain weight and grains per m^2 at a single environment, explaining 20% and 15% of the phenotypic variance for these yield components respectively. The wPt-2430 DArT marker locus also explained the highest percentage of phenotypic variance (up to 43%) for grain weight in Y18 at any one environment (Table 14).

Of the DArT loci that were not positioned in the genetic maps of Y14 and Y18, 28 and 37 respectively were found to be significantly ($p<0.05$) associated with grain weight and grains per m^2, and identified Yitpi alleles that were significantly ($p<0.05$) associated with an increase in these traits. Four (Y14) and 27 (Y18) of these loci were assigned to chromosomes 2B, 6D, 7A and 7B of Y14, and 1A, 2A, 2B, 3B and 6A of Y18 using the reference genetic maps and genotype matching (Appendix 5).

6.3.5 Effect of selection for high grain weight and grain yield performance on synthetic allele retention

In synthetic-derived family Y14, 32.5% of lines had significantly ($p<0.05$) heavier grains than Yitpi. These lines represented 89.8% and 89.2% of primary synthetic alleles detected

in this family at mapped and at all loci assayed respectively (Table 15). In family Y18, 21.7% of lines had significantly (p<0.05) heavier grains than Yitpi. These lines represented 71.9% and 74.9% of primary synthetic alleles detected in this family at mapped and at all DArT maker loci assayed respectively (Table 15). Both sets of high performing lines from Y14 and Y18 included all introgressed primary synthetic alleles that were significantly (p<0.05) associated with an increase in both grain weight and grains per m^2.

For grain yield, 16.3% of Y14 synthetic derivatives had results not significantly different (p>0.05) from that of Yitpi. These lines represented 66.8% and 71.2% of primary synthetic alleles detected in this family at mapped and at all loci assayed respectively (Table 15). The same level of grain yield performance in Y18 led to the selection of 41.3% of lines, which represented 96.8% of primary synthetic alleles detected in this family at both mapped (Table 15) and all loci assayed. All introgressed primary synthetic alleles of Y14 and Y18 that were significantly (p<0.05) associated with an increase in both grain weight and grains per m^2 were maintained in the selected lines for grain yield performance.

Of the lines within synthetic-derived families Y14 and Y18, 7.0% and 17.4% respectively had grain weights significantly (p<0.05) heavier than Yitpi, and grain yields not significantly different (p>0.05) to that of Yitpi. These lines represented 38.5% (Y14) and 61.3% (Y18) of all primary synthetic alleles detected in these families. These high performing lines represented 39.8% (Y14) and 57.2% (Y18) of the mapped DArT loci that displayed synthetic alleles in these families (Table 15). All introgressed synthetic alleles in Y18 that were significantly (p<0.05) associated with an increase in both grain weight and grains per m^2 were maintained in the lines subjected to the double selection. Across lines in Y14 double selected for their high grain weight and grain yield performance, three alleles that had significant (p<0.05) association for grain weight and grains per m^2 were not represented at DArT loci tPt-3714 (5B), wPt-9266 (1A) and wPt-7665 (5B), which had significant (p<0.05) association for these traits.

Table 15. Number of DArT maker loci of families Y14 and Y18 that would identify a primary synthetic allele in lines selected for high grain weight (significantly (p<0.05) heavier grains than Yitpi), high grain yield (not significantly different (p>0.05) grain yields of that of Yitpi) or both.

	Synthetic-derived families							
	Y14 Selection strategies				Y18 Selection strategies			
Chromosome	None	High grain weight	High grain yield	High grain weight and grain yield	None	High grain weight	High grain yield	High grain weight and grain yield
1A	31	31	23	23	34	34	34	9
2A	24	24	24	23	21	19	21	19
3A	18	18	8	1	20	20	20	20
4A	34	33	33	31	32	9	32	3
5A	4	3	3	1	3	3	3	1
6A	19	19	9	8	26	20	19	17
7A	26	19	14	6	26	11	26	11
1B	29	29	25	3	31	26	31	26
2B	42	29	20	8	37	12	37	12
3B	43	43	35	17	36	31	35	23
4B	17	11	14	8	13	10	11	5
5B	20	14	19	7	16	13	16	13
6B	35	35	35	20	29	20	29	15
7B	32	23	6	6	33	33	33	33
1D	30	30	10	0	20	5	17	4
2D	8	8	0	0	3	2	3	2
3D	8	6	0	0	4	3	4	2
4D	2	2	0	0	1	1	1	1
5D	0	0	0	0	7	4	7	2
6D	1	1	1	0	3	2	3	2
7D	17	17	16	14	14	14	14	14
Total	440	395	295	176	409	292	396	234

6.4 Discussion

6.4.1 DArT marker screening of synthetic-derivatives

DArT marker screening provided an efficient, high-throughput method of identifying large numbers of introgressed primary synthetic (donor parent) alleles, and recovered bread wheat (recurrent parent) alleles in two BC_1 synthetic-derived families of lines, Y14 and Y18. The number of polymorphic DArT marker loci identified in these families (>990) was similar to that identified in the ITMI Opata 85/Synthetic population (Van Deynze *et al.* 1995; Triticarte Pty Ltd 2008). Clustering of DArT marker loci was observed in the Y14 and Y18 genetic maps, inflating the number of informative polymorphic loci to some extent. This clustering has been observed in other genetic maps, particularly around the centromere, and hence this effect has flowed onto the Y14 and Y18 maps from the reference maps (Semagn *et al.* 2006b; Wenzl *et al.* 2006). Over 400 polymorphic DArT marker loci identified in Y14 and Y18 had not been mapped in the 17 reference genetic maps, suggesting that synthetic-derived populations could be generally useful to map DArT markers. As Y14 and Y18 were represented by few lines (<50), with many marker genotypes found not to provide discrimination (show recombination events), larger synthetic-derived populations would be required for such mapping. Hospital (2001) reported that to observe sufficient levels of recombination to identify introgression segments of 20cM in length in a BC_1 population, at least 330 lines need to be genotyped. In light of this, the use of reference maps to create the genetic maps of Y14 and Y18 was an appropriate method to provide practical information.

