Breeding Durum Wheat for South Australia

by

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For Jack

of Knaresborough

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Abstract

Durum wheat (*Triticum turgidum* L. var. *durum*) is a relatively new crop to South Australia, with commercial production commencing in 1991, based on the variety Yallaroi from New South Wales. Although the South Australian climate is characterized as Mediterranean, the environment for crop growing is highly variable. Early introductions were poorly adapted to this environment. Production is currently limited to the high rainfall zones of the state and for production to increase the crop needs to expand into more marginal areas. Much of the cereal belt in these areas is affected by high levels of soil boron (B), which has been identified as a major environmental factor limiting yield in hexaploid wheat (*T. aestivum*) and barley (*Hordeum vulgare*). Tolerance to B will therefore be a pre-requisite for improved adaptation of durum in South Australia. Success of the durum industry depends on continuity of supply, maintaining quality equivalent to premium grain from Canada, and improving quality attributes to have an advantage on the world market. This thesis examined: (i) genotype × environment (G×E) interaction to improve adaptation; (ii) breeding to increase B tolerance; and (iii) enhancing quality by improving dough strength.

Field studies showed a major factor influencing the grain yield of durum wheat in South Australia was seasonal rainfall. Durum wheat had a lower grain yield per unit of rainfall compared with the bread wheat variety Spear, which suggested durum had a lower water use efficiency. This is likely to be a reflection of edaphic variation (eg. intolerance to B toxicity and sodicity) in the root zone resulting in lower water and nutrient uptake, and therefore breeding should be for stress tolerance, not yield potential *per se*. G×E interaction accounted for most of the observed variation, and most was associated with the locations × years (L×Y) component, indicating that testing over a number of locations and years is necessary. Genotype × locations (G×L) and genotype × years (G×Y) components were relatively small, which suggests some consistency in the ranking of genotypes over locations and years, and a need for greater genetic diversity. Durum yielded poorly under unfavourable conditions. Yallaroi had the lowest overall rank for yield performance and was specifically adapted to

favourable environments, while the advanced breeder's line RH912025 had the highest rank and was well adapted to all environments. Yallaroi and RH912025 existed in two distinct and dissimilar genotypic domains on a Principal Component biplot, which will provide reference points from which stability and adaptation of new genotypes can be assessed in the future. Widely adapted lines are likely to be selected from within the domain of RH912025. Spatial Analysis classified four distinct groups of sites, one of which could be attributed to drought and another to B toxic locations.

To improve the B tolerance of Australian durum varieties, F_2-derived progeny from a cross with moderately B tolerant genotype AUS 14010 and sensitive Australian germplasm were selected at a high concentration of B, screened in a filter paper assay and evaluated under a range of B conditions in the field. The seedling root length of F_4 progeny showed significant differences in response when grown in toxic levels of B in a filter paper assay. Families with long roots in the filter paper bioassay had a grain yield advantage (11-19% over families with shorter roots) when grown under high B conditions (eg. when grain B concentrations > 3.0 mg kg^{-1}) in the field. This yield advantage did not occur at all locations, and other factors are likely to have contributed to yield differences among genotypes at these sites. In contrast to previous work with bread wheat, the concentration of B in whole shoots and in grain was not significantly correlated with root length at high B in the filter paper assay, or with grain yield at sites with a B effect. The concentrations of other elements in the grain were also determined, and these differed significantly among families. The concentrations of sodium (Na) indicated the levels for durum were generally higher than those reported for bread wheat, and above the value considered toxic for bread wheat.

To secure and maintain international markets, varieties with strong dough strength are necessary. To enhance quality of Australian durum, the association of alternative HMW glutenin subunits at the *Glu-1* loci (including *Glu-D1*) with physical dough properties and cooked pasta in a Yallaroi background was evaluated. The SDS-sedimentation (SDSS) test, which evaluates gluten strength, showed durum progeny lines had lower mean SDSS values

than the hexaploid controls. However, progeny lines with the substitution of chromosome 1D (with subunits 5+10) for chromosome 1A were associated with higher sedimentation volumes comparable to a hexaploid wheat with subunits 2+12. Significant differences occurred between the durum controls for mixograph rheological parameters mix time (MT), peak height (PR), time to peak bandwidth (TPBW) and peak bandwidth (PBW). In the progeny populations, subunits 2* or 13+16 increased MT, and the allele *Glu-A1V* produced higher PR. The introgression of desirable subunits 17+18 from hexaploid wheat into Yallaroi, unexpectedly, did not improve the quality parameters assessed. Cooked pasta firmness was highly significant and positively correlated with grain protein concentration and mixograph PR. No differences in firmness between the HMW glutenins occurred when protein concentration was used as a co-variate. Pasta resilience was independent of rheological parameters, but nevertheless varied with subunits 1, 2* or 13+16. Stickiness was negatively correlated to firmness, and influenced by protein concentration and subunit 2*.

In conclusion, the outcome of the yield evaluation trials conducted in this study was the identification of the high yielding and widely adapted line, RH912025, which was consequently released as the variety Tamaroi.` Development of B tolerant lines, with a grain yield advantage when grown under high B conditions in the field, means durum production will be able to expand into marginal areas where B toxicity occurs. Furthermore, by pyramiding genes for B tolerance and dough strength (ie. subunit 2*) into Tamaroi, the result should be widely grown germplasm, with premium quality for the international market, providing farmers with a financial reward.

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.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

SIGNED: ....

DATE: March 2004

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List of Abbreviations

ANOVA	analysis of variance
AWCC	Australian Winter Cereals Collection
AUS	AWWC accession number
BBD	bandwidth breakdown (of mixograph)
BC_xF_y	backcross (x times) filial generation y
B100	100 mg B L^{-1}
CIMMYT	Spanish acronym for International Maize and Wheat Improvement Center
CL%	cooking loss (of pasta)
CSIRO	Commonwealth Scientific & Industrial Research Organisation
G×E	genotype × environment
G×L	genotype × location(s)
G×Y	genotype × year(s)
HMW	high molecular weight
ICARDA	International Center for Agricultural Research in the Dry Areas
ICP	Inductively coupled plasma (for spectrometry)
LMW	low molecular weight
L×Y	locations × ycars
MT	mix time (of mixograph)
NDWIP	National Durum Wheat Improvement Program
NSW	New South Wales
OCT	optimum cooking time (of spaghetti)
PAGE	polyacrylamide gel electrophoresis
PBW	peak bandwidth (of mixograph)
PCA	principal component analysis
pers. comm.	personal communication
pers. obs.	personal observation
PR	peak resistance (of mixograph)

RBD	resistance breakdown (of mixograph)
RL	root length (mm)
SDS	sodium dodecyl sulphate
SDSS	SDS-sedimentation test
TPBW	time to peak bandwidth (of mixograph)

Chapter 1

Introduction

In the industrialized western world durum wheat (*Triticum turgidum* L. var. *durum*) is used predominantly for the manufacture of pasta, and to a minor extent, for baked raised breads in the southern Mediterranean. Alternatively, in areas of West Asia/North Africa, durum has traditionally been used in the production of single- and two-layered flat breads, burghul or bulgur, cous-cous and frekeh (Williams *et al.*, 1989). Although durum is ideally suited to the manufacture of pasta products (Cubadda, 1989), its inability to produce airy bread loaves has contributed to a lack of resources being invested into the research and breeding of this cereal crop.

With the advent of the Green Revolution, improved industrial technologies, changes in eating habits for a 'healthier' diet and new market opportunities, this ancient crop has seen a revival. There has been considerable growth in production but, the few resources applied to this crop in comparison to the inputs of research and breeding into bread wheat over the past 125 years, has left a large void in our knowledge of durum wheat. In retrospect, since these two cereal crops are closely related, the knowledge gained from bread wheat improvements can readily be applied to durum wheat.

The introgression of dwarfing genes into durum wheat during the Green Revolution produced yields higher than bread wheat under high input conditions (irrigation and fertiliser) (Breth, 1975; Brajcich *et al.*, 1983; Pfeiffer, 1996). However, world durum wheat productivity is low (1.7 t/ha), particularly in developing countries (1.2 t/ha) which account for 60% of the production area (Pfeiffer, 1996). Low productivity reflects semi-arid, marginal and low input growing conditions. Many constraints limit production in marginal areas eg. disease, insects, drought, salinity, and soil infertility, such that genetic progress in highly variable stressful environments is less efficient compared to that in high potential production conditions.

In Australia, durum wheat is a minor crop compared to bread wheat. Production was previously concentrated in northern New South Wales, where the average grain production per year was 175,000 t during the 1990s. In the past, South Australian farmers have been reluctant to grow durum, due to its reputation of having lower yields than bread wheat. However, the combination of demand from a local pasta manufacturer, modern varieties with increased yield potential, and a market price often higher than bread wheat, has made durum a promising and viable alternative crop. Large scale production of durum wheat in South Australia commenced in 1991 with the importation of the variety Yallaroi from New South Wales and currently 100,000 t is produced annually.

Durum breeding in Australia has been conducted by NSW Agriculture continuously since 1948, but only six varieties were released during the period 1948-1993. At the commencement of this study Yallaroi was the only variety recommended in South Australia. Yallaroi is resistant to cereal cyst nematode (*Heterodera avenae* Woll.), stem rust (*Puccinia graminis* f. sp. *tritici*), leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis*), but susceptible to black point (*Alternaria alternata*) and crown rot (*Fusarium* spp.). Little research has been conducted into growing durum in South Australia, and local growers and manufacturers have encountered some difficulties in production.

The areas of South Australia which have the greatest potential to grow durum wheat with high grain protein concentrations are mostly duplex and sodic soils (Rathjen *et al.*, 1996) and durum lines have been found to produce only half the grain yield of bread wheat in some field trials in these areas. In these soils, Yallaroi showed poor adaptation, in particular to boron (B) toxicity. For example, trial results from 1993 showed that the yield of Yallaroi was about 50-60% of Spear, a moderately B tolerant bread wheat, when grown at Two Wells, Rudall and Kimba (Rathjen and Brooks, 1994), areas where high concentration of B is a major yield limiting factor (Hollamby *et al.*, 1994). Therefore, there is an urgent need for B tolerant durum varieties to be developed for cultivation in these areas. Currently, most of the durum

is produced on the red-brown earths and related soils in high rainfall areas, where B toxicity is only a minor factor.

In the regions of southern Europe and the Americas, nearly all durum is used for pasta production and the surplus grain is exported. The premium price paid for good quality grain on the world market provides a financial incentive for farmers to grow the crop. Pasta producers dictate that grain with the characteristics required for processing into high quality goods is mandatory. Discrepancies in the quality of pasta produced by the same durum variety from the different production regions in Australia has been cause for concern in the local pasta industry. In southern Australia, cooler grain ripening conditions are associated with weaker gluten and dough strength. The traditional utilization of durum in pasta making has been associated with the uniqueness of the functional properties of its gluten. Gluten properties are influenced by the environment and by the genetic composition of the kernel. In the absence of genetic variation, the environment is the major factor contributing to dough strength. Breeders have the opportunity to manipulate protein composition of the grain in an attempt to improve durum dough quality.

This thesis examines the adaptation of durum wheat to South Australian growing conditions, and aims to identify or produce genotypes which overcome the initially recognized production constraints. First, the magnitude of genotype × environment (G×E) interactions for durum wheat was investigated (Chapter 3), and the response in durum was compared to bread wheat to identify appropriate levels of adaptation. A backcross population of durum was produced and progeny lines with moderate levels of tolerance were selected to assess B uptake and grain yield response when grown in the field (Chapter 4). Another backcross population was produced with variation for kernel storage proteins coding for HMW subunits associated with dough strength in bread wheat (Chapter 5). The genetic control of gluten strength, mixograph rheological and cooked pasta properties in durum wheat was investigated and compared with hexaploid wheat. The results of individual chapters are discussed separately while the overall results and conclusions are presented in a general discussion (Chapter 6).

Chapter 2

Review of the literature

2.1 Introduction

This chapter provides a brief description of durum wheat, and reviews the literature available on the objectives relevant to the improvement of the adaptation and quality of the crop in South Australia at the commencement of this study in 1993. The environment for crop growing in South Australia is variable and to release varieties which will be widely grown requires genotypes with broad climatic and edaphic adaptation to the state. Selection of lines which are broadly adapted is complicated by the occurrence of genotype × environment (G×E) interactions, where genotypes respond differently in different environmnents. In the current durum breeding program multi-environment trials are used to evaluate the performance of candidate varieties (genotypes) for a target population of environments. In this thesis, the evaluation of G×E interactions and their role in plant breeding is discussed.

Durum production in South Australia is currently limited to higher rainfall zones of the cereal-sheep zone and, to increase production, the crop needs to expand into more marginal areas. Much of the cereal belt in these areas is affected by high levels of soil boron (B) (Cartwright *et al.*, 1986) which is a major yield limiting factor (Cartwright *et al.*, 1984; Rathjen *et al.*, 1987). In these regions, the most widely grown varieties of almost all crop and pasture species are the most B tolerant available. Tolerance to B in bread wheat is under control of several major genes (Paull *et al.*, 1992a; Chantachume, 1995), and has been transferred into senstive varieties by deliberate backcrossing, culminating in the release of BT-Schomburgk (Rathjen *et al.*, 1993). The literature available attaining to B toxicity, and the feasibility of incorporating tolerance into Australian durum wheat is discussed.

The variable environment also influences the quality of the grain for end use, and precautions must be taken to buffer its effects to supply the market with consistent quality. Durum is ideally suited to pasta making, for which grain is milled to semolina, and a dough extruded and dried for pasta. This requires the growth of a durum with strong gluten for dough strength, so that pasta shapes retain their form after extrusion and cooking losses are minimized. Gluten properties are influenced by genetic composition of the variety and by the temperature during grain fill (Schipper *et al.*, 1986; Pogna *et al.*, 1988; Randall and Moss, 1990). This review will also examine the opportunities to manipulate protein composition of the kernel for improving durum dough quality.

2.2 Durum wheat

2.2.1 Classification

All wheats belong to the genus *Triticum* of the Family Gramineae. Botanically, cultivated wheats are classified into three groups according to their ploidy level. Durum wheat (*Triticum turgidum* L. var. *durum*) is an allotetraploid with genomes AABB [2n = 4x = 28]. The wild ancestor of durum, *T. dicoccoides* (Korn.) Thell., is derived from a hybrid between diploid *T. urartu* Thum. (comprising the A genome) (Dvorak, 1976; Chapman *et al.*, 1976; Dvorak *et al.*, 1993) and the diploid *Aegilops speltoides* (Tausch) Gren. or a closely related species (contributing the B genome) (Sarkar and Stebbins, 1956; Riley *et al.*, 1958; Dvorak *et al.*, 1989; Dvorak and Zhang, 1990; Feldmen *et al.*, 1995). Common or bread wheat (*T. aestivum* L. var. *aestivum*) is an allohexaploid, comprised of the A and B genomes from tetraploid wheat and the genome of *Ae. tauschii* L. (syn. *T. tauschii*, *Ae. squarossa*) which is designated as the D genome (Kihara, 1944; McFadden and Sears, 1946).

At the tetraploid level, two main species have been recognized, *T. timopheevi* (2n = 4x = 28, genome AAGG) and *T. turgidum* (genome AABB). *T. timopheevi* consists of three varieties, *araraticum*, *timopheevi* and *militinae*. Within *T. turgidum*, seven varietal groups are

recognized, including, *dicoccoides* (wild emmer), *dicoccum* (cultivated emmer), *durum, turgidum, polonicum, carthlicum* (syn. *persicum*) and *turanicum* (Feldman *et al.*, 1995). Durum wheat (*Triticum turgidum* L. var. *durum*) is the main modern tetraploid type cultivated. The remaining *T. turgidum* varieties constitute a rich gene resource of agronomically-important characters exploitable for durum wheat improvement (Feldman and Millet, 1993).

Emmer wheat is an east Mediterranean element, endemic to the 'Fertile Cresent' region of southwest Asia. It is locally abundant in the southwestern part of its distribution area (Palestine, Jordan, Syria and Lebanon) and less frequent in the northeastern part of its distribution area (southeastern Turkey, northen Iraq and southwestern Iran). The environmental conditions in these regions are similar to South Australia and the tetraploids which grow there are most likely to offer sources for improved adaptation.

2.2.2 Production and distribution

Durum wheat is produced on approximately 17 million hectares worldwide (Pfeiffer, 1996), and accounts for about 5.8% of the world's total wheat production (Clarke *et al.*, 1996). In the decade 1984-1993, total world durum production fluctuated between 20 and 34 million tonnes (International Wheat Council, 1994). The low world average yield (1.7 t/ha) of durum wheat reflects the tendency for a large proportion of the crop to be grown under semi-arid or low management inputs (Srivastava *et al.*, 1988; D. Leisle, pers. comm.). In marginal areas many constraints limit production, eg. disease, insects, drought, salinity, and soil infertility, such that genetic improvements (breeding to overcome each constraint) in adaptation to these highly variable stressful environments is less efficient compared to that in high potential production conditions with few constraints. Using the knowledge gained from other cereals grown in these regions, particularly bread wheat, will help improve the yields of the durum crop.

Over half of the world's durum wheat is cultivated in the Mediterranean Basin, from southern Europe through West Asia to northern Africa. Tetraploid wheats were domesticated in this region around 15,000 to 10,000 BC (Bozzini, 1988; Srivastava *et al.*, 1988). The Americas (Canada, North Dakota, Arizona, California, Mexico and Argentina) is the other major region of production.