The D genomes of the Y14 and Y18 genetic maps were poorly represented by marker loci, a phenomenon commonly reported in bread wheat populations (Röder *et al.* 1998; Chalmers *et al.* 2001). Due to the reliance on reference maps for the construction of the D genome chromosomes, this lack of polymorphism detection in the reference maps has also flowed on to Y14 and Y18. The inclusion of D genome markers from other marker platforms (simple sequence repeat (SSR) or amplified fragment length polymorphism (AFLP) markers, for example) could have improved marker representation on D genome chromosomes of Y14 and Y18. However, D genome markers developed in bread wheat may not be applicable for synthetic derivatives. Pestsova *et al.* (2000) reported that some D genome markers developed from *Ae. tauschii* accessions and the ITMI Opata 85/Synthetic mapping population could be absent from the D genome of bread wheat. Further use of *Ae. tauschii* populations to develop more D genome DArT markers may improve the

usefulness of this high-throughput technology to analyse the D genome of small synthetic-derived families of lines.

6.4.2 Patterns of excess parental allele introgression and recovery

Similar regions of excess primary synthetic allele introgression were detected at DArT marker loci on chromosomes 1A, 1B, 2A, 3B and 5B in both Y14 and Y18. Excess recovery of the Yitpi allele was also detected at similar regions on 2B, 3B, 4A and 4B in both families. These regions represented approximately half of all chromosomal regions where deviations of allele segregation from Mendelian expectations (for BC_1 material) were observed. This phenomenon may have been caused by several processes. Gamete survival and 'meiotic drive' elements have been shown to influence segregation distortion in *Drosophila melanogaster* and in populations of other organisms, including bread wheat (Lyttle 1991; Messmer *et al.* 1999; Semagn *et al.* 2006b). Competitive ability of the synthetic backcross lines during the bulk phase of the family development may have also caused target loci to be preferentially transmitted to future generations. Genotypes that produced more grain, had spring growth habits and were semi-dwarf in stature were preferentially selected during family development. Therefore, parental alleles at loci assayed here that promoted these characters provided a selective advantage. As Yitpi was the recurrent parent in both families, elements from Yitpi may have been the cause of segregation distortion at chromosomal regions that were similar in both families. Regions of excess parental allele representation that were not shared between Y14 and Y18 may have been influenced by the primary synthetic wheat parents. Segregation distortion has been observed in populations of both *T. turgidum* (Blanco *et al.* 1998; Kumar *et al.* 2007) and *Ae. tauschii* (Fritz *et al.* 1995; Faris *et al.* 1998); therefore it is not unreasonable to suggest that elements from the primary synthetic wheat parents of Y14 (S14) and Y18 (S18) were involved. Four other bread populations analysed with DArT markers have also shown deviations of allele segregation at the same marker loci as on 1A and 6B of Y14, and 1A, 2B, 7B and 7D of Y18 (Francki *et al.* 2009).

6.4.3 Beneficial parental alleles for increasing grain yield components

An aim of this investigation was to identify parental alleles within Y14 and Y18 that were significantly ($p<0.05$) associated with an increase in both grain weight and grains per m^2, at loci that had significant ($p<0.05$) association for these traits. Such alleles were identified in groups of these loci, possibly inferring the presence of QTLs. At the same or neighbouring DArT marker loci (<1cM away) from these groups on 1A, 2A and 4B in

Y14, and 1D, 2B, 3A and 6A in Y18, were previously reported QTL for grain yield, grain weight, grains per m^2, % screenings and hectolitre weight (McIntyre *et al.* 2010). Other previous reports stating the investigation of bread wheat germplasm with DArT and other marker systems have identified QTL for grain weight at loci <5cM from the loci groups assayed on 2A of Y14, and on 1A, 2A, 2B, and 3B of Y18 (Groos *et al.* 2003; Hai *et al.* 2008; Sun *et al.* 2009; Wang *et al.* 2009). Interestingly the beneficial primary synthetic (S18) alleles identified at loci grouped around tPt-9405 and wPt-1114 on 2A of Y18 appear to be situated between previously identified QTLs for grain weight (Huang *et al.* 2004; Sun *et al.* 2009). In Y14, beneficial primary synthetic (S14) alleles identified at loci grouped around wPt-6687 on 2A also appear to be situated between two previously reported QTL for grain weight on 2AL (Sun *et al.* 2009). Therefore, this study has shown that on the long arm of 2A of S14 and S18 primary synthetic wheat parents contributed beneficial alleles that increase grain weight and grains per m^2. This shows that the use of reference genetic maps and single marker regression can detect previously identified QTLs and potentially new QTLs in small families of lines. However, a p-value of < 0.05 may have not been stringent enough to exclude all false positives. The identification of few QTL outside of chromosome regions where QTL have been previously published was constructive, and hence a p-value of < 0.05 was considered appropriate for this exploratory study.

Of all DArT marker loci at which beneficial parental alleles were identified in Y14 and Y18, none were shared between both families. This was surprising for loci where beneficial Yitpi alleles were identified, as both families shared Yitpi as a common recurrent bread wheat parent. Interestingly most beneficial alleles at the putative QTL in Y18 were Yitpi derived, with all beneficial alleles at the putative QTL in Y14 being from its primary synthetic parent. This may help explain why Y18 lines were high grain yielding and the Y14 lines produced heavy grains. Primary synthetic wheats are known to be genetic resources for increased grain weight, but it appears here that alleles from the elite bread wheat parent were superior for high grain yield production. However, as nearly all primary synthetic and Yitpi alleles that had a significant ($p<0.05$) positive association for grain weight and grains per m^2 were retained in lines selected for high grain weight and grain yield performance, genetic contributions from the primary synthetic wheats were still important. This observation also supports the report by McIntyre *et al.* (2010), stating that selection for high grain yield performance can enrich the frequency of QTL that promote high grain yield in the selected lines.

6.5 Conclusion

Genetic material from two primary synthetic wheats was similarly introgressed in a common bread wheat background, but their effect on the major grain yield components was different. This study has demonstrated that DArT markers can identify large numbers of polymorphic loci in synthetic-derived material, assisting in the identification of introgressed parental alleles at putative QTL for grain yield components. Large synthetic-derived populations are recommended for future molecular and phenotypic studies, however small families were useful for assessing the genetic contributions of different primary synthetic wheats.

Chapter 7
General Discussion

The progenitor species of bread wheat, *T. turgidum* and *Ae. tauschii*, and their hybrid form, primary synthetic wheat, are sources of germplasm for bread wheat breeding. Initially primary synthetic wheats were created to identify these progenitor species in the 1940's, but in recent times they have become an important pre-breeding tool for broadening genetic diversity and improving agronomic traits in modern bread wheat germplasm. This resurgent interest in primary synthetics has come from the identification of genetic variation for agronomically important traits in this resource that is not readily observed in adapted breeding pools of bread wheat. The growing human population and the rising cost of crop maintenance have also driven the search for novel germplasm that can improve the health and productivity of bread wheat. The identification of primary and derived synthetics with superior performance, relative to modern bread wheat cultivars, has validated the desire to breed with this germplasm resource despite their expressions of deleterious traits. Investigations undertaken here focused on further determining the value of using primary and derived synthetics in Australian bread wheat breeding. This research was divided into three areas, 1) genetic diversity, 2) grain weight and grain yield in drought stressed environments, and 3) introgression of synthetic alleles. This chapter discusses the major findings of each of these areas and summarises the key conclusions.

7.1 Genetic diversity

Primary synthetic and Australian bread wheats were assayed in this study using DArT molecular markers, to determine the genetic variation within and among these germplasm pools. Although DArT markers had not been extensively used to assess primary synthetic wheat in the past, their use to evaluate genetic diversity in bread wheat had been validated (White *et al.* 2008). The DArT microarray platform allowed a large number of loci to be assayed simultaneous across the A, B and D genome chromosomes of the test genotypes. However, there were limitations to thoroughly assay the D genome of the bread wheats and the *Ae. tauschii* components of the primary synthetics. Interestingly this issue has been common to all molecular marker systems to date. The likely reason for this phenomenon is a low level of D genome polymorphism present in the bread wheat germplasm used to develop molecular makers. This study has strengthened the case to use *Ae. tauschii* populations more extensively to develop additional D genome markers, especially for the assessment of primary and derived synthetic wheats.

Genetic similarity, and conversely dissimilarity or genetic distance was observed between and within the A, B and D genomes of the primary synthetics and Australian bread wheats. Among the Australian cultivars this level was between what has been previously described (Paull *et al.* 1998; White *et al.* 2008). Further representation of the Australian bread wheat pool may have provided a different outcome, but the findings of the current study were within expectations.

Two results defined this body of work, the high level of dissimilarity between the primary synthetics and Australian bread wheats and their A and B genomes, and the lack of similarity between primary synthetics with the same recorded pedigree or those that shared common parents. The A and B genomes of the primary synthetics were derived from CIMMYT durum wheat cultivars or breeders lines, which represented a source of 'refined' genomes of *T. turgidum*. Some durum A and B genomes were bread wheat like, where as many others showed divergence from those of the bread wheats. Previous studies have noted divergence between bread and durum wheat. Hence, primary synthetic wheats used in the future to broaden the genetic diversity of the A and B genomes of Australian bread wheat may not need to be derived from wide *T. turgidum* sources, such as subspecies *dicoccoides*, *dicoccon* or *carthlicum*, which may express deleterious traits. The lack of genomic similarity between primary synthetic wheats with the same recorded pedigrees was unexpected, and raises issues of pedigree record keeping at CIMMYT, outcrossing among CIMMYT *Ae. tauschii* accessions or the primary synthetics themselves. The former seems most likely as this issue has been identified with other primary synthetics produced by CIMMYT that have been used in molecular studies. Better record keeping is therefore required in the future production of primary synthetic wheats. Seed from the exact parental *T. turgidum* and *Ae. tauschii* accessions used should be retained for molecular studies, a practise that in the past has not been commonly performed.