Durum production in Australia has expectedly increased rapidly, consistently rising in the last 20 years from 7,800 t in 1977 to nearly 650,000 t in 1999. Production commenced in South Australia in 1991 with 500 t, which rose to 54,000 t in 1996, and 100,000 t in 1999. The grain is destined for the manufacture of pasta, for which minimum quality specifications are required, and segregated into appropriate classes. The presence of a semolina mill and pasta manufacturer in the state ensures a consistent local annual demand for high protein grain (>11.5%) with strong gluten. Additionally, grain with protein concentrations greater than 13% is competitively sought by Italian pasta manufacturers.

2.2.3 Fundamental features

Durum wheat was developed as an economic crop in the Mediterranean basin in strict competition with hexaploid wheat (Bozzini, 1970). General agroclimatic conditions in this region include continental areas with low winter temperature, covering approximately 55% of the area sown with durum, while temperate areas with mild winters cover about 35%, mainly in the coastal and southern latitudes of North Africa. Both zones experience dry, hot summers, sometimes together with hot winds (sirocco). Rainfall is winter dominant, and the growing period extends for approximately 8 months (from November to June). Drought may occur alone or in combination with extreme thermal stress at various stages of crop development. Originally the soils were favourable for agricultural production, but now after two millennia of cropping, the soils are often infertile, with low organic carbon contents. Under these conditions durum wheat landrace varieties were more productive than local bread wheats and this provides one reason for the survival of tetraploid wheats (Bozzini, 1970).

Additionally, the cultural preference of durum wheat in developing countries for traditional consumption of local food products (cous-cous, burghul, frekeh, single- and two-layered flat breads) and raised bread in Italy, and as an industrial crop for pasta products in developed countries, will continue its demand in the future (Brajcich *et al.*, 1983).

2.3 Genotype × environment interaction

2.3.1 The environment of the South Australian wheat belt

South Australia is characterized by a Mediterranean climate with wet, cool but not severe winters (Adelaide mean July maximum 15.1°C) and warm, dry summers (Adelaide mean January maximum 28.7°C). This differs to the climate in the Mediterranean Basin where extreme thermal stresses (cold and heat) occur more often. The predominant crop in South Australia is bread wheat and the major areas of production are the Mid-north, the upper Yorke Peninsula, the Eyre Peninsula and the Murray Mallee. The dry margin for the wheat belt receives approximately 300 mm average annual rainfall, while the wetter boundary is 500-550 mm. About 80% of precipitation falls during the crop growing season, April to November. Year to year fluctuations in rainfall are quite substantial and largely influence dry matter production. Production is based on a late autumn - early winter sowing of spring wheats, and harvest in early summer under warm, dry conditions. Yield potential is often constrained by poor root growth throughout the profile reducing access to available soil water.

Despite South Australia having a Mediterranean climate, the environment is distinct in that different edaphic constraints to production have been recognised and overcoming these constraints is now included as breeding objectives. Soil types across the wheat belt are variable. In the western and eastern regions the soils are a sandy surface with a clay or clay-loam B horizon, and in the central and much of the south-eastern region there is a predominance of fertile clay loams. Soils are often low in organic matter, nitrate, sulphur and in their original condition, deficient in phosphorus and some trace elements (manganese, zinc,

copper, molybdenum), with an excess of boron and sodium, and pH in the B horizon greater than 9.0.

The major disease and pest problems encountered in the state are cereal cyst nematode (CCN) (*Heterodera avenae*), root lesion nematode (*Pratylenchus neglectus*, *P. thornei*), crown rot (*Fusarium* spp.), black point (*Alternaria* spp.) stripe rust (*Puccinia striiformis*), stem rust (*Puccinia graminis tritici*), leaf rust (*Puccinia triticina*), septoria leaf blotch (*Septoria tritici*), yellow leaf spot (*Pyrenophora tritici-repentis*) take-all or haydie (*Gaeumannomyces graminis*), rhizoctonia (*Rhizoctonia solani*), common root rot (*Bipolaris sorokiniana*) and barley yellow dwarf virus (BYDV).

Contrary to the historic scenario in the Mediterranean basin, in South Australia bread wheat is more productive than durum (Rathjen and Brooks, 1994) despite the similar climatic patterns of the two regions. A similar observation has been made between the predominance of barley in the drier areas of West Asia (van Oosterom and Acevedo, 1992) and comparative lack of adaptation of barley to bread wheat in South Australia (Jefferies *et al.*, 1999b). This has led to the need to investigate the importance of the interaction between genotypes and environmental constraints to production in South Australia in an effort to identify and select superior yielding varieties.

2.3.2 Effect of genotype × environment interaction on grain yield in plant breeding

The wheat growing environment of South Australia is variable and unpredictable due to variation in climate, soils and farming practices (Hollamby, 1996). Plant breeders widely use multi-environment trials to evaluate the relative performance of genotypes for a target population of environments. The process of sampling environments is generally associated with testing the genotypes at a number of sites for several years. Therefore, environments are commonly defined as particular location × year combinations. Genotype × environment (G×E) interactions occur when genotypes respond differently in different environments. Selection of

genotypes with superior performance for broad adaptation, based on genotype mean yield over all environments, is complicated by the incidence of G×E interactions. The nature and causes of G×E need to be understood to utilize and exploit them in selection for specific adaptation (Finlay and Wilkinson, 1963; Rathjen, 1994; Rathjen *et al.*, 1999). Analysis of data from multi-environment trials shows that G×E interactions are ubiquitous, and these are often large compared with genotype main effects (DeLacy *et al.*, 1996). Since durum wheat is only a relatively new crop to South Australia, there is a need to examine the response of varieties and advanced lines in the environments of current and proposed production areas to define the limitations to adapatation in these tetraploids.

Plant breeding programs improve quantitative characters by selecting among genotypes, based on their phenotypic performance. The phenotype is affected by genetic (genotypic) and non-genetic (environmental) influences (Comstock and Moll, 1963), and selection only exploits the genetic components of phenotypic variability. When a phenotypic effect for a gene under selection pressure is observed repeatably, achieving a response to selection is relatively simple. However, variation for quantitative characters is often under the control of many genes and the contribution of these can often differ between environments (DeLacy *et al.*, 1996). This differing contribution of genes is the biological basis of G×E interaction, and to detect these by statistical tests is the objective of much experimental work (Rathjen, 1994).

Variation between genotypes associated with G×E interactions complicates selection, as it introduces a degree of uncertainty into the measurement of superiority of any given genotype. This uncertainty increases with the magnitude and complexity of the responses contributing to G×E interaction variation compared to the main effects. If G×E interactions are present, their effects must be taken into account when selecting for performance within a target population of environments. The expectation is that breeding methodologies which identify and reduce this uncertainty should result in more rapid genetic improvement of the crop (DeLacy *et al.*, 1996). Plant breeders need to increase their understanding of the nature and causes of interactions to utilise and exploit genetic variation effectively through breeding programs.

There are three ways of dealing with G×E interaction in a breeding program: ignore, avoid or exploit G×E effects in breeding objectives (Eisemann *et al.*, 1990). The first approach is unacceptable when G×E interactions are a significant feature of genotypic performance. The second involves the characterization of interactions and the design of breeding strategies and selection procedures to minimize impact on the products of the breeding program by subdividing the target area into coherent subregions (Romagosa and Fox, 1993). One approach to this is to identify similar environments via cluster analysis (Fox *et al.*, 1990). Such an approach may be useful to exploit specific adaptation. However, if wide adaptation is sought, clustering would not be a viable approach. To exploit G×E effects necessitates a much more detailed analysis and interpretation of biophysical factors (eg. water, nutrition, temperature, disease) to identify genotypic and environmental differences that trigger interactive behaviour, enabling a directed breeding strategy. This is the local approach, demonstrated by the developing of boron tolerant bread wheat varieties (Paull *et al.*, 1992a; Moody *et al.*, 1993). Where the variation attributed to G×E interactions is large relative to that for genotypic effects, a combination of avoidance and exploitation may be the appropriate compromise in many circumstances, with the decision strongly influenced by the repeatability of the causes of G×E interactions (Cooper *et al.*, 1993). Where the measure of repeatability is low, it provides an indication of the merit of selection for broad adaptation, and if repeatability is high for specific adaptation.

Plant breeders use field trials for multi-environment evaluation of genetic material to simultaneously quantify similarities and differences in the adaptive reactions of genotypes, to identify the environments in which these occur, and to determine, if possible, the basic genetic and environmental causes. This requires a systematic analysis of the biological basis of G×E interactions to predict appropriate combinations of genes. There has also been an emerging concept of better defining the E in G×E to accomodate repeatable G×E interactions which can be exploited by selection, ie. breeding for specific adaptation (Eisemann *et al.*, 1990). This involves defining the environmental limitations that are contributing to the

interactions, and addressing the issue of genetic improvement to overcome the constraint to production. For the last three decades in South Australia researchers have successfully defined numerous causes of G×E interactions in wheat breeding (Rathjen, 1994; Rathjen *et al.*, 1999). These include leaf diseases, especially Septoria; root pests, eg. cereal cyst nematode and root lesion nematodes; root diseases, eg. crown rot; soil toxicities, eg. boron, sodicity and high pH; and soil deficiencies, eg. manganese, zinc and copper. This allows the breeder to focus on searching for relevant genetic variation for resistance/tolerance to the defined constraints. The consequence is development of improved genotypes with increased understanding of the causes of superiority.

2.3.3 Methodology for the analysis of data from multi-environment trials

There are a number of methodologies for analysis of data collected from multi-environment trials. The effectiveness of these different methods depends on how they depict the G×E interaction. Methods include analysis of variance (ANOVA), stability analysis, including joint linear regression (Yates and Cochran, 1938; Finlay and Wilkinson, 1963), ordination including Principal Component Analysis (PCA) (Eisemann, 1981; Kempton, 1984), clustering (Abou-El-Fittouh *et al.*, 1969), pattern analysis (Mungomery *et al.*, 1974), spatial analysis (Gilmour *et al.*, 1997) and the Ward-Modified Location Model (Ward-MLM) (Franco and Crossa, 2002).

Yield trials frequently have both significant main effects and a significant G×E interaction. The customary statistical analyses applied to yield trials have inadequacies in effectively treating a complex data structure. The ANOVA has been used extensively for the analysis of data from multi-environment trials. It is an additive model, which identifies the G×E interaction as a source of variation, but does not analyse it. Partitioning the component of variation attributable to G×E interactions into sub-components is useful if some sub-components account for substantial proportions of the total interaction and may be indicative of their cause. A common partition is based on the cross classification of years and locations,

enabling the detection of genotype × location (G×L), genotype × year (G×Y) and genotype × location × year (G×L×Y) interactions (Comstock and Moll, 1963; Nyquist, 1991). This partition reflects the way that environments are sampled, and therefore needs to be an adequate, representative subsample of the target population of environments in multi-environment trials. Frequently the sample is too small and biased.

Linear regression analysis is only able to effectively analyse interaction terms where the pattern fits a specific regression model. Finlay and Wilkinson's (1963) technique has also been criticised, due to the juxtaposition of ecologically different environments with similar mean yields on the x-axis which can mask linear relationships for individual factors (Knight, 1970). PCA is a multiplicative model, where ordination is used to represent the essential variation from a two-way G×E matrix in a few dimensions (Eisemann, 1981; Kempton, 1984), which however, contains no sources for additive genotype or environment main effects. Spatial analysis has been used to account for local trend (adjoining plots and across the whole trial) in either complete block or incomplete block designs to provide more accurate and precise estimates of genotype effects (Lill *et al.*, 1988; Gilmour *et al.*, 1997). Of particular importance is the accomodation of unbalanced data. Pattern analysis, the joint use of classification and ordination methods, has been recommended for the description of G×E interactions and to explore relationships intrinsic to the data studied, eg. for soybean (Mungomery *et al.*, 1974), wheat (Byth *et al.*, 1976) and triticale (Fox *et al.*, 1990). However, any prediction requires careful consideration of both the adequacy of the sample and the populations of both genotypes and environments for which recommendations are made. The Ward-MLM is a two-stage sequential clustering strategy using all variables, continuous and categorical, and it tends to form more homogeneous groups of genotypes than other clustering strategies (Crossa and Franco, 2004). The sequential clustering strategy can be applied to three-way data comprising genotypes × environments × traits.

The ideal analysis is the integration of methodologies which enables compensation for each of their inadequacies. As no ideal model is forthcoming, and since the developed models still

attract criticism, the solution is to use the most suitable model and complement this by applying alternative model(s) to compensate for weaknesses. This study will utilize ANOVA as the principal method of analysis, with complementary use of regression, PCA and Spatial Analysis to examine G×E interactions encountered by durum wheat grown in South Australia.

2.3.4 The case for wide adaptation

Wide adaptation is considered a primary objective by many national and international breeding programs. Most of the evidence identifying wide adaptation comes from wheat and rice breeding where the use of photoperiod and vernalization insensitive genes allow the cultivation of individual genotypes over a wide geographical area. In this sense it is a demonstration of wide adoption rather than adaptation. In relation to the insensitivity genes, the term 'wide adaptation' has been used in a geographical, rather than in an environmental sense, whereas the concept underlying wide adaptation is high yield even in marginal conditions. The proposition that high yield potential CIMMYT germplasm incorporated into Australian programs has increased yields has been expounded by Brennan (1986). In fact, the adoption of 'widely' adapted CIMMYT-derived materials in the marginal environments of Australia has been negligible. The major contribution of CIMMYT germplasm to increasing grain yield has been through dwarfing genes increasing harvest index (althougth this may be combined with a pleiotropic effect on the number of grains per spikelet), rather than improved adaptation. It is argued here that accumulation of tolerance/resistance to the climatic and edaphic stresses is the key to wide adaptation.

2.3.5 Handling G×E in the bread wheat breeding programs in South Australia

Yield is the prime breeding objective of most Australian breeding programs (Hollamby *et al.*, 1983; Rathjen and Pederson, 1986). In general, Australian farmers will not grow a new bread wheat variety unless it is higher yielding on their farms despite other improvements. Because of large significant interactions between genotype, location and year in South Australia

(Hollamby, 1973) selection must be carried out across several sites and seasons. Reference varieties to represent the tolerable limits of maturity, quality characters, and height are included in trials with varieties currently recommended for sowing (Hollamby *et al.*, 1983).

Hollamby *et al.* (1983) believe trial sites need to be chosen for their geographical location, to represent soil and climatic variation, and for their farming practice so that different farming systems, such as fallow, conventional ley and minimum tillage systems, are involved. Ideally, the selected locations will be representative of the whole cereal zone (Rathjen and Pederson, 1986). This wide spectrum of trial sites is essential to cover the effects of G×E interaction existent in South Australia. For the same reasons, including more sites is preferred to increased replication at a site (Hollamby *et al.*, 1983). A successful selection program relies on the maximization of the correlation between the yields of the lines in plots and their yield in commercial conditions (Rathjen and Pederson, 1986). This correlation tends to be higher when the lines are grown in commercial conditions, than when grown in specialized circumstances.

The routine Roseworthy Campus technique is a pedigree method modified to overcome the criticisms of the classical method (Hollamby *et al.*, 1983, 1994). Lines that yield in the top 20% over all sites are retained and to these are added those lines which were in the top 20% at any one site. This method retains specific as well as widely adapted lines. Complex statistical procedures are not necessary. A more intense selection pressure in a single year does not allow for G×Y effects (Hollamby *et al.*, 1983).

A problem arises as to how to handle specifically adapted lines. Are they specifically adapted to the location or to the L×Y interaction? Selection under L×Y interaction is likely to result in lines that are more sensitive to year to year variation in the environment at a location. Varieties which consistently rank highly for yield are more likely to be specifically adapted to the location. Subsequently, identifying the contributing factor to adapation can be sought.

In the Waite Campus program, the aim is to identify the biological factors affecting yield, eg. CCN tolerance, resistance to *Septoria tritici*, B tolerance and more recently tolerance to *Pratylenchus neglectus*, trace element efficiency and tolerance to sodicity. The biological explanations for important G×E interactions in South Australia have their origins in the performance of contrasting genotypes under field conditions, ie. the role of boron tolerance came from the contrasting behaviour of Oxley and Halberd. It has proven to be a more productive approach of applying selection pressures than based on statistical methods alone. Low selection intensities are applied, retaining 20% of the population from one generation to the next (Rathjen *et al.*, 1999). The progeny method of breeding is used and in fact can assist in identifying the biological factors. Then more rapid and genetically precise methods of backcrossing can be used to incorporate resistance/tolerance genes once the G×E factor has been biologically identified and relevant genetic systems understood. Using this method means that once a new biological factor affecting bread wheat yields is found, the findings can be readily applied to durum wheat. This concept is being applied in this study by incorporating a gene conferring B tolerance into durum.

2.4 Boron toxicity

2.4.1 Introduction

Widespread areas of the wheat growing environment of South Australia have high concentrations of boron (B) in the soil. The adverse effect of B toxicity on grain yield in South Australia was first recognized in barley in 1983 (Cartwright *et al.*, 1984). Subsequent studies have highlighted substantial genetic variation in response to B toxicity in important economic crop and pasture species, including bread wheat, barley, field peas and annual pasture medics (Paull *et al.*, 1988a; Bagheri *et al.*, 1992; Paull *et al.*, 1992a). As soil amelioration is not feasible, the use of tolerant varieties is the best option to overcome the problem of B toxicity (Rathjen *et al.*, 1987). Since the mid-1980's, the incorporation of a higher level of B tolerance into otherwise adapted Australian varieties has been a major

objective of the breeding programs in southern Australia. This has lead to the release of the bread wheats BT-Schomburgk (Rathjen *et al.*, 1993), Barunga and Frame. This section examines if tolerance to B toxicity can be incorporated in durum wheat in an attempt to increase yields when grown at high B sites.