7.2 Grain weight and grain yield under drought stressed environments

In this study synthetic backcross lines were evaluated in drought stressed environments in southern Australia, to identify synthetic derivatives that could achieve superior grain yields compared to their Australian recurrent bread wheat parent. Their major grain yield components (grain weight and grains per m^2) were also investigated. The magnitude of grain yield improvements achieved, and the major grain yield component responsible, changed with each environment type. Grain yield BLUPs up to 12.0% higher than that of

Yitpi (recurrent parent) were achieved by the synthetic backcross lines in the highest yielding environments, with grain yield BLUPs up to 43.8% higher identified under the lowest yielding environments. These significant grain yield improvements were due to significant increases in grain weight in the highest yielding environments, whereas significant increases in grains per m^2 were commonly responsible in the lowest yielding environments, without significant penalty to grain weight. Computer simulation models have predicted a similar mechanism of grain yield improvement in Australian environments (Asseng *et al.* 2002). In light of this pattern Australian bread wheat breeders may be able to apply additional selection pressure to increase grain weight in higher yielding environments without significant negative effects on grain yield. What synthetic backcross lines can provide is an incremental increase in grain yield without penalty to grain quality (grain weight), particularly under drought in low yielding environments.

It is a challenge to select bread wheat genotypes that can achieve increased grain yields and maintain and/or increase grain quality (grain weight) in the low rainfall conditions experienced in Australia. There is a need in Australia to increase bread wheat productivity in times of drought as such meteorological events are set to become more prevalent in the future (Hennessy *et al.* 2007). As some of the highest grain yielding synthetic backcross lines identified here performed well in a subsequent experiment conducted by Australian Grain Technologies (AGT) Pty Ltd in 2008, which experienced less water stress, this suggests that using synthetic derived material in bread wheat breeding in the future may lead to more improved and stable yields in Australian bread wheat. Synthetic backcross lines from this subsequent experiment have been incorporated into AGT's South Australian wheat breeding program.

A second key aim of this investigation was to investigate the potential of different primary synthetic wheats to increase grain yield, and its major components, of an Australian bread wheat cultivar. This was achieved by evaluating synthetic backcross lines as families, each created from a different primary synthetic parent and a common recurrent Australian bread wheat cultivar. Differences in genetic contributions from the primary synthetic parents were evident as the mean grain yield of lines within families was commonly significantly different between the worst and best performing families. Superior grain yielding synthetic backcross lines were identified in about half of the families evaluated. This shows that primary synthetic wheats can be used to develop superior grain yielding derivatives but the choice of primary synthetic parent can affect the potential to identify such material.

However, as not all superior grain yielding synthetic backcross lines were identified in the highest performing families it was still beneficial to assess a range of primary synthetic wheats as donor parents. Identifying primary or derived synthetic germplasm that achieves high performance with respect to grain yield or its major components provides a breeding resource that may broaden the genetic diversity of a breeding pool whilst maintaining the commercial potential of the pool.

This study has demonstrated that primary and derived synthetics would be useful to improve grain weight and grain yield performance of Australian bread wheat under drought stressed environments. Breeding programs in Australia are likely to continue to favour crossing adapted genotypes to develop new cultivars, and CIMMYT is likely to continue to develop and pre-screen synthetic germplasm that may be useful for Australia. The current study however does encourage the development and screening of synthetic-derived germplasm in Australia, in particular for the identification of superior genotypes under southern Australian environments. Collaborative research between the Australian Winter Cereals Collection (AWCC) and the wheat breeding companies in Australia have the capacity to achieve this.

7.3 Introgression of synthetic alleles

The underlying principle for introgressing genetic material from diverse sources, such as primary synthetic wheats, into modern bread wheat cultivars is to capture desirable genes and alleles that have commercial worth. In the past the main focus for exploiting the progenitor species of bread wheat was to obtain simply inherited traits, such as disease resistance. However, the efficient implementation of large numbers of molecular markers has facilitated investigations into the genetic control of important quantitative traits. Identifying primary synthetic alleles that have a positive association with increasing grain weight and grains per m^2 may therefore lead to the tracking and maintenance of synthetic alleles in breeding programs through marker assisted selection. To identify such alleles two families of synthetic backcross lines (Y14 and Y18) were investigated here. This involved combining phenotypic data from their field evaluation (Chapter 5) with molecular marker data from their DArT marker analysis (Chapter 6).

With the use of reference genetic maps and the application of single marker regression to evaluate the small synthetic-derived families of lines, it was possible to detect previously reported QTL for grain yield and its components. In the current study these QTL were

observed as groups of linked loci with significant (p<0.05) associations for both grain weight and grains per m^2. These techniques differ from those traditionally used for QTL mapping in large populations of lines. Due to introgressed primary synthetic alleles, potentially new QTLs for increasing grain weight and grains per m^2 were identified on 2AL in both Y14 and Y18. This demonstrates that primary synthetic wheats can provide potentially novel genes and alleles to a modern bread wheat cultivar. Upon selecting for high grain weight and/or grain yield performance, these beneficial synthetic alleles on 2AL and most others identified were represented in the selections. Therefore it is conceivable that genetic improvement of grain yield over time can be achieved through the enrichment of beneficial alleles within a breeding program (McIntyre *et al.* 2010). Although a large population size is preferable for QTL mapping the use of reference maps and single marker regression could be a strategy to identify putative QTL in other small families of lines within a breeding program. However, the use of many molecular makers would be required. Future studies to further validate the value and effect of primary synthetic alleles at the putative 2AL QTL's may be performed through the phenotypic and genotypic evaluation of near-isogenic lines (NIL) that have the 2AL synthetic introgressions.

7.4 Conclusions

Bread wheat breeding is essential for the ongoing development of improved cultivars, which meet changing human requirements and field environments. In Australia these environments are set to become dryer with the incidence of severe drought predicted to increase (Hennessy *et al.* 2007). This study has shown that many, but not all primary synthetic wheats can be a germplasm source to increase the grain yield and grain quality (grain weight) of a modern Australian bread wheat under drought stressed environments in southern Australia (Chapter 5). The identification of potentially novel synthetic alleles from two primary synthetic wheats, on 2AL that increased grain weight and grain per m^2 under drought (Chapter 6) may prompt future investigations of the use of these synthetic alleles in Australian breeding programs. Finally, whilst maintaining or increasing the commercial worth of Australian bread wheat breeding pools, primary synthetic wheats also have the potential to further broaden the genetic diversity of these pools (Chapter 4). These findings warrant the continued assessment of primary and derived synthetic wheats in Australian bread wheat breeding programs.

Chapter 8
Conclusions

This research focused on evaluating primary and derived synthetics for their value and potential use in Australian bread wheat breeding. The research involved identifying the genetic similarity between and among primary synthetic wheat and modern Australian bread wheat cultivars, evaluating the grain yield performance of families of synthetic backcross lines in drought stress environments in southern Australia, and the major grain yield components (grain weight and grains per m^2) responsible for their performances, the tracking of synthetic wheat alleles introgressed into the background of a modern Australian bread wheat, and the identification of introgressed synthetic alleles associated with increasing grain weight and grains per m^2. This thesis presents the following conclusions:

1. Primary synthetic wheats are a source of genetic variation for Australian bread wheat breeders as greater dissimilarity was observed between the 44 primary synthetic wheats and the nine modern Australian bread wheat cultivars investigated here than that identified within these germplasm groups (Chapter 4). One hundred and sixty eight synthetic alleles were identified across the 44 primary synthetic wheats that could be novel to modern Australian bread wheat germplasm. The CIMMYT durum wheat component (A and B genomes) of the 44 primary synthetic wheats investigated was substantially different compared to the A and B genomes of the nine modern Australian bread wheats evaluated. The D genomes of the primary synthetic wheat were genetically divergent compared to those of the bread wheats, however more D genome markers representing polymorphisms in *Ae. tauschii* need to be added to the DArT array to sufficiently examine this genome.

2. Synthetic backcross (BC_1) lines can achieve superior grain yields under drought stressed environments in southern Australia, compared to their recurrent Australian bread wheat parent (Chapter 5). Grain yields up to 12.0% higher than Yitpi (recurrent parent) were achieved by synthetic backcross lines in the highest yielding environments, with grain yields up to 43.8% higher identified in the lowest yielding environments. The grain yield component responsible for these increases was grain weight (up to 108.1% of that of Yitpi) in the highest yielding environments, whereas grain per m^2 (up to 154.3% of that of Yitpi) was commonly responsible in the lowest yielding environments. High performing synthetic backcross lines from this study have been incorporated into the bread wheat breeding program of Australian Grain Technologies (AGT) Pty Ltd in South Australia.

3. Primary synthetic wheats differ in their potential to increase the grain yield, and its major components, of an Australian bread wheat cultivar (Chapter 5). Out of 27 primary synthetics evaluated through 27 associated families of synthetic backcross lines, 14 primary synthetics (S01, S05, S06, S09, S11, S12, S13, S14, S15, S18, S36, S37 and S40) were the donor parents to lines that produced superior grain yields. Genetic contributions from primary synthetic wheat S18 was most superior for grain yield as its associated family of lines Y18 produced the highest estimated family grain yield in every environment. For the improvement of grain weight, genetic contributions from S12, S14 and S43 were most superior as their families of lines (Y12, Y14 and Y43 respectively) had higher estimated grain weights than the recurrent Australian bread wheat parent in every test environment.

4. Similar regions of excess primary synthetic and Yitpi allele introgression were detected in synthetic derived families of lines Y14 and Y18 (Chapter 6). Regions on chromosome 2A were found to be associated with grain weight and grains per m^2, in drought stressed environments, with the favourable allele(s) from the primary synthetic parents. These regions were near DArT marker loci wPt-6687 in Y14, and tPt-9405 and wPt-1114 in Y18. These seem to be different locations than the QTL on 2A identified by Huang *et al.* (2004) and Sun *et al.* (2009), however a p-value of <0.05 that was used may have not been stringent enough to exclude this finding as a false positive. Selection of high grain weight and grain yielding lines in families Y14 and Y18 did represent these beneficial regions on 2A, therefore primary synthetic wheats S14 and S18 may be a source of novel alleles for improving grain weight and/or grain yield performance under drought stressed environments in southern Australia.

Appendices

Appendix 1. Additional information of primary synthetic wheat used in this study.