2.4.2 B in the soil

The total B in soils originates from the soil minerals, and varies according to the B content of parental rock materials. Sedimentary rocks of marine origin have a higher amount of total B than igneous rocks (Norrish, 1975). High B levels are found in soils derived from marine shales, loess and alluvium, with all being essentially fine-textured deposits (Fleming, 1980). The geological history of South Australia has resulted in marine sediments being widespread, and therefore, naturally high concentrations of B occur in the soil. Once B is released from soil minerals, due to its non-ionic nature, it can be readily leached (Gupta, 1979) and deficiency commonly occurs in plants. Conversely, where there is limited leaching from the profile, B accumulates in the lower soil horizon.

2.4.3 B toxicity in the soils of South Australia

The symptoms of black spotting of the tips and margins of barley leaves, common in South Australia (Cartwright *et al.*, 1984), were confirmed as being caused by high concentrations of B in soils (Cartwright *et al.*, 1986: Rathjen *et al.*, 1987; Paull, 1990; Holloway and Alston, 1992). In semi-arid areas, such as southern Australia, B accumulates naturally. The concentration of B in affected soils increases with depth and reaches a maximum usually 30-100 cm from the soil surface (Cartwright *et al.*, 1986). Many soil types in the cereal belt contain concentrations of extractable B greater than 20 mg kg^{-1}, and up to 100 mg kg^{-1} in the subsoils.

Soils with high concentrations of B are generally sodic and alkaline, and these include a number of soil types such as calcareous earths, calcareous sands, grey clays and red-brown earths often with some level of salinity (Cartwright *et al.*, 1986). In sodic soils, poor physical conditions and correspondingly poor soil aeration are major constraints for crop production (Marschner, 1986), and these may correlate with Na and B toxicity associated with accumulation due to limited leaching.

A significant yield reduction of 17% due to high levels of B in the soil and plants has been reported for barley (Cartwright *et al.*, 1984), and the widespread distribution in southern Australia (Plate 2.1) of high levels of B in plant samples (Cartwright *et al.*, 1986) indicates that B toxicity is a major factor affecting yield. The extensive areas of B toxic soils, low annual rainfall and high irrigation costs preclude amelioration of high levels of soil B by leaching. Therefore, the identification and breeding of varieties tolerant to high concentrations of B offers the best practical solution for limiting loss of production.

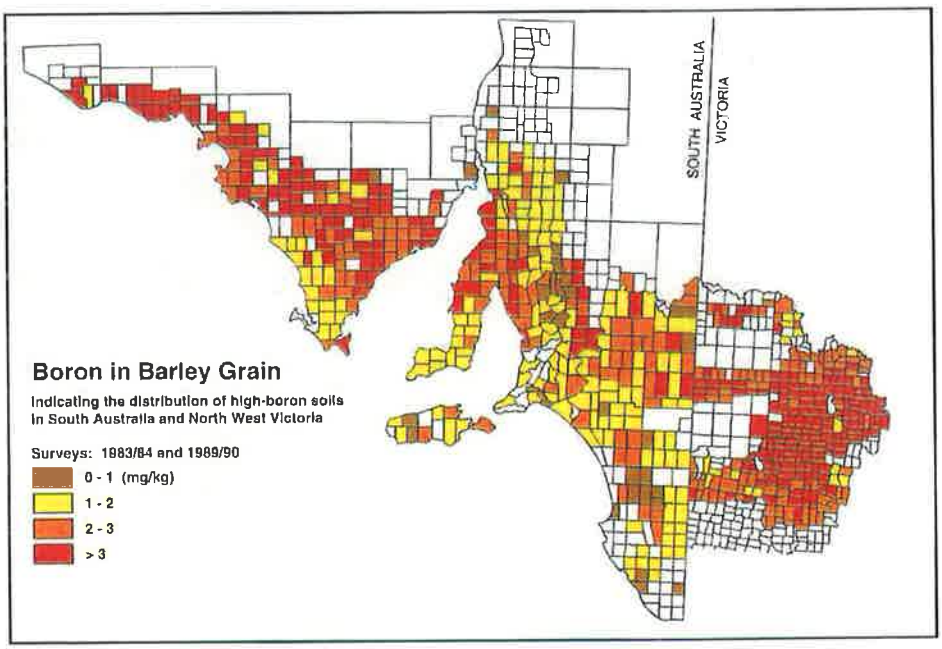


Plate 2.1. Boron concentration in grain of barley (surveyed 1983/84 and 1989/90) indicating the distribution of high B soils in South Australia and North West Victoria.

2.4.4 Physiological effects of B toxicity

Boron is transported in the transpiration stream and accumulates at the site of evapo-transpiration (Oertli and Kohl, 1961). Symptoms of B toxicity are tissue chlorosis and necrosis progressing from the leaf tip, or margin, to the base of leaves; symptoms are most severe in oldest leaves. In some plant species, such as barley and medic, distinctive brown lesions form within the chlorotic tissue. Genetic variation exists for symptom expression in barley, bread wheat and durum wheat (Nable, 1988; Paull *et al.*, 1990, 1991; Yau *et al.*, 1994; Jamjod, 1996). Other deleterious effects of B on the growth of barley, bread wheat and durum plants include reduced vigour, which is reflected in reduced height of plants, reduced root growth and delayed development (Paull *et al.*, 1990; Chantachume, 1995; Jamjod, 1996; Yau and Saxena, 1997; Yau, 2002). The delay in development is most pronounced with respect to tillering, rather than development of the primary culm.

2.4.5 Physiological control of B tolerance

The mechanism of B tolerance in bread wheat as well as in barley, peas and medics is a reduced accumulation of B in both roots and shoots (Cartwright *et al.*, 1987; Rathjen *et al.*, 1987; Paull *et al.*, 1988a; Nable, 1988; Paull *et al.*, 1992b). Results from barley genotype uptake kinetics (Nable *et al.*, 1990a) suggest that the control of B uptake is a non-metabolic process. Huang and Graham (1990) found that, in bread wheat genotypes, tolerance to B appears to be regulated at the level of the cell wall or cell membrane, rather than being a function of root structure or mediated by the shoots.

Tolerance to high levels of B for bread wheat is associated with reduced accumulation of B in plant tissue and in grain (Cartwright *et al.*, 1987; Paull *et al.*, 1988a). The concentration of B in grain of high yielding lines in a field experiment was significantly less than in the lower yielding lines (Cartwright *et al.*, 1987). Seven contrasting lines were subsequently selected and studied at a range of B treatments in a pot experiment by Paull *et al.* (1988a). Both the dry

matter production and grain yield of lines with the lowest grain concentrations of B in the field experiment were those least affected by high levels of soil B, and these also contained the lowest concentration of B in shoots. Other studies have also shown tolerant genotypes have a lower concentration of B in the shoots (Moody *et al.*, 1988; Paull *et al.*, 1988b). Furthermore, grain yield was correlated with the concentration of B in the vegetative tissues and grain (Rathjen *et al.*, 1987).

Evidence for the mechanism of B tolerance in durum wheat is controversial. Yau and co-workers (1994, 1997a, 1997b) classified durum accessions with the least severe symptoms as tolerant, although the symptom scores were higher than Halberd (a tolerant bread wheat check). As the tolerant durums had higher shoot B concentrations than Halberd, they suggested durum may be more tolerant to higher shoot B concentrations than bread wheat, which conflicts with the findings of an exclusion mechanism controlling tolerance to B in other crop species. Foliar symptom score was, however, not correlated with shoot B concentrations (Yau *et al.*, 1995), and may therefore not be a suitable selection criteria. Nevertheless, Jamjod (1996) found tolerant durum genotypes displayed less severe leaf symptoms of B toxicity and lower B concentrations in shoots than sensitive durum genotypes. When grown in soil with high B, the tolerant durums had grain yields comparable to Halberd (Yau *et al.*, 1997a). However, this is more likely to be a function of late maturity in the durums than tolerance *per se*, since yield data for the treatment with no added B were not presented. Yau *et al.* (1997a) believe durum utilizes a different mechanism of B tolerance to bread wheat, whereby durum localises B in leaf tips, rather than by restricting uptake. However, they failed to observe from the literature that all species translocate B in leaf tissue, resulting in chlorosis. In a subsequent study in which sensitive durums were included and relative yields available, durums previously classified as tolerant were less tolerant to high B than Halberd (Yau *et al.*, 1997b). Further analysis of the data they presented showed there was a negative relationship between relative grain yield and shoot B concentration ($r=-0.658$, $n=9$; $P<0.05$), which suggested that the mechanism of tolerance is reduced accumulation of B in shoots. This is consistent with the findings of Jamjod (1996).

2.4.6 B tolerance in Australian wheats

There is a wide range in response to B among Australian bread wheat varieties released during the twentieth century (Moody *et al.*, 1988). Distinct differences also occur in the response of varieties selected in different regions. Those from northern New South Wales and Queensland, and most of those from Western Australia were moderately intolerant, with a response similar to Condor. Varieties which have dominated wheat production in South Australia and Victoria were descendants of Federation (released in 1901) and Currawa (1912), both of which are classified as moderately tolerant to B (Paull *et al.*, 1986). Halberd (1969), a moderately tolerant descendant (Moody *et al.*, 1988), accounted for more than 70% of the total delivery of wheat to silos in areas of Eyre Peninsula and the Murray Mallee in the 1970s and early 1980s (Rathjen and Pederson, 1986), the regions of South Australia where high concentrations of B have been detected in barley grain (Cartwright and Hirsch, 1986). The dominance of tolerant varieties in specific regions indicates tolerance is an important feature in the adaptation of cereal varieties to southern Australia.

In constrast to Australian bread wheats, there is a narrow range of tolerance to B among Australian durum wheat genotypes. Durum varieties and advanced lines selected by the National Durum Wheat Improvement Program (NDWIP), based in Tamworth, NSW, were classified as sensitive to very sensitive to B when grown at a high B site in South Australia (Brooks, 1991). These findings were confirmed by Jamjod (1996) in laboratory and glasshouse analyses. The lack of tolerance to B in Australian durum varieties compared to that in other crops is likely to arise from two factors. First, these durum genotypes were developed in northern NSW, where B toxicity has not been reported. Second, this material has a narrow genetic base due to the high proportion of one variety, Kamilaroi, being used as a parent. Therefore, there is an urgent need to improve the B tolerance in durum for growing in areas of South Australia where high B is a major yield limiting factor. To increase the level

of B tolerance in South Australian durum wheat varieties, a suitable range in genetic variation needed to be identified from exotic sources.

2.4.7 Genetic variation in tolerance to B

To breed crops for adaptation to an environmental stress, it is necessary to identify the range for variation of response. This requires a screening method which is simple, rapid, reliable, economic and able to reflect the crop situation to provide breeders with a tool to select plants of the desired genotype.

Screening method

Response to B can be screened by several methods. At Waite Institute, the methods used are based on the correlation between response under controlled conditions and B accumulation in the grain under field conditions (Cartwright *et al.*, 1984; Paull, 1990). Two techniques have been adopted, namely growing plants in a high B soil in a glasshouse or growing seedlings in filter papers soaked in a high B solution. In the glasshouse screening, seedlings are sown in a large box containing soil with B added up to a concentration of 100-150 mg kg^{-1}. Plants are rated for tolerance to B by comparing their growth to that of the control genotypes at 4 to 6 weeks after sowing with respect to vigour, leaf symptom expression and tiller development (Moody *et al.*, 1988). Chantachume *et al.* (1995) developed a filter paper method to assess B tolerance in bread wheat. Seedlings were sown in a sheet of filter paper soaked with B solution and root growth was measured after 12 days. Tolerant genotypes produced significantly longer roots than sensitive genotypes and the rankings were consistent with those obtained by Moody *et al.* (1988) in high B soil.

Chantachume (1995) found the filter paper method to be more appropriate than soil screening to discriminate between the more tolerant hexaploid wheat genotypes. Jamjod (1996) reported the response of durum to high concentrations of B was independent of seed size, and

both root and shoot growth showed positive correlations with relative growth. She concluded that root length in the filter paper bioassay with a high B treatment was appropriate for discriminating between tolerant and sensitive durum genotypes. In contrast to the correlation coefficient reported in bread wheat (r=0.78**) by Chantachume *et al.* (1995), Jamjod (1996) found for durum wheat a relatively low correlation (r=-0.56*) between root length of seedlings grown at high B on filter paper and expression of leaf toxicity symptoms in seedlings grown in high B soil.

Extent of genetic variation

A large range in genetic variation for response to B has been reported in a number of crops, including bread wheat (Moody *et al.*, 1988), barley (Nable, 1988), peas (Bagheri *et al.*, 1992) and annual medics (Paull *et al.*, 1992a). In bread wheat the range in response to high concentrations of B is rated from the very sensitive, eg. Kenya Farmer, to tolerant, eg. G61450. South Australian bread wheat varieties are in the range moderately sensitive, eg. Schomburgk, to moderately tolerant, eg. Halberd. The deliberate transfer of tolerance from Halberd or Dagger into sensitive but otherwise well-adapted recurrent parents has seen the release of moderately tolerant varieties such as BT-Schomburgk, Barunga and Frame.

In contrast, Brooks (1991) found small, but significant, variation among commercial and landrace durum genotypes. The durum genotypes were classified as sensitive to very sensitive to B. Yau *et al.* (1995) also reported a narrow range of response in durum. Examination of a large collection of durums by Jamjod (1996) revealed limited genotypic variation among the varieties of durum from Australia, the Mediterranean Basin and other modern breeding programs. However, she identified landrace accessions from West Asia and China which were moderately tolerant or tolerant to B. The existance in durum wheat of genetic variation for response to high concentrations of B provides a means to incorporate tolerance into otherwise adapted varieties. An understanding of the genetic control of tolerance facilitates the breeding methodology for incorporation of this tolerance into local genotypes.

2.4.8 Genetic control of B

The genetic control of tolerance to B has been extensively studied for a number of crops. Generally, response to B was found to be controlled by several major genes, interacting additively, in durum wheat (Jamjod, 1996), bread wheat (Paull *et al.*, 1992a; Chantachume, 1995), barley (Jenkin, 1993) and peas (Bagheri *et al.*, 1992). Inheritance of B tolerance in durum wheat is under the control of three partially dominant major genes, namely *Bo$_T$1* and *Bo$_T$2*, which have been located on chromosome 7B, and *Bo$_T$3* (Jamjod, 1996). B tolerance in bread wheat is controlled by a series of partially dominant major genes (at least four) (Paull, 1990; Paull *et al.*, 1991; Paull *et al.*, 1992a), two of which have been located on chromosomes 4A (Paull *et al.*, 1988b, 1991) and 7B (Chantachume *et al.*, 1993). Chromosome 7D has also been implicated as another probable location of genes for tolerance to B (Paull, 1990). The smaller range of response to B in durum wheat may be due to response being controlled by fewer genes than in bread wheat (Jamjod, 1996), a likely consequence of the different ploidy levels of these cereals.

2.4.9 Incorporation of B tolerance

The transfer of B tolerance into otherwise adapted Australian varieties has been a major objective of wheat breeding programs in southern Australia in the past decade. For example, the incorporation a single major gene (*Bo1*) into B sensitive bread wheat by backcrossing identified a yield advantage in tolerant lines of up to 11% when compared to sensitive derivatives grown under high B conditions at a number of sites in South Australia (Moody *et al.*, 1993). Incorporation of two major genes (*Bo1* and *Bo3*) further increased the yield advantage of tolerant genotypes to as much as 25% more than sensitive genotypes (Paull, 1990). The findings for bread wheat suggest a yield advantage is also likely to be achieved for durum by incorporating tolerance into sensitive varieties. Identification that tolerance to B

for durum is under control of major genes (Jamjod, 1996) suggests backcrossing may be employed to achieve this goal.

2.5 Quality

2.5.1 Introduction

The distinctive characteristics of protein and starch composition, and the rheological properties of dough made from durum wheat semolina, make it particularly well suited to the manufacture of pasta products, such as spaghetti and macaroni. The hardness and protein concentration of durum wheat enable it to command the highest premium on the world wheat market, which provides a financial incentive for farmers to grow the crop. However, durum grain which does not reach minimum receival standards can not be included in hexaploid wheat classes except as feed, resulting in a high price penalty to the grower. Therefore, consistent production of high grade durum grain is paramount importance to the industry.

2.5.2 Definition of quality

Wheat quality is a very broad term and its definition depends on whether it is being assessed for the nutritional or processing purposes of a given product. In the present context the term 'quality' refers to the functional properties of durum semolina for processing into pasta and its bread-making capabilities. In terms of seed endosperm components affecting pasta and bread-making quality, emphasis will be placed on those contributing to gluten strength, since this is a major pre-requisite for processing, and selection for this factor can be made early in a breeding program.

Several small scale tests have been used to predict pasta and bread quality. Initial assessment of durum gluten strength on small samples in early generations of the breeding program can be performed using the sodium dodecyl sulphate (SDS) sedimentation test (Dick and Quick,

1983). The sedimentation volume is strongly correlated with bread-making quality (Axford *et al.*, 1979) as well as with spaghetti cooking quality (Dexter *et al.*, 1981). Physical dough testing (including farinograph, alveograph, mixograph, viscoelastograph and extensograph) and end product assessment are performed on larger samples available from advanced generations of the breeding program.