Primary synthetic	Australian Winter Cereals Collection accession number	CIMMYT Wheat Pedigree Management System			Wheat Genetics Resource Center accession number [3]
		Cross number and selection history	CID [1]	SID [2]	
S1	AUS 29642	CIGM90.525-0SY	159559	7	TA 4152-26
S2	AUS 29643	CIGM90.525-0SY	159559	7	TA 4152-26
S3	AUS 29637	CIGM87.2771-1B-0PR-0B-0SY	88726	24	TA 4152-9
S4	AUS 29641	CIGM88.1249-0B-0SY	159551	8	
S5	AUS 29638	CIGM86.955-1M-1Y-0B-0PR-0B-0SY	159538	7	TA 4153-4
S6	AUS 29668	CIGM90.881-0SY	161595	1	
S7	AUS 29655	CIGM89.545-0Y-0SY	160208	2	
S8	AUS 29663	CIGM89.567-1B-0SY	161079	35	TA 4152-54
S9	AUS 29664	CIGM89.567-0SY	161079	36	
S10	AUS 29636	CIGM88.1194-0B-0SY	152421	32	TA 4152-8
S11	AUS 29680	CIGM93.233-0SY	161730	1	
S12	AUS 29653	CIGM90.534-0SY	160194	2	TA 4152-37
S13	AUS 29676	CIGM93.203-0SY	161706	1	
S14	AUS 29681	CIGM93.242-0SY	161739	1	
S15	AUS 29677	CIGM93.207-0SY	161709	1	
S16	AUS 29678	CIGM93.211-0SY	161711	1	
S17	AUS 29652	CIGM88.1353-0B-0SY	152422	11	
S18	AUS 29682	CIGM93.267-0SY	161751	1	
S19	AUS 29684	CIGM93.306-0SY	161775	4	TA 4152-95
S20	AUS 29640	CIGM88.1237-0B-0SY	159549	15	
S21	AUS 29649	CIGM90.583-0SY	159579	16	
S22	AUS 29650	CIGM90.586-0SY	159582	1	
S23	AUS 29669	CIGM90.909-0SY	161093	8	
S24	AUS 29673	CIGM92.1742-0SY	161682	1	

Appendix 1. *Continued.*

Primary synthetic	Australian Winter Cereals Collection accession number	CIMMYT Wheat Pedigree Management System			Wheat Genetics Resource Center accession number[3]
		Cross number and selection history	CID [1]	SID [2]	
S25	AUS 29672	CIGM92.1700-0SY	161662	1	
S26	AUS 29685	CASW94Y00064S-0SY	181742	1	
S27	AUS 29657	CIGM90.603-0SY	160220	7	
S28	AUS 29674	CIGM92.1750-0SY	161688	1	
S29	AUS 29648	CIGM88.1335-0B-0SY	159578	12	
S30	AUS 29667	CIGM90.873-0SY	161592	5	
S31	AUS 29666	CIGM90.846-0SY	161589	20	TA 4152-63
S32	AUS 29675	CIGM93.180-0SY	161696	1	
S33	AUS 29670	CIGM90.910-0SY	161609	5	TA 4152-74
S34	AUS 29651	CIGM88.1351-0B-0SY	159585	8	
S35	AUS 29644	CIGM88.1288-0B-0SY	159567	7	TA 4152-29
S36	AUS 29659	CIGM89.543-3B-0SY	161078	15	
S37	AUS 29660	CIGM89.559-0SY	160213	25	TA 4152-44
S38	AUS 29661	CIGM89.559-0SY	160213	25	TA 4152-45
S39	AUS 29662	CIGM89.559-2B-0SY	160213	26	
S40	AUS 29656	CIGM89.561-0Y-0SY	160215	7	TA 4152-47
S41	AUS 29645	CIGM88.1297-0B-0SY	159571	2	
S42	AUS 29654	CIGM89.538-0Y-0SY	160202	26	TA 4152-41
S43	AUS 29646	CIGM88.1313-0SY	159573	54	TA 4152-30
S44	AUS 29647	CIGM88.1313-0SY	159573	54	TA 4152-31

[1] CID, Cross identification number
[2] SID, Selection identification number
[3] Kansas State University, data obtained from W. J. Raupp, personal communication, 2005.

Appendix 2. *Aegilops tauschii* Coss. accessions that were recorded as the male parent for one or more of the 44 primary synthetic wheats used in this study.

CIMMYT Wheat Pedigree Management System [1,2]	Country of Origin [1,3,4]	Taxonomic Group [5]	Australian Winter Cereals Collection accession number [1,4]	CSIRO Plant Industry accession number [1,4]	Wheat Genetics Resource Center accession number [1,2]	Kyoto University accession number [1,3]
WX 218 (315; 628; 890)	Iran	strangulata	AUS 24140 (18905)	CPI 110798	TA 2463	KU 2088
WX 220 (321; 630; 897)	Iran	strangulata	AUS 24147 (18908)	CPI 110805	TA 2470	KU 2096
WX 247 (818)	Afghanistan	typica	AUS 24067	CPI 110725	TA 2389	KU 2012
WX 256 (829)	Afghanistan	typica	AUS 24078	CPI 110736	TA 2400	KU 2023
WX 258 (831)	Afghanistan	typica	AUS 24080 (18894)	CPI 110738	TA 2402	KU 2025
WX 264 (836)	Afghanistan	typica	AUS 24086	CPI 110744	TA 2408	KU 2031
WX 269 (841)	Afghanistan	typica	AUS 24091	CPI 110749	TA 2413	KU 2036
WX 309 (881)	Iran	strangulata	AUS 24131	CPI 110789	TA 2454	KU 2078
WX 326 (902)	Iran	typica	AUS 24152 (18988)	CPI 110810	TA 2475	KU 2102
WX 332 (908)	Iran	meyeri	AUS 24158 (18993)	CPI 110816	TA 2481	KU 2108
WX 369	Iran	typica			TA 2528	KU 2159
WX 385 (969)	Iran	typica	AUS 24219	CPI 110877	TA 2549	KU 2629
WX 390 (978)	Afghanistan	typica	AUS 24228	CPI 110886	TA 2558	KU 2638
WX 783	Japan	strangulata	AUS 24032	CPI 110690	TA 1695	KU 751
WX 882	Iran	strangulata	AUS 24132	CPI 110790	TA 2455	KU 2079
WX 890 (218)	Iran	strangulata	AUS 24140	CPI 110798	TA 2463	KU 2088
WX 895	Iran	strangulata	AUS 24145	CPI 110803	TA 2468	KU 2094

[1] Data obtained from W. J. Raupp, personal communication, 2005.
[2] Data obtained from Mujeeb-Kazi *et al.* (2000).
[3] Data obtained from Lubbers *et al.* (1991).
[4] Data obtained from using data base program OZGrip v2.0 (Skovmand *et al.* 2000)
[5] *Ae. tauschii* subps. *tauschii* var. typical, anathera, meyeri and subsp. *strangulate* (Eig) Tzvelev

Appendix 3. Summary of random and spatial effects fitted as fixed covariates in models for the generation of best linear unbiased predictions (BLUPs) for grain weight (GW), number of grains produced per m^2 (GN) and grain yield (GY) of each genotype, per environment.

	Environment														
	2006						**2007**								
Spatial effects	**Minnipa**			**Roseworthy**			**Minnipa**			**Pinnaroo**			**Roseworthy**		
Fixed covariates	**GW**	**GN**	**GY**	**GW**	**GN**	**GY**	**GW**	**GN**	**GY**	**GW**	**GN**	**GY**	**GW**	**GN**	**GY**
Sowing error [a]				Yes	Yes	Yes									
Emergence [b]				Yes	Yes	Yes									
Crown rot symptom Severity [c]				Yes	Yes	Yes							Yes	Yes	Yes
Random															
Linear range									Yes			Yes			
Linear row		Yes	Yes		Yes	Yes			Yes				Yes	Yes	
Random range	Yes						Yes	Yes		Yes	Yes		Yes		
Random row							Yes	Yes	Yes			Yes			
Range AR1 [d]	0.46	0.12	0.22	0.39	0.54	0.53	0.11	0.44	0.46	0.49	0.31	0.16	0.35	0.34	0.43
Row AR1 [d]	0.51	0.52	0.50	0.36	0.70	0.78	0.13	0.55	0.68	0.63	0.62	0.66	0.34	0.77	0.79

[a] Estimated percent of plot that was missing due to sowing complications.
[b] Estimated percent of plot that failed to emerge.
[c] Crown rot symptoms were observed and scored (1 to 9) during grain fill (1= no presences of white spikes, and 9 = all spikes within a plot been white before maturity).
[d] AR1, autogressive process level 1

Appendix 4. Simple linear correlation coefficients (*r*) for associations among grains per m^2, grain weight and grain yield of 27 synthetic-derived families and all lines across all families at Minnipa in 2006 and 2007, Pinnaroo in 2007 and Roseworthy in 2006 and 2007.

Synthetic-derived families and lines within	Grains per m^2 v grain weight		Grain yield v grains per m^2		Grain yield v grain weight	
	r	**df**	***r***	**df**	***r***	**df**
Minnipa 2006/07						
All lines	-0.157**	443	0.936**	454	0.146*	454
Y01	0.013	12	0.968**	12	0.241	12
Y03	0.048	25	0.947**	25	0.295	25
Y05	-0.472	8	0.757**	8	0.151	8
Y06	0.335	13	0.943**	13	0.596*	13
Y07	0.207	11	0.954**	11	0.450	11
Y09	0.292	13	0.954**	14	0.529*	14
Y10	-0.835**	6	0.965**	6	-0.736*	6
Y11	0.200	20	0.950**	20	0.437*	20
Y12	-0.207	21	0.948**	22	0.059	22
Y13	-0.455	13	0.964**	13	-0.238	13
Y14	0.112	34	0.941**	34	0.393*	34
Y15	-0.455*	19	0.958**	19	-0.247	19
Y16	-0.637*	9	0.738**	10	-0.033	10
Y18	-0.319	36	0.968**	38	-0.140	38
Y19	-0.199	22	0.941**	23	0.075	23
Y20	-0.083	14	0.986**	14	0.047	14
Y26	0.143	3	0.748	4	0.751	4
Y27	-0.460	9	0.970**	9	-0.265	9
Y31	0.775**	14	0.960**	14	0.896**	14
Y33	-0.197	20	0.947**	20	0.058	20

Appendix 4. *Continued.*

Synthetic-derived families and lines within	Grains per m^2 v grain weight		Grain yield v grains per m^2		Grain yield v grain weight	
	r	**df**	***r***	**df**	***r***	**df**
Minnipa 2006/07						
Y35	-0.644*	9	0.751**	9	-0.044	9
Y36	-0.080	14	0.947**	16	0.119	16
Y37	-0.531*	13	0.942**	15	-0.281	15
Y40	-0.189	6	0.957**	6	0.081	6
Y41	-0.118	2	0.961*	2	0.163	2
Y42	-0.383	16	0.916**	16	-0.026	16
Y43	-0.5	9	0.785**	9	0.083	9
Pinnaroo 2007						
All lines	-0.300**	649	0.776**	649	0.312**	649
Y01	-0.194	20	0.701**	20	0.521**	20
Y03	0.096	34	0.856**	34	0.493**	34
Y05	0.001	20	0.876**	20	0.411	20
Y06	-0.258	21	0.727**	21	0.415	21
Y07	0.349	16	0.892**	16	0.688**	16
Y09	0.141	23	0.886**	23	0.519**	23
Y10	-0.468	14	0.781**	14	0.168	14
Y11	0.050	26	0.765**	26	0.645**	26
Y12	-0.449*	25	0.728**	25	0.221	25
Y13	-0.446	17	0.708**	17	0.231	17
Y14	-0.558**	44	0.748**	44	0.075	44
Y15	-0.100	23	0.857**	23	0.365	23
Y16	-0.433	17	0.737**	17	0.238	17