2.5.3 Pasta-making quality

The end product of durum, which is predominantly pasta in the western world, has quality parameters unlike those assessed in baking quality of bread wheat flour. Selection of semolina for pasta processing is based on factors affecting dough development and the quality factors of the finished products, such as colour and cooking performance. Assessing the cooking quality of durum wheat pasta is difficult because it is a perceived quailty. An overview of the quality requirements of pasta (Hare, 1994; Clarke *et al.*, 1998) is presented in Table 2.1.

Rheological properties and state of the surface of cooked pasta are the two main parameters on which the overall cooking quality of pasta depends. These are not directly related, so must be estimated separately (Menger, 1974; Feillet and Abecassis, 1976; D'Egidio *et al.*, 1979; Dexter *et al.*, 1983; Feillet, 1984; Autran *et al.*, 1986; D'Egidio *et al.*, 1993). The involvement of protein concentration in determining pasta quality has been widely documented, with concentrations greater than 13% associated with satisfactory cooking quality (Matveef, 1966; Damidaux and Feillet, 1978; McKenzie, 1994). However, it has been observed that the same protein concentration can be present in pasta samples with contrasting rheological and cooking qualities, indicating that other gluten characteristics are important in transformation processes (Liu *et al.*, 1996). Protein quality or gluten strength, on the other hand, is highly correlated with cooking quality (Grzybowski and Donnelly, 1979; Damidaux *et al.*, 1980b).

Table 2.1. Parameter and criteria basis of cooked pasta quality.

Selection Parameter	Selection Criteria
colour	bright yellow
	low browning
rheological properties	retain firmness
	retain chewiness
	high elastic recovery (*al dente*)
state of the surface	low stickiness
	low surface disintegration
	low solid loss to cooking water
cooking time	(varies according to strand diameter)
	reduce
swelling	low water absorption
	low cooked weight
taste	flavour
	pleasant or attractive aroma

Strong gluten properties are an essential factor contributing to pasta cooking quality. Gluten firmness and elastic recovery were correlated with SDS sedimentaion (Autran *et al.*, 1986). Firmness of cooked pasta was related to gluten strength, as measured by farinogaph or alveograph (Matsuo and Irvine, 1970; Grzybowski and Donnelly, 1979; Dexter and Matsuo, 1980). Cooking loss was negatively correlated with gluten strength (Grzybowski and Donnelly, 1979). Cooked pasta resilience measured using the viscoelastograph demonstrated that varieties which have strong gluten with high elastic recovery exhibit good cooking quality, whereas those having weak gluten with low elastic recovery results in poorer cooking quality (Feillet *et al.*, 1977; Kovacs *et al.*, 1995b). Kovacs *et al.* (1997) reported that mixing peak height values obtained using a mixograph were the best predictors for chewiness and firmness. Consequently, gluten strength is recognised to be one of the most important quality criteria in durum breeding.

The type of gluten present, rather than the total gluten content, also has a pronounced influence on cooking quality (Matsuo and Irvine, 1970; Grzybowski and Donnelly, 1979). Gluten is a complex mixture of proteins classified in two groups, the gliadins and the glutenins, on the basis of their solubility or insolubility in aqueous alcohols, respectively. Superior pasta firmness was related to a high glutenin to gliadin ratio (Walsh and Gilles, 1971).

From the perspective of a breeding program aimed at producing new durum varieties from which premium quality pasta can be manufactured, the rheological properties of semolina are characteristics that can be evaluated (using the SDS sedimentation test or mixograph) and selected for in relatively early generations, increasing the proportion of good quality genotypes in the later generations.

2.5.4 Bread-making quality of durum

In the southern regions of Italy, in addition to pasta production, durum flour is used for numerous types of breads. Durum breads have several characteristics which customers find appealing: unique taste and smell, yellowish colour, fine and uniform crumb structure and long shelf life (Quaglia, 1988). However, the use of durum wheat for commercial bread production elsewhere has been restricted due to the inability to produce bread comparable to that made from hexaploid flour. The generally weaker gluten strength of durum compared to hexaploid wheat produces a smaller loaf volume (Kaltsikes *et al.*, 1968; Kerber and Tipples, 1969).

Good bread-making flour is associated with strong gluten which is capable of producing an extensive viscoelastic matrix during dough formation, along with good physical handling properties (see review by MacRitchie, 1984). Although various rheological tests are used to indicate the potential of wheat flour for bread-making, a baking test is still considered to be

the final and most reliable test. Durum wheat is classified as having poor bread-making quality on the basis of baking performance (Boyacioglu and D'Appolonia, 1994).

Durum wheat milling is designed to produce semolina, and durum clear flour is a by-product of semolina milling. The durum mill usually extracts 60-65% good quality semolina from the milling system, with 13-18% durum flour and approximately 22% mill offal (bran and pollard). Currently, durum flour is of little economic value, as it is legally prohibited for use in pasta manufacture in countries such as Italy, France, Greece and Turkey, and generally not considered suitable for bread. Durum first clear flour is often blended for use in generic noodles, while durum second flour, which has high ash, is specky and has a dull colour, is used for manufacturing pet or stock feed. However, if durum flour had increased gluten strength, it could be utilized in bread-making by blending with bread wheat, thus developing a high priced, value added product.

With the development of durum with strong gluten in Italy, the United States and Canada during the 1980's, the prospects of utilizing durum directly for bread-making increased. In Italy, Boggini and co-workers (Boggini, 1985a; 1985b; Boggini *et al.*, 1988; 1995; Boggini and Pogna, 1989) reported that loaf volume of durum bread was correlated significantly with flour protein content, SDS-sedimentation value and parameters measured by mixograph (time to peak height, peak height and bandwidth) and farinograph (development time and mixing stability), and hence can be selected for in the breeding program. Boggini and Pogna (1989) found Italian durum genotypes differed in their loaf volume. Dexter *et al.* (1981) found some Canadian varieties approached acceptable quality for bread-making, but superior durums only matched the baking performance of weak hexaploid wheats. Other studies (Josephides *et al.*, 1987; Dick, 1988; Boyacioglu and D'Appolonia, 1994) report that strong durums were superior in baking tests to weak durums, but none were as good as the bread wheat control. Boggini *et al.* (1995) demonstrated durum loaf volume was correlated positively with alveograph W (dough strength) and negatively to P/L ratio, with a ratio of tenacity (P) to extensibility (L) less than one producing high loaf volume. The current bread-making ability

of durum wheat is relatively poor, but there is scope for improvement through selection for desirable predictive characteristics.

2.5.5 Factors contributing to gluten strength

The two major influences on gluten composition are genetic composition and the environment, and the interaction between the two. Genetic composition is purely a reflection of genotype, whereas the growing environment varies from year to year and between sites due to seasonal and regional fluctuations.

Association between environment and dough properties

Effect of the environment

Panozzo and Eagles (2000) reported that environmental variation was greater than cultivar variation for dough rheological characters in hexaploid wheat. The environment is largely responsible for seasonal variation in grain protein content at a given location (Mangels, 1925). There is also potential to modify the gluten composition during grain fill, namely, the extent of gene expression, the resulting ratio of high molecular weight (HMW) to low molecular weight (LMW) glutenin subunits, the overall proportion of aggregated glutenin polypeptides to non-aggregating gliadin protein, and the proportion of very large aggregates of glutenin polypeptides (Wrigley, 1994), which will influence the final dough strength properties.

Randall and Moss (1990) concluded that temperature and sulphur deficiency during grain filling were potenially the most important factors modifying dough properties of bread wheat relevant to Australia. McKenzie (1994) and Alvino (pers. comm.) found that durum grain from South Australia produced a weaker dough compared to grain from northern NSW at similar protein contents. Similar observations have been reported for samples of the bread wheat variety Condor having the same protein concentration (Archer and O'Brien, 1987), and

were attributed to differences in mean maximum temperature during grain development (Moss *et al.*, 1986). Panozzo and Eagles (2000) found a highly significant *Glu-D1* × environment interaction for Rmax, which they were able to attribute to a greater response of *Glu-D1a* than *Glu-D1d* to high temperatures during the first 14 days of grain filling. Since temperature is highly variable, within and between regions and seasons, further examination of its effect on dough properties in durum wheat is warranted.

The effect of temperature on dough properties

Annual crop reports of the Australian Wheat Board have indicated that wheat produced in the northern regions of the cereal belt (where growth temperatures tend to be higher) has greater dough strength than wheat from the southern regions. The areas in South Australia which consistently produce high durum yields tend to be located in regions with cool ripening conditions. Studies have shown that low temperatures (below 30°C) during grain filling decrease dough strength (Schipper *et al.*, 1986; Randall and Moss, 1990; Graybosch *et al.*, 1994; Salinger *et al.*, 1995). Hence, areas which are relied upon climatically for a consistent supply of grain in South Australia are likely to produce grain with weaker dough strength, compared to durum produced in northern NSW.

Episodes of high temperature (over 35°C) during grain fill also contribute to weakened dough properties. Blumenthal *et al.* (1991a, 1991b) attributed this to a higher ratio of gliadin to glutenin in grain protein. However, it was later indicated that dough strength is determined more by the size of glutenin polymers than gliadin : glutenin ratio (Blumenthal *et al.*, 1998; Panozzo and Eagles, 2000). Genotypes with the *Glu-D1a* allele are also affected more by high temperatures than those with the *Glu-D1d* allele (Blumenthal *et al.*, 1995; Panozzo and Eagles, 2000).

Australian varieties of durum wheat currently produce strong doughs due to the Tamworth (NSW) breeding program actively selecting for genotypes with desirable alleles and

mixograph and farinograph parameters. Advanced lines from Tamworth exhibit higher dough strength than durums from most other countries when grown under South Australian conditions (Liu and Rathjen, 1994), suggesting that further selection for greater dough strength within modern durums is limited and alternative strategies are required. To reduce the environmental effect on grain in South Australia producing intrinsically weaker dough compared to that from northern NSW, the improvement of the genotypic component is a major objective. One approach is manipulation of the grain gluten composition by allelic variation to improve dough strength, particularly from novel sources.

Genetic composition: Association between storage protein polypeptides and dough properties

Protein quality is a highly heritable character only partly influenced by the environment. A wheat variety with better bread or pasta-making attributes maintains this property over a wide range of protein concentrations, whereas a poor quality variety would not perform satisfactorily even at high protein concentrations (Finney and Barmore, 1948). The protein quality of a particular variety is generally believed to be controlled by the nature of the alleles present at the various loci controlling the gluten proteins, namely, the glutenins and gliadins.

Gliadins, LMW glutenin subunits and durum quality

Durum varieties with good quality have been associated with the electrophoretic pattern of the *Gli-B1* locus which encodes for γ-gliadin 45 proteins (Damidaux *et al.*, 1978, 1980a, 1980b; Kosmolak *et al.*, 1980; du Cros *et al.*, 1982). Strong gluten, as determined by the viscoelastograph, is due to the LMW-2 banding pattern encoded by the *Glu-B3* locus (Pogna *et al.*, 1988), which is highly linked to the *Gli-B1* locus (Payne *et al.*, 1984b). In contrast, the LMW-1 banding pattern, which is linked to γ-gliadin 42 proteins, is associated with dough weakness. Breeding programs worldwide have subsequently placed emphasis on selecting

genotypes, particularly parents, possessing the γ-gliadin 45 banding pattern to increase the proportion of strong gluten lines.

The strong relationship between LMW glutenin subunits and gluten quality in durum wheat, contrasts to the situation in bread wheat where HMW glutenin subunits are well correlated with the bread-making qualities of flour (Payne, 1987). Payne (1987) established within each locus a ranking with an arbitrary score assigned to alleles based on their contribution to flour quality, with a higher sum of the alleles associated with better quality. The HMW glutenin subunits in durum wheat have been either poorly or not consistently correlated with flour/semolina quality (du Cros, 1987; Autran and Galterio, 1989; Kaan *et al.*, 1993).

HMW glutenin subunits and durum wheat quality

Early studies showed HMW glutenin subunits had no effect on pasta quality (du Cros *et al.*, 1982; Vallega 1986). Later studies, however, found allelic variation at the *Glu-B1* locus had a minor additive effect (Autran and Feillet, 1987; Pogna *et al.*, 1990; Turchetta *et al.*, 1995), and the *Glu-A1* alleles (encoding 1 or 2*) increased the SDS-sedimentation volume associated with better cooked pasta quality (du Cros, 1987; Ciaffia *et al.*, 1991; Kann *et al.*, 1993; Turchetta *et al.*, 1995). The HMW glutenin subunit genes on chromosome 1B appeared to make a larger contribution to durum quality parameters compared to genes on chromosome 1A (Josephides *et al.*, 1987). *Glu-B1* alleles, coding for subunits 7+8, 6+8 or 6+17 versus subunits 20 or 13+16, and only one *Glu-A1* allele, subunit 2* versus the null, were found to be weakly correlated with improved gluten rheological properties and surface properties of cooked pasta (Autran and Feillet, 1987; du Cros, 1987). In a recent study conducted in South Australia by Liu and Rathjen (1994), the HMW glutenin subunits, ranking in the order 13+16>7+8>6+8>20, were highly correlated with SDS sedimentation values. However, no relationship was found between *Glu-A1* allele subunit 1 and dough strength as expected on the basis of findings in bread wheat (Payne *et al.*, 1987).

When examining previous studies two factors must be considered. First, commercial durum wheat varieties have a very narrow range of genetic variability (Vallega and Zitelli, 1973), thus data related to the importance of the HMW subunits can be misinterpreted due to lack of random association between subunits. Second, the HMW glutenin subunits which are predominant in durum have *Glu-1* alleles associated with low Payne scores (ie. mostly null at the *Glu-A1* locus and a high frequency of subunits 20 or 6+8 at *Glu-B1*) (Vallega, 1988; Branlard *et al.*, 1989; Kaan *et al.*, 1993; Liu, 1994). Thus, it is not unexpected to find no association reported between allelic HMW glutenin subunits and durum quality in the studies reviewed above. Liu and Rathjen (1994) suggested increasing variation at the *Glu-A1* locus in durum as they expect the results obtained in bread wheat (reviews of Payne, 1987; MacRitchie *et al.*, 1990) by incorporating desirable functional alleles (Payne *et al.*, 1987, 1988) could be used to develop a strategy for increasing the dough strength of durum wheat.

2.5.6 Approaches to quality improvement in durum wheat

The low gluten strength of modern durum wheat varieties is probably due to inactive alleles at the *Glu-A1* locus and a high frequency of alleles at the *Glu-B1* locus associated with low Payne scores. Modification of the protein composition of grain by genetic means is a fundamental approach to improving durum dough quality. Although there was emphasis on selection of durums with improved gluten strength during the 1970s and 1980s in Canada, Italy and the USA, the durums produced still had much lower gluten strength than hexaploid wheat. Since there is a lack of genetic variability for kernel storage proteins among commercial durum varieties worldwide (du Cros, 1987; Vallega, 1988; Branlard *et al.*, 1989), it would be of interest to incorporate kernel storage protein alleles with high *Glu-1* Payne scores from landraces combined with those existing in current varieties (eg. *Glu-A1* subunits 1 or 2* and *Glu-B1* subunits 13+16 or 7+8) in an attempt to increase the dough strength of durum wheat. In addition, other alleles could be transferred, such as *Glu-B1i* (subunits 17+18), which has a strong influence on bread-making quality in bread wheat, but does not occur in durum.

The poorer dough quality of durum compared to bread wheat is due to a combination of low Payne scores for the HMW glutenin subunits present, and the absence of *Glu-D1* alleles (Liu *et al.*, 1994a, 1994b; Liu, 1995a, 1995b). Thus, introducing desirable 1D alleles may be a more efficient approach than selection within tetraploids for improving the strength of durum gluten.

2.5.7 Introduction of genes from wheat chromosome 1D by substitution

The effects on rheological properties by introducing chromosomes from the D-genome into durum were reported by Joppa *et al.* (1983). Gluten strength increased when chromosome 1D of hexaploid Chinese Spring was substituted for chromosome 1B of durum variety Langdon, suggesting that chromosome 1D may have a greater role in influencing strength than 1B, even when carrying the poorer bread-making quality allele *Glu-D1a* (2+12) from Chinese Spring (Moonen *et al.*, 1983; Payne *et al.*, 1984a).

Results of Liu *et al.* (1995) showed that only chromosome 1D substitutions in the genetic background of durum var. Langdon gave a significant beneficial effect on rheological properties, resulting in stronger dough, compared to other D-genome chromosome substitution lines. Substituting chromosome 1D from Chinese Spring for chromosome 1A in Langdon resulted in a two-fold increase in quality parameters (SDS sedimentation, mixograph mix time and peak resistance) (Liu *et al.*, 1994a). A combination of substitution 1D and LMW-2 gave the highest values for all parameters, contributing positively to dough strength, indicating that there were cumulative effects of these genetic factors. The 1D(1A) substitution lines had comparable gluten strength to that of medium-strength hexaploid wheat, as measured by SDS-sedimentation (Liu *et al.*, 1994b). These results imply 1D(1A) substitution types might have the potential for improving both pasta-making and bread-making quality of durum.

The specific HMW glutenin subunits 5+10 (coded by *Glu-D1d*) have been shown to contribute more to the improved baking characteristics of bread wheat than subunits 2+12 (coded by *Glu-D1a*) (Burnouf and Bouriquet, 1980; Moonen *et al.*, 1983; Payne *et al.*, 1984a). Introduction of the *Glu-D1d* allele (subunits 5+10) instead of the *Glu-D1a* allele (subunits 2+12) from hexaploid wheat into durum wheat should further increase gluten strength. Results have shown that lines with subunits 5+10 had higher SDS sedimentation values than those with subunits 2+12 (Liu, 1995a), supporting the above hypothesis.

2.6 Conclusions and objectives

Genotype × environment interactions are observed as changes in the relative performance of genotypes over different environments. The growing environments in South Australia are variable, and a high concentration of B in the soil has been identified as one factor causing reduced crop yields. Environmental effects can also influence the functional quality of wheat flours, and grain produced in South Australia has been associated with weaker dough strength compared to that from New South Wales.

Durum wheat was introduced into South Australian agriculture in 1991, and was based on the New South Wales bred variety Yallaroi. Little information was available on the nature of G×E interactions encountered by this crop in South Australia or its adaptation to high concentrations of B in these areas. In addition, there was a lack of field data on the role of HMW glutenin subunits in kernel storage proteins in assessing dough strength in this species.

With the importance of cereal production to the primary industry of South Australia, this project has three basic objectives. The first was to examine the G×E interactions for durum wheat in the regions of cereal production and to determine appropriate breeding methodologies and performance evaluation. The second objective was to establish whether increasing the level of tolerance to B in Australian durum varieties would result in higher yields at locations with high B in the soil. The third objective was to determine whether

gluten properties in the durum wheat variety Yallaroi could be improved by incorporating HMW glutenin subunits that are associated with dough strength in bread wheat.

Chapter 3

Genotype × environment interaction of durum wheat in South Australia

3.1 Introduction

The final stages in a plant breeding program involve performance evaluation of candidate breeding lines (the genotypes) in a set of environments. Two goals of the durum wheat breeding program in South Australia are increased yield and wide adaptation of varieties to the cereal belt in the state, and to this end multi-environment testing occurs. Breeders need to know how much of the selection progress made in one environment can be carried over to another environment. Selection for yield is based on phenotype, which is influenced considerably by the interaction between genotype and environment (G×E interaction). G×E interactions are observed as changes in the relative performance of genotypes over environments. The G×E interaction encountered in multi-locational trials is a challenge for plant breeders to overcome, and can hinder the progress in breeding widely adapted varieties. An understanding of the causes of G×E interaction will enable the breeder to exploit positive components of specific adaptation to widen adaptation.

Future progress in analyzing differences in genotypic adaptation in crop improvement requires plant breeders to pay attention to influences of environmental factors (Eisemann *et al.*, 1990). Relatively little information is available on the performance and stability of durum wheat genotypes across the widely different environments of Australia, let alone southern Australia. Such basic information is necessary to identify genotypes useful as parents, and to determine the best way to evaluate the performance of material in multi-location trials without losing valuable germplasm. This chapter examines the nature of G×E interaction encountered for durum wheat in South Australia using four analytical techniques, to identify (a) genotypes useful as parents or to release as varieties with improved adaptation and (b) the components contributing to G×E interactions in an effort to determine breeding objectives.

3.2 Materials and methods

3.2.1 Genotypes

Fifty-three genotypes of durum wheat (*T. turgidum* var. *durum*) and two varieties (genotypes) of hexaploid wheat (*T. aestivum*) were used in this study. The durum lines comprised three varieties and 27 advanced breeding lines from the National Durum Wheat Improvement Program (NDWIP), Tamworth; ten genotypes with a range of boron tolerance, derived from backcross populations made at Waite (see Chapter 4); two advanced lines from Waite; elite lines from CIMMYT, Mexico, and ICARDA, Syria; and varieties from the USA and Italy. Seeds of the Australian durum varieties and advanced lines were obtained from Dr. R.A. Hare, NDWIP, Tamworth, NSW; the CIMMYT elite lines and AUS 19764 from the Australian Winter Cereals Collection (AWCC), Tamworth; the ICARDA elite lines from Dr. M. Nachit, ICARDA; the USA variety, Kronos, from Cultivaust Pty Ltd; the Italian variety, Simeto, from Dr. D. Lafiandra, University of Tuscia, Viterbo, Italy; and the hexaploid varieties from the Wheat Breeding Unit, Waite Campus, University of Adelaide. The name, origin and pedigree of each of the genotypes are shown in Table 3.1. The experiments were conducted over three years, 1994 - 1996.

3.2.2 Environments

Description

A total of twelve locations in the South Australian wheat belt were used. These sites are used for hexaploid wheat evaluation trials to measure response to the range of yield limiting factors encountered across the state. Soil types and constraints are described in Table 3.2 and rainfall data are shown in Appendix A. Rainfall was recorded by the Bureau of Meteorology, Roseworthy Campus and Minnipa Research Station, and by the farmers at other locations.

Table 3.1. Pedigree, country of origin and description of durum and bread wheat genotypes grown at least once in the period 1994 - 1996.

Genotypes		Pedigree	Origin	Description
#	**Durum wheat**			
1	Yallaroi	Guillemot seln. No.3/Kamilaroi sib	NSW	Semi-dwarf variety
2	Kamilaroi	Durati/Leeds	NSW	Semi-dwarf variety
3	Wollaroi	TAM1B-17/Kamilaroi sib//Rokel Sel./ Kamilaroi sib	NSW	Semi-dwarf variety
4	Altar 84	Ruff/Flamingo//Mexicali 75/3/Shearwater	CIMMYT	High yielding semi-dwarf variety
5	Kronos	Complex cross of Canadian, Italian and CIMMYT germplasm	Arizona, USA	Semi-dwarf variety
6	Simeto	Capeiti/Valnova	Italy	Semi-dwarf variety
7	WLYY9/1/2/2/1	Wol/3/AUS 14010/2*Yal//RH880009	Waite	Moderately B sensitive
8	WLYY9/1/3/4	Wol/3/AUS 14010/2*Yal//RH880009	Waite	Moderately B sensitive
9	WLYY9/2/L1/1	Wol/3/AUS 14010/2*Yal//RH880009	Waite	Moderately B sensitive-moderately B tolerant
10	WLYY9/3/1/5	Wol/3/AUS 14010/2*Yal//RH880009	Waite	Moderately B tolerant
11	WLYY9/3/2/1	Wol/3/AUS 14010/2*Yal//RH880009	Waite	Moderately B sensitive
12	WLYY9/3/2/3	Wol/3/AUS 14010/2*Yal//RH880009	Waite	Moderately B sensitive
13	WLYY9/3/2/4	Wol/3/AUS 14010/2*Yal//RH880009	Waite	Moderately B sensitive
14	WLYY9/3/2/5	Wol/3/AUS 14010/2*Yal//RH880009	Waite	Moderately B sensitive-moderately B tolerant
15	WLYY9/1/4/4/S	Wol/3/AUS 14010/2*Yal//RH880009	Waite	Moderately B tolerant
16	WLYY9/2/6/3	Wol/3/AUS 14010/2*Yal//RH880009	Waite	Moderately B tolerant
17	BB 94059/-aL1	AUS 14010/2*Yal//RH880009/3/2*WLYY9	Waite	Advanced breeding line
18	(LYY9/-1a*Yal)/4	AUS 14010/2*Yal//RH880009/3/Yal	Waite	Advanced breeding line
19	RH900218	TAM1B-17/Kamilaroi/3/Rokel'S'/2/ Kamilaroi/4/TAM1B-17/Kamilaroi/3/ Winged'S'//Kamilaroi	NSW	Advanced breeding line
20	RH900453	Altar 84/4/Guillemot'S'/Kamilaroi'S'/3/ Wells/56111//Guillemot'S'	NSW	Advanced breeding line
21	RH911840	Scoter'S'/3/BD1814//BD1708/BD1543/4/ Rokel'S'	NSW	Advanced breeding line
22	RH911913	Altar 84/3/Guillemot'S'/Kamilaroi'S'// Rokel'S'/Kamilaroi'S'	NSW	Advanced breeding line
23	RH911926	TAM1B-17/Kamilaroi/3/Rokel'S'/2/ Kamilaroi/4/HD4530	NSW	Advanced breeding line

Table 3.1. continued.

#	Genotypes	Pedigree	Origin	Description
24	RH911996	Altar 84/4/Guillemot'S'/Kamilaroi'S'/3/ Wells/56111//Guillemot'S'	NSW	Advanced breeding line
25	RH912023	Altar 84/4/Guillemot'S'/Kamilaroi'S'/3/ Wells/56111//Guillemot'S'	NSW	Advanced breeding line
26	RH912025	Altar 84/4/Guillemot'S'/Kamilaroi'S'/3/ Wells/56111//Guillemot'S'	NSW	Advanced breeding line
27	RH920274	Sterna/Sula'S'	NSW	Advanced breeding line
28	RH920276	Sterna/Churrilla'S'	NSW	Advanced breeding line
29	RH920314	TAM1B-17/Kamilaroi/3/Rokel'S'/2/ Kamilaroi/4/Yallaroi//TAM1B-17/Kamilaroi	NSW	Advanced breeding line
30	RH920318	TAM1B-17/Kamilaroi/3/Rokel'S'/2/ Kamilaroi/4/Yallaroi//TAM1B-17/Kamilaroi	NSW	Advanced breeding line
31	RH920325	TAM1B-17/Kamilaroi/3/Rokel'S'/2/ Kamilaroi/4/Yallaroi//TAM1B-17/Kamilaroi	NSW	Advanced breeding line
32	RH920326	TAM1B-17/Kamilaroi/3/Rokel'S'/2/ Kamilaroi/4/Yallaroi//TAM1B-17/Kamilaroi	NSW	Advanced breeding line
33	RH920334	TAM1B-17/Kamilaroi/3/Rokel'S'/2/ Kamilaroi/4/Yallaroi//TAM1B-17/Kamilaroi	NSW	Advanced breeding line
34	RH920351	TAM1B-17/Kamilaroi/3/Rokel'S'/2/ Kamilaroi/4/Yallaroi//TAM1B-17/Kamilaroi	NSW	Advanced breeding line
35	RH920356	TAM1B-17/Kamilaroi/3/Rokel'S'/2/ Kamilaroi/4/Yallaroi//TAM1B-17/Kamilaroi	NSW	Advanced breeding line
36	RH920405	Yallaroi//TAM1B-17/Kamilaroi/4/TAM1B-17/Kamilaroi/3/Rokel'S'//Kamilaroi	NSW	Advanced breeding line
37	RH920528	TAM1B-17/Kamilaroi/3/Rokel'S'/2/ Kamilaroi/4/TAM1B17/Kamilaroi/3/ Winged'S'//Kamilaroi	NSW	Advanced breeding line
38	RH920532	TAM1B-17/Kamilaroi/3/Rokel'S'/2/ Kamilaroi/4/TAM1B-17/Kamilaroi/3/ Winged'S'//Kamilaroi	NSW	Advanced breeding line
39	RH920615	TAM1B-17/Kamilaroi/3/Winged'S'// Kamilaroi/4/Yallaroi//TAM1B-17/Kamilaroi	NSW	Advanced breeding line
40	RH920616	TAM1B-17/Kamilaroi/3/Winged'S'// Kamilaroi/4/Yallaroi//TAM1B-17/Kamilaroi	NSW	Advanced breeding line
41	RH920618	TAM1B-17/Kamilaroi/3/Winged'S'// Kamilaroi/4/Yallaroi//TAM1B-17/Kamilaroi	NSW	Advanced breeding line

Table 3.1. continued.

#	Genotypes	Pedigree	Origin	Description
42	RH920621	TAM1B-17/Kamilaroi/3/Winged'S'// Kamilaroi/4/Yallaroi//TAM1B-17/Kamilaroi	NSW	Advanced breeding line
43	RH920672	TAM1B-17/Kamilaroi/3/Rokel'S'/2/ Kamilaroi/4/TAM1B-17/Kamilaroi/3/ Winged'S'//Kamilaroi	NSW	Advanced breeding line
44	RH920680	TAM1B-17/Kamilaroi/3/Rokel'S'/2/ Kamilaroi/4/TAM1B-17/Kamilaroi/3/ Winged'S'//Kamilaroi	NSW	Advanced breeding line
45	RH920777	891602/Wollaroi	NSW	Advanced breeding line
46	AUS 19764	Pelicano'S'/Ruff//Gaviota'S'/Waha	Egypt	Breeding line
47	Yavaros 'S'	Jori'S'/Anhinga'S'//Flamingo'S'	CIMMYT	Semi-dwarf variety
48	Cormorant	Jori'S'/3/Lakota Enano/Langdon390// Chapala67/4/Crane'S'/Ganso'S'	CIMMYT	Semi-dwarf variety
49	EDYT18-10	Ren'S'//Dack'S'/Teal'S'	CIMMYT	Advanced breeding line
50	EDYT18-19	Yavaros'S'/Tezontle'S'	CIMMYT	Advanced breeding line
51	EDYT19-14	Melianopus69/Huitle'S'/Somorgujo'S'	CIMMYT	Advanced breeding line
52	Daki=Cyn	Dackiye/Gediz//USDA575	ICARDA	Advanced breeding line
53	Hagla	Crane//T.dic.V.Vernum/Grulla/3/Jori	ICARDA	Advanced breeding line
	Bread wheat			
54	Spear	Sabre/Mec3//Insignia	South Australia	Moderately B tolerant, semi-dwarf, ASW[a] variety
55	Molineux	Pitic 62/Festiguay//2* Warigal	South Australia	Semi-dwarf, AH[b] variety

[a] Australian Standard White

[b] Australian Hard

Table 3.2. Location, soil type descriptions, constraints and soil classification of the trial sites.

Locality	Soil Type Description	Key Constraints	Soil Classification[a]
Roseworthy	Calcareous solonized brown soil	Leaf diseases, B	Calcarosols
Palmer (Eichler)	Sandy solonized brown soil	Root diseases	Calcarosols
Palmer (Krause)	Calcareous brown earth	Crown rot, B, Na	Calcarosols
Lowbanks	Friable red duplex soil	Low rainfall, root diseases	Calcarosols
Walker Flat	Red calcareous earth	High pH, calcareous	Calcarosols
Kapunda 1994, 1995	Dark brown cracking clay	Leaf diseases, low pH	Sodosols
Kapunda 1996	Red brown earth	Leaf diseases, low pH	Chromosols
Jamestown	Clayey red brown earth	Increasing pH down profile	Chromosols
Mallala	Calcareous solonized brown soil	Na	Calcarosols
Two Wells	Red duplex soil	Leaf disease, Na, B, HCO_3^-	Sodosols
Winulta 1994, 1995	Grey duplex soil	B, root diseases	Sodosols
Winulta 1996	Calcareous red earth	B, root diseases	Calcarosols
Rudall	Red duplex soil	Zn & Mn deficiency, Na, B, root diseases	Sodosols
Minnipa	Calcareous solonized grey brown soil	B, Na	Calcarosols

[a] After Isbell (1996).

Rainfall during the months April to October, inclusive, is defined as the growing season rainfall in South Australia (French and Schultz, 1984).

The trials at Roseworthy were conducted on the farm of the University of Adelaide, Roseworthy Campus; at Minnipa on the Minnipa Research Centre; at Walker Flat, Jamestown, Mallala, Two Wells, Winulta, Kapunda, Palmer, Rudall and Lowbanks, the trials were on farmers' properties (Plate 3.1). Private farms are used extensively as selection sites by the South Australian breeding programs, because yield results obtained from such sites are, in general, a better predictor of the regional performance of varieties than results from research stations (Rathjen and Pederson, 1986).

Plate 3.1. Map showing locations where field trials were conducted 1994 - 1996.

Experimental design and field layout

Eight trials were sown per year and designed as completely randomized blocks, re-randomized every year, but each location had the same randomization within each year. The number of durum genotypes sown was 37 in 1994, 34 in 1995 and 35 in 1996. Genotypes were not consistent over time due to ongoing elimination or promotion of material to advanced breeders' line status. Two bread wheat varieties, Spear and Molineux, were grown every year. There were five replicates in all trials in 1995 and 1996, but in 1994 (Trials 1 to 8) there were six replicates. For analysis over years, except for the spatial analysis, the last replicate from 1994 was omitted and only five replicates were analysed. All trials had a grid of plots of the durum variety Yallaroi sown every seventh plot. Borders were sown at each end of the trial.

Each plot was four drill rows wide (15 cm row spacing) and 6 m long, which was reduced to 4.2 m after the pathways were sprayed out with herbicide (Roundup®) in spring. Adjacent plots were separated by one missing row, or 30 cm. The plots were arranged in bays and each experiment was 15 bays deep (90 m). Sowing rate for durum wheat was 40 g/plot, equivalent to sowing at 80 kg/ha. Bread wheat varieties were sown at a rate of 30 g/plot, equivalent to 60 kg/ha. A higher sowing rate was used for durum to compensate for its larger seed size (approximately 40 mg/seed) compared to bread wheat (approximately 30 mg/seed) and to conform with local farming practice. The plots were sown using a modified fourteen row drill and three plots were sown simultaneously.