Appendix 4. *Continued.*

Synthetic-derived families and lines within	Grains per m^2 v grain weight		Grain yield v grains per m^2		Grain yield v grain weight	
	r	**df**	***r***	**df**	***r***	**df**
Pinnaroo 2007						
Y18	-0.406	45	0.754**	45	0.254	45
Y19	-0.413*	32	0.863**	32	0.036	32
Y20	-0.182	27	0.903**	27	0.207	27
Y26	0.036	16	0.916**	16	0.415	16
Y27	-0.614**	17	0.909**	17	-0.274	17
Y31	-0.543**	21	0.699**	21	0.165	21
Y33	-0.393*	26	0.783**	26	0.158	26
Y35	-0.352	16	0.930**	16	-0.047	16
Y36	0.004	23	0.770**	23	0.602**	23
Y37	-0.286	21	0.694**	21	0.419	21
Y40	0.044	12	0.880**	12	0.495	12
Y41	-0.848**	7	0.304	7	0.088	7
Y42	-0.231	23	0.903**	23	0.158	23
Y43	-0.578*	11	0.731**	11	0.121	11
Roseworthy 2006/07						
All lines	-0.184**	645	0.817**	645	0.387**	645
Y01	0.056	20	0.905**	20	0.460**	20
Y03	-0.285	34	0.755**	34	0.377*	34
Y05	-0.768**	20	0.815**	20	-0.301	20
Y06	0.240	21	0.895**	21	0.606**	21
Y07	-0.129	16	0.912**	16	0.268	16

Appendix 4. *Continued.*

Synthetic-derived families and lines within	Grains per m^2 v grain weight		Grain yield v grains per m^2		Grain yield v grain weight	
	r	**df**	***r***	**df**	***r***	**df**
Roseworthy 2006/07						
Y09	0.091	23	0.797**	23	0.653**	23
Y10	-0.714**	14	0.681**	14	-0.015	14
Y11	0.450*	26	0.896**	26	0.785**	26
Y12	-0.421*	25	0.752**	25	0.258	25
Y13	-0.114	17	0.818**	17	0.405	17
Y14	-0.318*	43	0.730**	43	0.392*	43
Y15	0.108	23	0.943**	23	0.387	23
Y16	-0.539*	17	0.963**	17	-0.322	17
Y18	-0.361*	45	0.713**	45	0.365*	45
Y19	-0.239	32	0.880**	32	0.188	32
Y20	-0.08	27	0.935**	27	0.252	27
Y26	0.406	16	0.920**	16	0.691**	16
Y27	-0.100	17	0.885**	17	0.357	17
Y31	0.156	21	0.554**	21	0.901**	21
Y33	-0.538**	25	0.773**	25	0.100	25
Y35	-0.003	16	0.900**	16	0.417	16
Y36	-0.333	23	0.901**	23	0.075	23
Y37	-0.408	21	0.814**	21	0.189	21
Y40	-0.376	12	0.822**	12	0.208	12
Y41	0.356	6	0.813*	6	0.809*	6
Y42	-0.245	23	0.588**	23	0.593**	23
Y43	-0.446	10	0.666*	10	0.358	10

df = degrees of freedom; *, ** significant at the 0.05 and 0.01 levels of probability, respectively

Appendix 5. DArT marker loci not arranged on the genetic maps of synthetic-derived families of lines Y14 (figure 17) and family Y18 (figure 18), which highlighted primary synthetic wheat and Yitpi alleles that had significant (p<0.05) positive associations with both grain weight and grains per m^2. Chromosomes stated reflect either chromosome assignment from 16 reference maps or an inferred chromosome assignment through genotype matching.

Synthetic-derived families							
Y14				Y18			
Primary synthetic allele		Yitpi allele		Primary synthetic allele		Yitpi allele	
Chromosome	Marker	Chromosome	Marker	Chromosome	Marker	Chromosome	Marker
4B	wPt-5497, wPt-6123, wPt-6887, wPt-8856, wPt-8892	2B	wPt-3504	1B	wPt-3571	1A	wPt-0128, wPt-1792, wPt-1906, wPt-4408, wPt-4886, wPt-5660, wPt-8172, wPt-8569
5B	tPt-3714	6D	wPt-8729	2B	wPt-4002		
		7A	wPt-0494	3A	wPt-1688, wPt-2478		
		7B	wPt-6276	3D	wPt-5215		
		Unknown	wPt-0011, wPt-0059, wPt-0886,wPt-0914, wPt-1103,wPt-1349, wPt-1412,wPt-1440, wPt-1446, wPt-1958, wPt-2788, wPt-3041, wPt-4062, wPt-4151, wPt-6530,wPt-6797, wPt-6811, wPt-7498, wPt-7784, wPt-8301, wPt-8686, wPt-8838, wPt-9820, wPt-9892	7A	wPt-4877	2A	tPt-1041, wPt-4197, wPt-5647, wPt-6570
				Unknown	wPt-0568, wPt-0797, wPt-0836, wPt-2070, wPt-2178, wPt-6217, wPt-6711, wPt-8068, wPt-8155	2B	wPt-0094, wPt-3504, wPt-5044, wPt-7715
						3B	wPt-1804, wPt-1940, wPt-2491, wPt-3327, wPt-4608, wPt-5452, wPt-6020, wPt-7737, wPt-9432, wPt-9510
						6A	wPt-3262
						Unknown	wPt-0011,wPt-0115, wPt-0906, wPt-1709, wPt-2448, wPt-4091, wPt-6530, wPt-6654, wPt-7152, wPt-8915

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