Trial management

The management of field experiments, including field preparation, was in accordance with local district practices. As previous crops for the paddocks in which experiments were conducted varied, these practices varied according to the rotations for the individual farms and research stations. Topfos® double strength super-phosphate (16% P) at a rate of 100

kg/ha was drilled with the seed at a depth of 5 cm during sowing of each experiment. No artificial nitrogen fertiliser was applied. Paddock rotations of selected trials in 1994, 1995 and 1996 are presented in Appendix B.

Sowing dates varied according to the time of the autumn break. (ie. the arrival of sufficient rain to prepare a seedbed and allow sowing, germination and establishment).

1994: Lowbanks 14th June, Palmer-Eichler 18th June, Two Wells 10th June, Rudall 17th June, Kapunda 20th June, Mallala 13th June, Roseworthy 30th June, Winulta 29th June.

1995: Rudall 2nd June, Palmer - Krause 22nd May, Walker Flat 26th May, Two Wells 18th May, Kapunda 19th June, Mallala 1st June, Roseworthy 15th June, Winulta 31st May.

1996: Minnipa 26th June, Jamestown 14th June, Walker Flat 10th June, Two Wells 14th June, Kapunda 19th June, Mallala 12th June, Roseworthy 15th July, Winulta 3rd July.

The time of sowing was optimal for the locations used in 1995, whereas in 1994 and 1996 sowing in the majority of locations, with the exceptions of Mallala and Two Wells, were after the optimum time due to a late opening rain.

Weed control was achieved by application of herbicides and was conducted by the farmer as part of the normal management practices for the paddock. In general, however, the crops recieved a broadleaf herbicide in each season.

Plots were harvested at maturity (December), using harvestors designed and built at Waite Campus. All four rows per plot were harvested.

3.2.3 Statistical analysis of the data

The main statistical procedures used were analysis of variance (ANOVA), adaptation analysis, principal component analysis (PCA) and spatial analysis. Yield data for ANOVA and PCA were analysed using Genstat 5 (1987) software on DEC station 5000/240. Coefficients of variation for individual locations were determined.

Analyses of G×E interaction for grain yield were performed on a number of environment sets. A summary of number of genotypes within each set, the type of analysis performed on each set and trials in each set is provided in Table 3.3. This allowed examination of different combinations for the environment components, locations and years, to identify which was contributing a larger effect to the interaction. However, not all the same genotypes and locations were used in each of the three years, resulting in unbalanced data sets. Therefore, environmental Sets 4 and 5 contain the genotypes grown repeatedly at the same locations between seasons to obtain balanced data sets. To compare genotypes in Sets 4 and 5 at a larger number of environments, without considering the effect of location between seasons, all locations over two and three years were pooled to produce Sets 6 and 7, respectively. A total of 24 environments in which 55 genotypes, not all in common, were included in Set 8.

Analyses were performed on Sets 4 - 8 in an attempt to elucidate which grouping provided the most information appropriate for assisting with the identification of genotypes suitable for release to farmers, or for use as parents in the breeding program.

Analysis of variance

Each environmental set (except Set 8) was subjected to an ANOVA combining data from the respective locations. There was no attempt to sub-divide the G×E interaction into components, due to linear regression and deviations from linearity.

Table 3.3. Number of durum genotypes, the type of analysis performed and trials in each environment set.

Environment Set	No. of Durum Genotypes	Analysis Performed	Trials*
1	37	ANOVA	1 to 8, 8 locations in 1994
2	34	ANOVA	9 to 16, 8 locations in 1995
3	35	ANOVA	17 to 24, 8 locations in 1996
4	29	ANOVA PCA	11 to 16 & 19 to 24, same 6 locations in 1995 and 1996
5	19	ANOVA PCA	5 to 8, 13 to 16 & 21 to 24, same 4 locations in 1994, 1995 and 1996
6	29	ANOVA PCA Regression	9 to 24, all 16 locations, 1995 and 1996
7	19	ANOVA PCA Regression	1 to 24, all 24 locations, 1994, 1995 and 1996
8	53	Spatial	1 to 24, all 24 locations, 1994, 1995 and 1996

*Details of trials are provided in Appendix C.

The mathematical model underlying the analysis is:

$$Y_{ijk} = \mu + G_i + E_j + GE_{ij} + R_{jk} + e_{ijk}$$

where Y_{ijk} = the yield of genotype (i) at environment (j) and replicate (k)

μ = mean of all genotypes over all environments and replicates

G_i = mean effect of genotype (i)

E_j = mean effect in environment (j)

GE_{ij} = effect of genotype (i) at environment (j)

R_{jk} = effect of replicate (k) within environment (j)

e_{ijk} = error of genotype (i) at environment (j) and replicate (k).

The appropriate analysis of variance for this model is shown in Table 3.4. The varieties are treated as fixed, and environments as random samples.

The environmental effect can further be divided into location, year and L×Y effects, with the concurrent division of G×E interaction into G×L, G×Y and G×L×Y components. The appropriate analysis of variance is shown in Table 3.5, where location and year are treated as random samples with genotype as fixed.

Table 3.4. Analysis of variance for a series over environments (McIntosh, 1983).

Source	d.f.	m.s.	F pr.
Genotype (G)	g - 1	M_1	M_1/M_3
Environments (E)	e - 1	M_2	M_2/M_4
G × E	(g - 1)(e - 1)	M_3	M_3/M_5
Replicates within E	e(r - 1)	M_4	M_4/M_5
Residual error	e(r - 1)(g - 1)	M_5	

Table 3.5. Analysis of variance for a series over locations and years (McIntosh, 1983).

Source	d.f.	m.s.	F pr.
Location (L)	l - 1	M_1	M_1/M_3
Years (Y)	y - 1	M_2	M_2/M_3
L × Y	(l - 1)(y -1)	M_3	M_3/M_4
Replicates in L & Y	ly(r-1)	M_4	
Genotype (G)	g - 1	M_5	$(M_5+M_8)/(M_6+M_7)$
G × L	(g - 1)(l - 1)	M_6	M_6/M_8
G × Y	(g - 1)(y - 1)	M_7	M_7/M_8
G × L × Y	(g - 1)(l - 1)(y - 1)	M_8	M_8/M_9
Residual error	ly(r - 1)(g - 1)	M_9	

Bartlett's test for homogeneity of variance was used to test whether the experimental error was homogeneous between environments (Steel and Torrie, 1980). Since it is generally expected locations will not be homogeneous, those with coefficients of variation (c.v.) greater than 20% were excluded from the combined analysis according to the recommendation of Gomez and Gomez (1984).

Regression - adaptation analysis

The term 'adaptation' is used to indicate a regression analysis where an environmental index based on a quantitative score is assigned to each environment. Generally, site mean yield is used as the environmental index (Finlay and Wilkinson, 1963). However, in this study, the yield of the durum variety Yallaroi relative to that of the bread wheat variety Spear was used as the environmental index. This environmental index is used rather than site mean yield as, under unfavourable conditions, durum performs relatively poorly compared to bread wheat (Brooks, 1991). At present, a major objective of durum production is to reduce the yield difference between durum and bread wheat in lower yielding environments, therefore, an indicator of this is the yield of durum relative to the current check in bread wheat trials, Spear. Only Yallaroi was used in the comparison because exotic durums were less adapted to South Australian conditions (Brooks, 1991). The yields of individual durum varieties relative to the well adapted bread wheat Spear were used as the dependent variate against the environmental index. In this analysis, a regression coefficient of 0, with a relative value approximating 100% is desirable, since this indicates yielding behaviour consistent with Spear, whereas a regression coefficient of 1.0 indicates the same yield stability as Yallaroi. Regression was performed for genotypes in environment Sets 6 and 7 to examine response of lines grown repeatedly over at least two years.

Principal Component Analysis

PCA was conducted on environmental Sets 4 - 7 to determine relationships between genotypes from a display of genotypic domains. For each set a two-way genotype × environment matrix was formed with a single value of yield (g/plot) for each genotype at each environment. Eigenvectors and Eigenvalues were calculated from a variance-covariance matrix. The PC values (Eigenvalues) for each genotype are weighted according to the environment loading (Eigenvectors).

When the majority of the variation in varietal responses is accounted for by the first two principal components, a plot of varieties on these two axes (a biplot) provides a succinct description of the data (Kempton, 1984). The distance between points represented in two dimensional Euclidean space provides a measure of dissimilarity.

The mathematical model underlying the analysis is:

$$Y_{ge} = \mu + \sum_{n=1}^{N} \lambda_n \zeta_{gn} \eta_{en} + \theta_{ge}$$

where Y_{ge} = the yield of genotype (g) in environment (e)

μ = the grand mean

λ_n = the eigenvalue of the PCA axis, n

ζ_{gn} = the genotype PCA score for the PCA axis, n

η_{en} = the environment PCA score for the PCA axis, n

N = the number of PCA axes retained in the model

θ_{ge} = the residual.

The bread wheats Spear and Molineux were not included in the principal component analyses, as their inclusion in initial biplots demonstrated that the bread wheats were greatly dissimilar from the durums, whilst the durums were clustered together.

Principal components were examined with genotypic response to boron (root length at high concentration of B - Chapter 4), and environment characteristics, rainfall (April – October), sowing date and soil pH (Appendix B), to identify if correlations existed

Spatial Analysis

Spatial analysis was performed on Environmental Set 8, using the method developed by Gilmour *et al.* (1997). In this analysis, there are two main statistical components to consider. First, there is a possibility that every individual trial has some significant spatial effects, and that these effects are different for each trial. Second, to find relationships between each trial by examining the relationships between varietal performances among trials. Varieties are assumed to come from a single population with a single genetic variance, hence, there is no correlation between varieties in the model.

1. Spatial effects on individual trials

There are two types of spatial effects. Natural spatial effects are the relationships between adjoining plots within a trial. Extraneous spatial effects are those that occur in the form of trends across the whole trial.

The statistical model considered for each site is:
Mean yield = Variety + Extraneous error + Natural error + Noise.

The extension of this model across multiple trials is:
Mean yield for variety (j) at site (i) = site (i) + site (i):Variety (j) + Extraneous error site (i) + Natural error site (i) + Noise site (i).

All variety means across all sites are estimated and site by variety interaction investigated.

2. Relationships between trials

The relationships between varietal performances between each trail are called the correlations between trials. These correlations are examined using the methods proposed by Smith *et al.* (2001). A correlation can be found between each pair of trials. A matrix of correlations is produced, where the size is determined by the number of trials examined. Smith *et al.* (2001) proposed a method of factoring a large matrix into vectors, of length determined by the number of trials, containing loadings. The first set of loadings generated is representative of correlations of each trial site with an average site. A site with a high correlation with the average site (ie. close to 1.0) performs expectedly for a site from the group of trials analysed. The second set of loadings produced are related to the environmental effect. Environmental loadings must be measured against the correlation with average site. The environmental loading indicates how much the site departs from the average performance.

Common effects of variety can be related to the first set of environment loadings. This is the yield component describing the relationship between the predicted yields at all the sites. Positive deviations from 0 indicate higher yield than average. The second set of varietal common effects deal with the effects due to site (or environment). A variety that has 0 common effect is stable across all sites, and large deviations from 0 in either direction indicate that the variety changes ranking throughout the sites.

3.3 Results

Analyses (ANOVA, regression - adaptation analysis, PCA and spatial) were carried out for grain yield on the genotypes within the environmental sets. Trials 3 (Two Wells, 1994) and 4 (Rudall, 1994) were abandoned due to plot losses from crown rot (*Fusarium* spp.) and frost, respectively, and so were excluded from analyses. Trial 10 (Palmer - Krause) was severely infected with crown rot, however, grain yield was recorded.

Bartlett's test for homogeneity of experimental error in the different environments indicated highly significant heterogeneity in all years. Trial 2 (Palmer - Eichler) in 1994 and Trial 10 (Palmer - Krause) in 1995 had coefficients of variation greater than 20%, and additional analyses were conducted with these locations excluded.

For the 22 sites, a significant correlation existed between April - October rainfall and mean yield of durum genotypes ($r=0.877$, $P<0.01$). The line of regression for mean yield (g/plot) of durum wheat genotypes ($y=-240.94+2.70x$) was lower, and significantly different ($P<0.01$) from the line of regression for Spear ($y=-192.01+3.02x$, $r=0.876$). As rainfall increased, there was a yield increase for durum and the bread wheat variety Spear, but the magnitude for durum was lower. When the regressions for individual durum genotypes were calculated, Yallaroi ($y=-267.41+2.66x$, $r=0.862$) was significantly ($P<0.01$) lower yielding than Spear, whereas RH912025 ($y=-259.47+2.94x$, $r=0.858$) (which had the highest mean yield over three years) was not significantly different from either Spear or Yallaroi (Figure 3.1).

There was no relationship between rainfall and mean grain yield of durum wheat relative to Spear ($r=0.407$, n.s.) (Figure 3.2). The large range of relative yields at the low rainfall locations is likely to be a reflection of the larger relative differences between yields under poor growing conditions. Nevertheless, at the two locations where the mean durum yield was less than 55% of Spear, the contributing factors were identified: at Palmer - Krause in 1995 the plots were infected with *Fusarium* crown rot, and Rudall in 1995 had boron toxicity (see Chapter 4).

No significant relationship was found between sowing date and yield (data not shown).

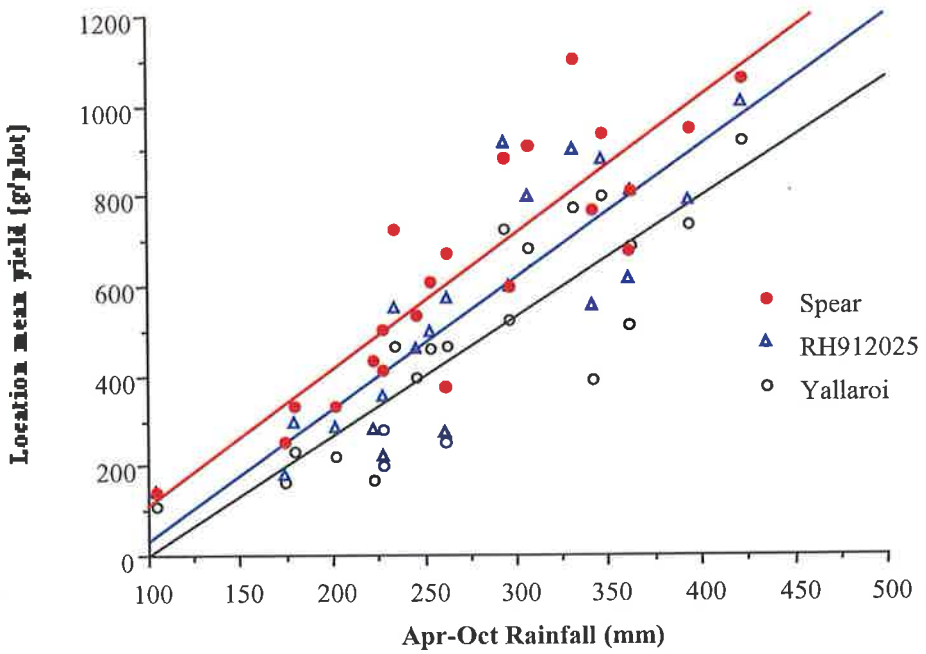


Figure 3.1. Relationship between yield of durum genotypes, Yallaroi and RH912025, and bread wheat Spear and April - October rainfall at 22 locations during the period 1994 -1996.

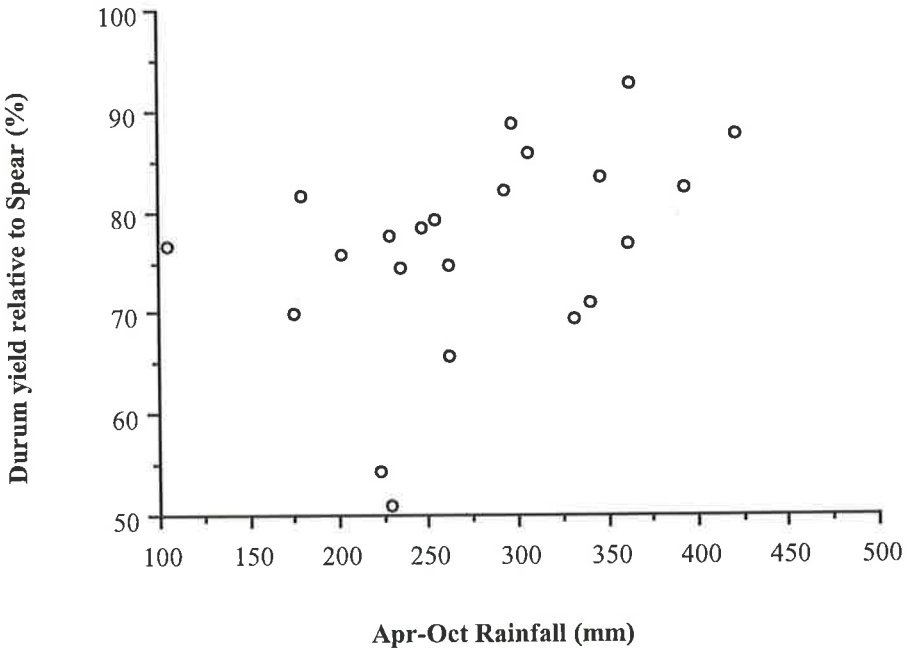


Figure 3.2. The relationship between the mean yield of durum relative to Spear and April - October rainfall at 22 locations during the period 1994 -1996.

3.3.1. Analysis of variance

Genotype × environment interactions were highly significant in all analyses. The analyses for the different environment sets are presented in Tables 3.6, 3.9, 3.10 and 3.11; Set 1 (farm locations 1994), Set 2 (farm locations 1995), Set 3 (farm locations 1996), Set 4 (same genotypes and locations 1995-1996), Set 5 (same genotypes and locations 1994-1996), Set 6 (same genotypes at all farm locations 1995-1996) and Set 7 (same genotypes at all farm locations 1994-1996). The bread wheats Spear and Molineux were dropped from the ANOVA as initial analysis showed their inclusion reduced the variation observed between durum genotypes.

Sets 1 - 3

ANOVA for Sets 1 – 3 showed location variances were highly significant, and accounted for most of the variation observed (Table 3.6). Mean site yields were highly variable in the seasons 1994-1996 (Table 3.7), ranging from 109 to 541 g/plot (equivalent of 0.36 to 1.80 t/ha), 212 to 931 g/plot (0.71 to 3.11 t/ha), and 250 to 788 g/plot (0.83 to 2.63 t/ha) in 1994, 1995 and 1996, respectively. Generally, low yields were obtained in 1994, primarily due to drought conditions, while higher yields occurred in 1995 and 1996.

There were significant differences between the mean grain yields of the durum genotypes (Table 3.8). Grain yields ranged from 247 to 334 g/plot (0.82 to 1.11 t/ha) in 1994, 463 to 636 g/plot (1.54 to 2.12 t/ha) in 1995, and 559 to 692 g/plot (1.86 to 2.31 t/ha) in 1996. The commercial variety Yallaroi was ranked lowest in 1994, second lowest in 1995, and relatively low in 1996. Highest yielding lines were an advanced line, RH920615, from Tamworth in 1994, Kronos, a variety from the USA in 1995, and a boron tolerant advanced line, WLYY9/2/6/3, in 1996. Nevertheless, the bread wheat variety Spear yielded more than the durums in every season. These results highlight the poor adaptation of Yallaroi to the South Australian environment compared to the other durum germplasm tested. Higher yielding alternatives to Yallaroi exist, although at present they are still not as good as Spear.

Table 3.6. Summary of mean squares (m.s.) and the significance levels from ANOVA of environmental Sets 1 - 3.

Source	Set 1 - 1994			Set 1[a] - 1994			Set 2 - 1995			Set 2[b] - 1995			Set 3 - 1996		
	d.f.	m.s.		d.f.	m.s.		d.f.	m.s.		d.f.	m.s.		d.f.	m.s.	
Genotype (G)	36	15370	***	36	15956	***	33	49055	***	33	46039	***	34	53057	***
Location (L)	5	5327439	***	4	5803937	***	7	11628223	***	6	10301755	***	7	6493640	***
G × L	180	5577	***	144	6233	***	231	11065	***	198	11599	***	238	12282	***
Replicates within L	30	485	ns	25	563	ns	32	9962	*	28	11307	*	32	9396	*
Residual error	1080	1710		900	1784		1056	6181		924	6630		1088	5324	

[a] excluding Trial 2 (Palmer - Eichler)

[b] excluding Trial 10 (Palmer - Krause)

*** $P<0.001$ * $P<0.05$ ns $P>0.05$

Table 3.7. Mean yields (t/ha) of durum wheat at trial locations for environmental Sets 1 - 3.

Locality	1994		1995		1996	
	Trial	Yield	Trial	Yield	Trial	Yield
Lowbanks	1	0.36				
Palmer - Eichler	2	0.59				
Rudall			9	0.71		
Palmer - Krause			10	0.79		
Minnipa					17	0.83
Jamestown					18	1.69
Walker Flat			11	1.40	19	1.62
Two Wells			12	1.77	20	1.74
Kapunda	5	1.31	13	3.10	21	2.44
Mallala	6	0.85	14	1.83	22	2.63
Roseworthy	7	0.91	15	2.62	23	2.51
Winulta	8	1.80	16	2.56	24	2.61
LSD (0.05)		0.05		0.07		0.08

Table 3.8. Mean performance of durum and bread wheat genotypes averaged across locations in Sets 1 - 3.

Genotype	Yield (t/ha)				
	1994		1995		1996
	Set 1	Set 1[a]	Set 2	Set 2[b]	Set 3
Durum wheat					
Kamilaroi	0.98	1.07	1.78	1.91	1.87
Yallaroi	0.82	0.88	1.70	1.86	1.94
Wollaroi	0.98	1.05	1.83	1.98	2.00
Altar 84	1.03	1.12	1.88	2.00	
Kronos			2.12	2.28	1.93
Simeto					1.96
WLYY9/1/2/2/1					2.02
WLYY9/1/3/4			1.97	2.12	1.99
WLYY9/2/L1/1			1.93	2.09	2.11
WLYY9/3/1/5			1.90	2.06	2.06
WLYY9/3/2/1			1.94	2.11	2.16
WLYY9/3/2/3			1.93	2.09	2.12
WLYY9/3/2/4			1.98	2.16	2.16
WLYY9/3/2/5			2.04	2.20	2.16
WLYY9/1/4/4/S					2.14
WLYY9/2/6/3					2.31
BB 94059/-aL1					1.94
(LYY9/-1a*Yal)/4					2.08
RH900218	1.00	1.08			
RH900453	0.94	0.99	1.84	1.98	2.10
RH911840	0.93	1.00			
RH911913	1.00	1.06	1.73	1.87	1.94
RH911926	1.00	1.09	1.90	2.02	1.98
RH911996	0.99	1.08	1.92	2.06	2.07
RH912023	1.00	1.08	1.85	2.01	
RH912025	1.01	1.09	2.02	2.17	2.24
RH920274	0.89	0.98			
RH920276	0.85	0.93			
RH920314	0.92	0.99	1.78	1.94	1.86
RH920318	1.10	1.18	1.93	2.08	2.01
RH920325			1.73	1.90	1.77
RH920326	0.89	0.95			
RH920334	1.05	1.14	1.75	1.92	1.76
RH920351	0.89	0.96	1.74	1.92	1.96
RH920356	0.89	0.94	1.80	1.97	2.00
RH920405	1.01	1.10			
RH920528	1.03	1.11	1.82	1.94	1.98
RH920532	0.94	1.00	1.72	1.85	1.90
RH920615	1.11	1.21	1.92	2.07	1.95
RH920616	0.97	1.02			
RH920618	1.01	1.09	1.93	2.07	2.04
RH920621			1.86	2.00	1.95
RH920672	1.03	1.11	1.77	1.91	1.97
RH920680	1.03	1.11	1.76	1.88	1.87
RH920777	1.02	1.09			
AUS 19764	1.01	1.09			
Yavaros 'S'	0.88	0.95			
Cormorant	1.04	1.14	1.78	1.95	
EDYT18-10	0.93	1.00	1.72	1.85	
EDYT18-19	0.97	1.06			
EDYT19-14	0.87	0.92	1.54	1.69	
Daki=Cyn	0.99	1.07			
Hagla	0.91	0.99			
LSD[#] (0.05)	0.12	0.13	0.15	0.17	0.16
Bread wheat					
Spear	1.27	1.36	2.43	2.57	2.47
Molineux	1.20	1.28	2.13	2.26	2.14

[a] excluding Trial 2 (Palmer - Eichler), [b] excluding Trial 10 (Palmer - Krause)

[#] calculated for durum only

Sets 4 and 5

Analyses were carried out for the 29 durum genotypes in Set 4 (two years data) and 19 durum genotypes in Set 5 (three years data). The L×Y effects accounted for most of the variation observed in the G×E interaction (Table 3.9). G×L and G×Y showed little effect (mostly non-significant) which suggests that genotypes yielded consistently over both locations and years in these sets.

Site and genotype mean yields in Sets 4 and 5 were significant and are provided in Appendix D. The highest yielding durum genotype in both Sets 4 and 5 was an advanced line, RH912025, originating from the NDWIP in Tamworth. The variety Yallaroi had a low ranking in Set 4 and was ranked lowest in Set 5.

Table 3.9. Summary of mean squares (m.s.) and the significance levels from ANOVA of environmental Sets 4 and 5, with location and year components.

Source	Set 4			Set 5		
	d.f.	m.s.		d.f.	m.s.	
Location (L)	5	6489579	ns	3	1703540	ns
Years (Y)	1	3185	ns	2	17777900	**
L × Y	5	1477644	***	6	986536	***
Replicates in L & Y	48	12985	**	48	12632	**
Genotype (G)	28	74611	**	18	42918	**
G × L	140	13907	ns	54	7422	ns
G × Y	28	15641	ns	36	15611	*
G × L × Y	140	10552	***	108	9493	***
Residual error	1344	6874		864	5824	

*** P<0.001 ** P<0.01 * P<0.05 ns P>0.05

Sets 6 and 7

When all the locations over two years (Set 6) and three years (Set 7) were included in single ANOVA with the sub-set of genotypes grown repeatedly over the seasons, environmental variance remained highly significant and accounted for most of the variation observed (Tables 3.10 and 3.11), which agrees with the analyses of individual seasons (ie. Sets 1 - 3).

Differences between the mean yields of the genotypes were highly significant (Table 3.10, 3.11; Appendix D5 and D6). The pooled analysis for Set 6, sixteen locations over two years, and Set 7, 22 locations over three years, demonstrated that Yallaroi was one of the lowest ranked genotypes, while the best performing genotype was RH912025, which was subsequently registered as Tamaroi (Hare, 1996). Site mean yields were also significantly different and are presented in Appendix D.

Table 3.10. Summary of mean squares (m.s.) and the significance levels from ANOVAs of environment Set 6.

Source	Set 6			Set 6[b]		
	d.f.	m.s.		d.f.	m.s.	
Genotype (G)	28	73840	***	28	72190	***
Environments (E)	15	7105000	***	14	6344000	***
G × E	420	11270	***	392	11480	***
Replicates within E	64	9799	**	60	10410	**
Residual error	1792	5700		1680	5860	

[b] excluding Trial 10 (Palmer - Krause)

*** P<0.001 ** P<0.01

Table 3.11. Summary of mean squares (m.s.) and the significance levels from ANOVAs of environment Set 7.

Source	Set 7		Set 7[a,b]	
	d.f.	m.s.	d.f.	m.s.
Genotype (G)	18	44810 ***	18	43100 ***
Environments (E)	21	5092000 ***	19	4740000 ***
G × E	378	9441 ***	342	9760 ***
Replicates within E	88	5354 ns	80	5804 ns
Residual error	1584	4645	1440	4880

[a] excluding Trial 2 (Palmer - Eichler)

[b] excluding Trial 10 (Palmer - Krause)

*** $P<0.001$ ns $P>0.05$

3.3.2. Principal Component Analysis

Set 4

The first three components of the ordination of Set 4 accounted for 45%, 14% and 12% of the total genotypic variation, respectively. Genotype Principal Component 1 (Eigenvalue 1) was significantly correlated to genotype mean yield ($r=-0.970$, $P<0.01$), and can therefore be considered as a main effect. No relationships were found between response to boron and Genotype Principal Component 2, although genotypes that were observed to be late maturing had Eigenvalues close to zero. Environment Principal Component 1 (Eigenvector 1) was significantly correlated with site mean yield ($r=-0.604$, $P<0.05$) and with April - October rainfall ($r=-0.614$, $P<0.05$). There were no correlations between Environment Principal Component 2 and sowing date or soil pH.

When the data were plotted, natural groupings of genotypes were apparent. Boundaries were drawn around these groups, using genotypic pedigrees and the proximity for the first two dimensions of ordination to assist with the inclusion of genotypes (Figure 3.3). Three major groups of genotypes were distinguished. Groups 1 and 2 were predominantly lines with a

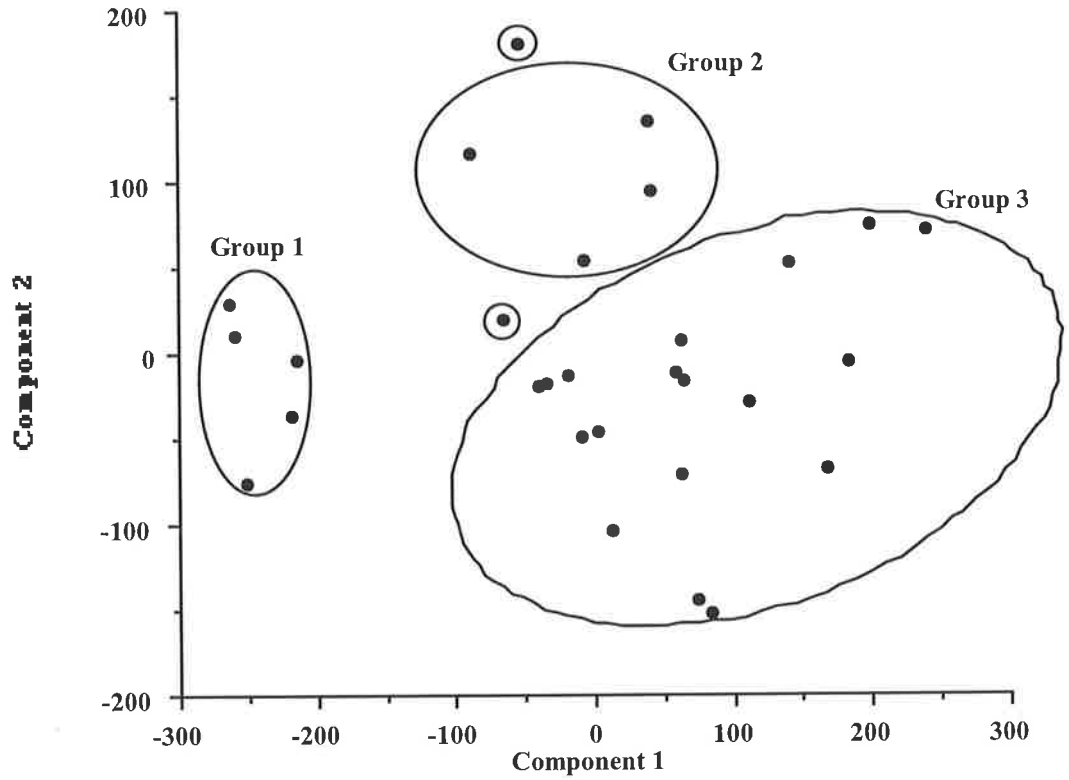
range of tolerance to boron (Chapter 4), while genotypes in Group 3 originated from the NDWIP based in Tamworth, NSW. These form two broad populations which show the influence of parentage or origin in response to the environment. This likely to be a reflection of adaptation of South Australian selected material to shallow soils with low field capacity and NSW material to deep soils which are able to store more water. The two groups, Groups 1 and 2, formed by the backcross population from Chapter 4 were based on maturity rather than on response to boron.

The six environments from the 1995 trials compared to 1996 trials did not form a distinct group, and were not localised on the biplots for Environment Principal Component 2 versus Environment Principal Component 1 (Figure 3.4). The presentation of Environment Principal Component 1 provided limited information since all environments had negative values, although main effects did exist. Environment Principal Component 2 shows there was a contrast between seasons for the trials at Walker Flat, Two Wells, Kapunda and Mallala. Due to contrasting results between seasons for the same locations, it is difficult to target breeding for specific adaptation. In such conditions it would be advantageous to produce widely adapted varieties which are buffered against seasonal fluctuations. This would require testing lines over as many years as possible.

Set 5

The first three components of the ordination of Set 5 accounted for 37%, 22% and 15% of the total variation, respectively. Genotype Principal Component 1 was significantly correlated to genotype mean yield ($r=-0.886$, $P<0.01$). Environment Principal Component 1 was significantly correlated with site mean yield ($r=-0.644$, $P<0.05$) and with April - October rainfall ($r=-0.628$, $P<0.05$).

a)



b)

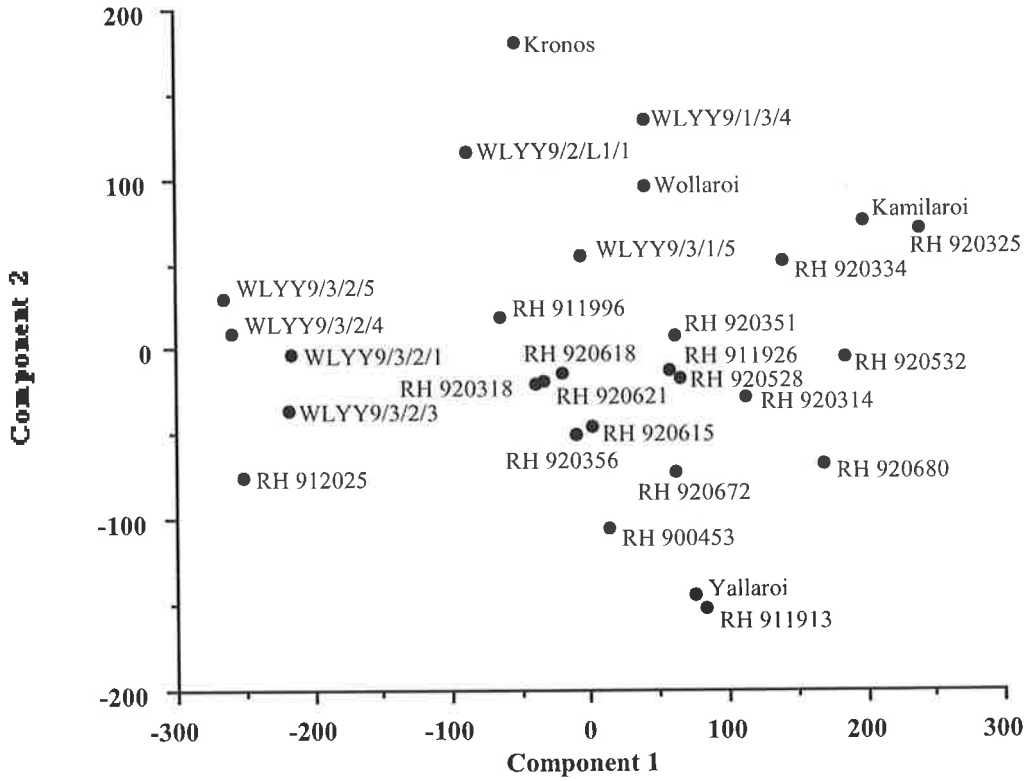


Figure 3.3. Two dimensional biplot of principal components for genotypes in Set 4.

a) Grouped.

b) With genotype labels.

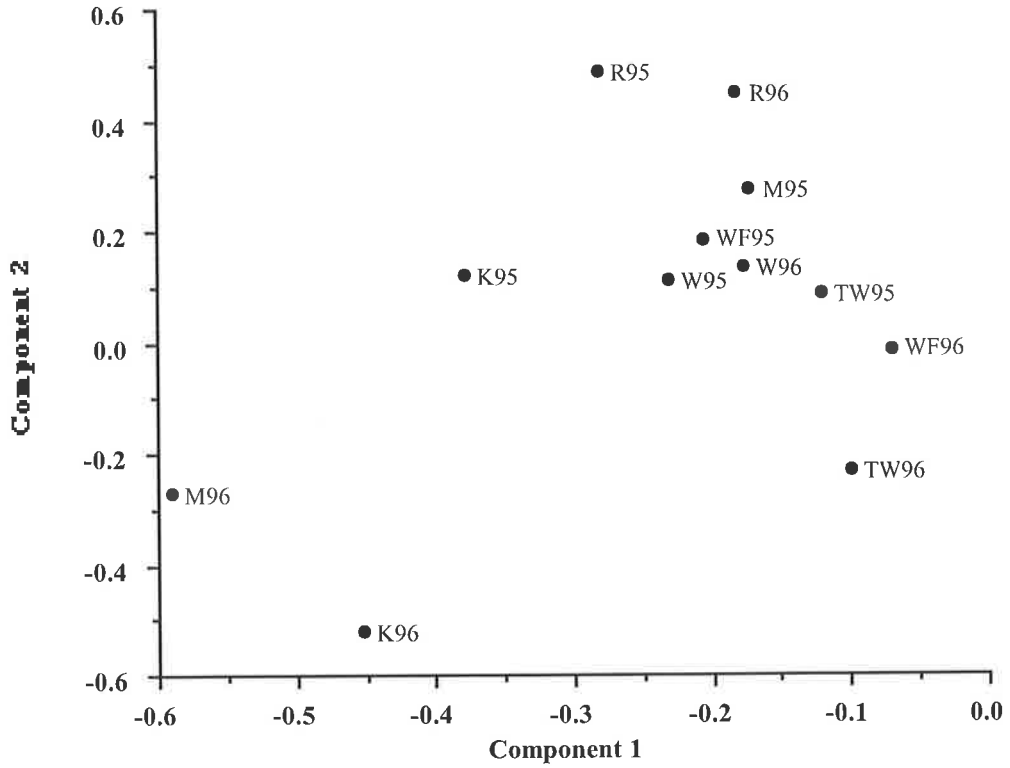
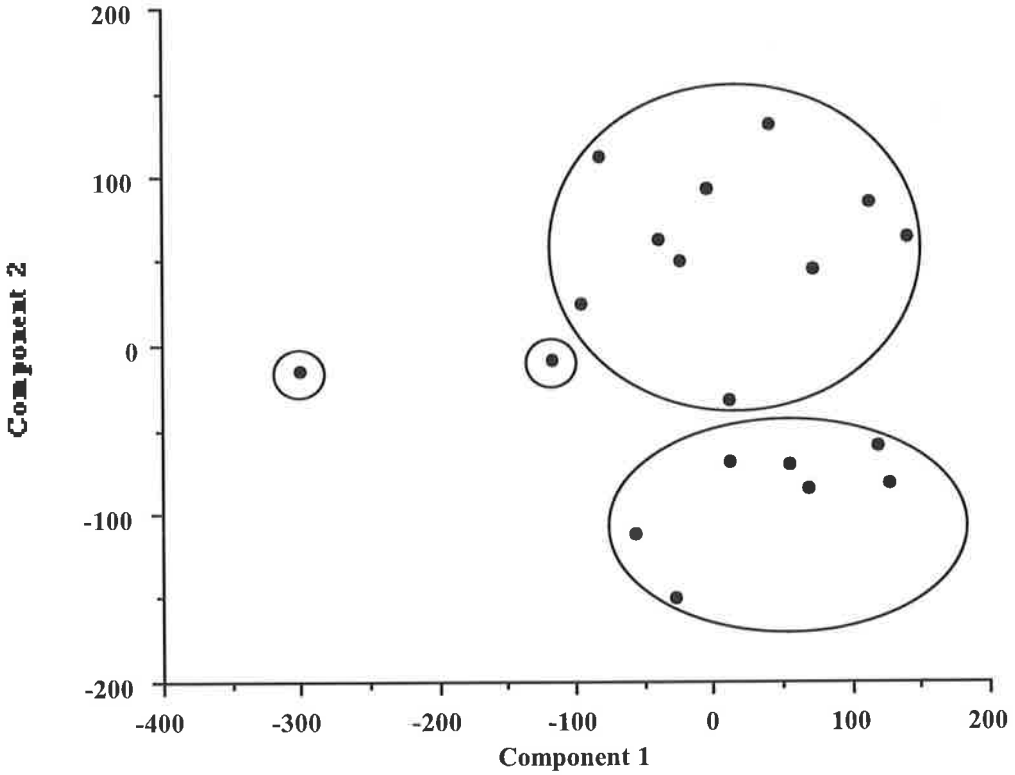


Figure 3.4. Two dimensional biplot of principal components for environments in Set 4. Location codes as in Appendix C.

The absence in Set 5 of the locally bred and selected genotypes compared to Set 4 (see Appendix D3) has resulted in less genotypic differentiation on which to draw group boundaries (Figure 3.5). Principal Component 1 was correlated with yield, and therefore, considered a main effect. Generally, Set 5 was similar to Set 4. However, data from an additional year resulted in some contrasting changes in response along the Principal Component 2 axis. For example, in Set 4 Yallaroi contrasted with Wollaroi (Figure 3.3), whereas in Set 5 the two varieties have positive Eigenvalues and are closer (Figure 3.5). Principal Component 2 may be based on inheritance patterns, since most lines have common parents from a pool of three crosses. The pedigrees of these crosses basically only differ for the three CIMMYT varieties Guillemot, Rokel and Winged. The positive and negative directions of the two groups on Principal Component 2 may be based on segregation of traits from one or a combination of the CIMMYT varieties. The derivatives of Altar 84, a high yielding line from CIMMYT, did not group but nevertheless tended to centre around 0 on the Principal Component 2 axis.

a)



b)

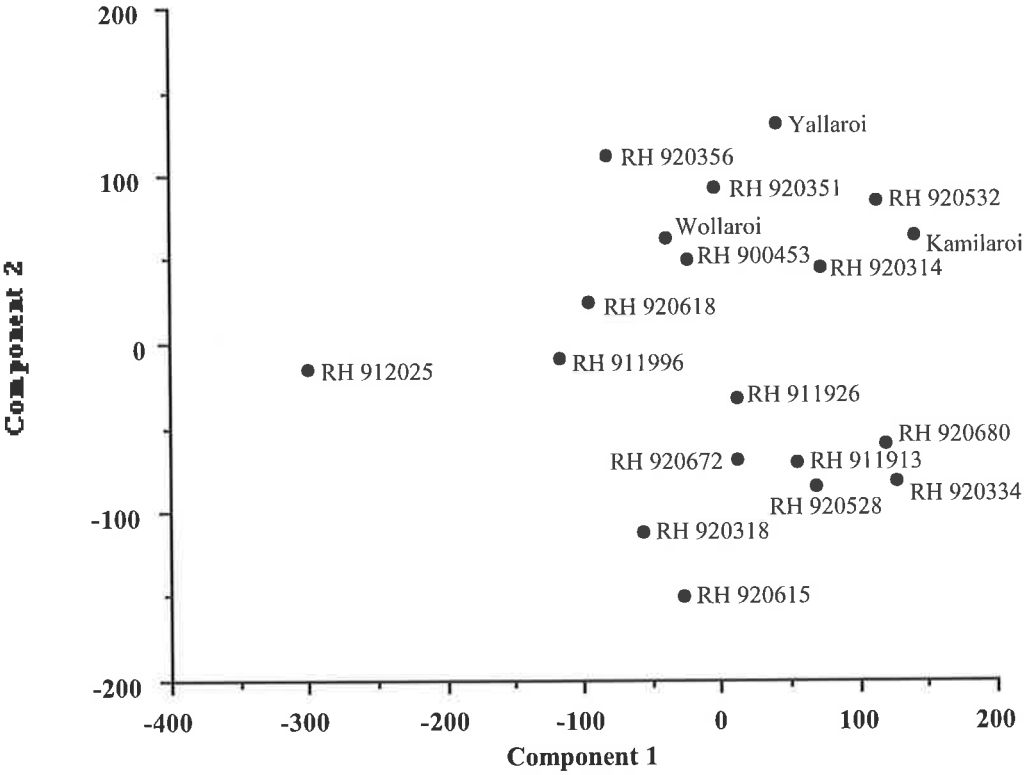


Figure 3.5. Two dimensional biplot of principal components for genotypes in Set 5.

a) Grouped.

b) With genotype labels.

The four locations from 1994 to 1996 did not form a distinct group between the seasons, but four groups are evident on the biplot for Environment Principal Component 2 versus Environment Principal Component 1 (Figure 3.6). There is general grouping of environments in the centre of the biplot. However, Winulta in 1994 and Kapunda in 1994 compared to Winulta in 1995 and Kapunda in 1996, respectively, indicates that a location can be a significantly different environment in different seasons. The marked differences between locations in 1994 compared with 1995 or 1996 are likely to be a reflection of drought in 1994 and a change in genotypic rankings due to water stress.

Set 6

The first three components of the ordination of Set 6 accounted for 41%, 13% and 11% of the total variation, respectively. Genotype Principal Component 1 was significantly correlated to genotype mean yield ($r=-0.950$, $P<0.01$), and Genotype Principal Component 2 with the regression coefficient (b) from adaptation analysis for Set 6 (Appendix D5) ($r=0.628$, $P<0.01$). Environment Principal Component 1 was significantly correlated with site mean yield ($r=-0.720$, $P<0.05$) and with April - October rainfall ($r=-0.725$, $P<0.05$).

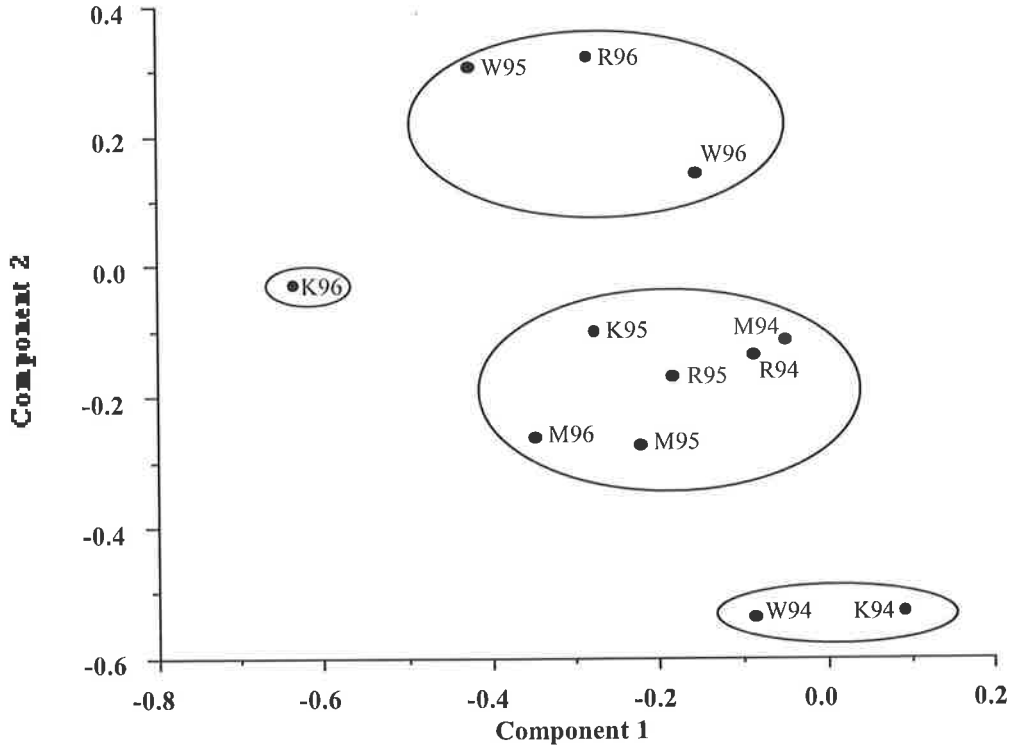


Figure 3.6. Two dimensional biplot of principal components for environments in Set 5. Location codes as in Appendix C.

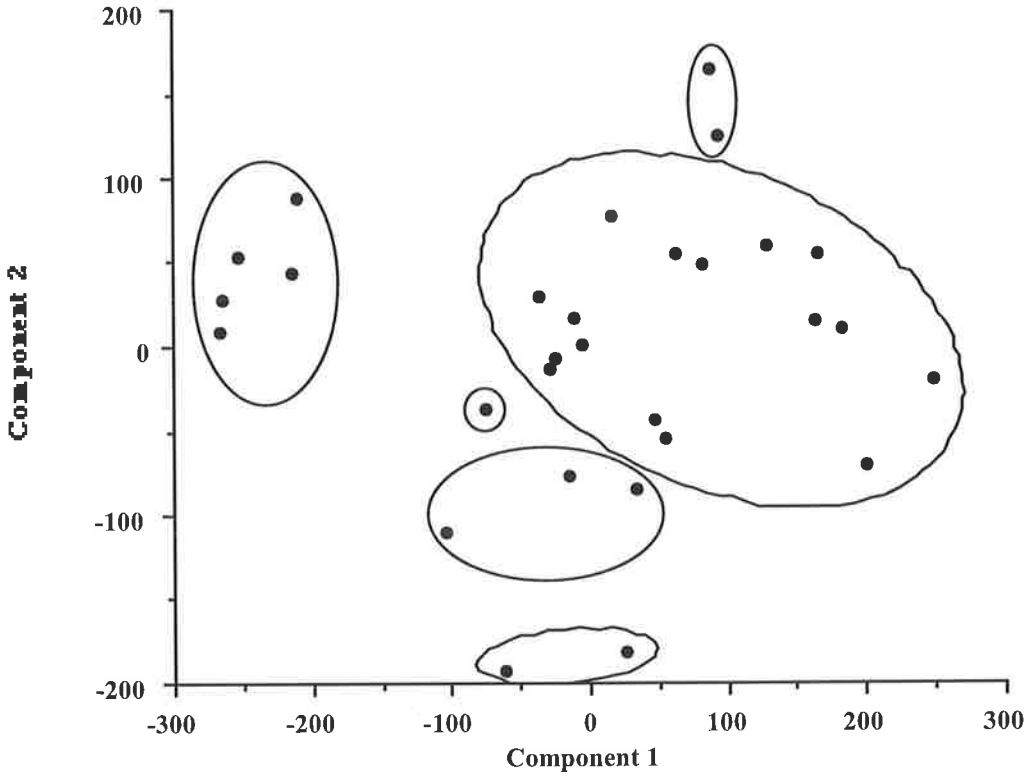
The biplot of genotypes for Set 6 (Figure 3.7) was similar to that for Set 4, although Genotype Principal Component 2 reversed the sign (all positive) of its co-ordinates. The symmetry therefore retained the two distinct populations originating from South Australia and from New South Wales. This indicates that the genotypes from NSW are responding differently to the environments of South Australia, compared with genotypes selected locally. This demonstrates selection of genotypes in NSW will not adequately predict lines suitable for growing in South Australia. Kronos and WLYY9/1/3/4, which flower very early, had large negative scores on the Principal Component 2 axis. These two lines also had non-significant regressions (after excluding Trial 10) in the regression analyis.

The inclusion of all sixteen environments for Set 6 did not dramatically alter the display of the biplot (Figure 3.8) compared to Set 4 (Figure 3.4). Large differences in main effects (Environment Principal Component 1) occurred at Mallala, whilst the largest contrasts along Environment Principal Component 2 remained between the seasons at Kapunda and at Mallala.

Set 7

The first three components of the ordination of Set 7 accounted for 31%, 17% and 14% of the total variation, respectively. Genotype Principal Component 1 was significantly correlated to genotype mean yield ($r=-0.900$, $P<0.01$), and Genotype Principal Component 2 was significantly correlated with the regression coefficient (b) from adaptation analysis for Set 7 (Appendix D6) ($r=0.726$, $P<0.0.1$). Environment Principal Component 1 was significantly correlated with site mean yield ($r=-0.625$, $P<0.05$) and with April - October rainfall ($r=-0.633$, $P<0.05$).

a)



b)

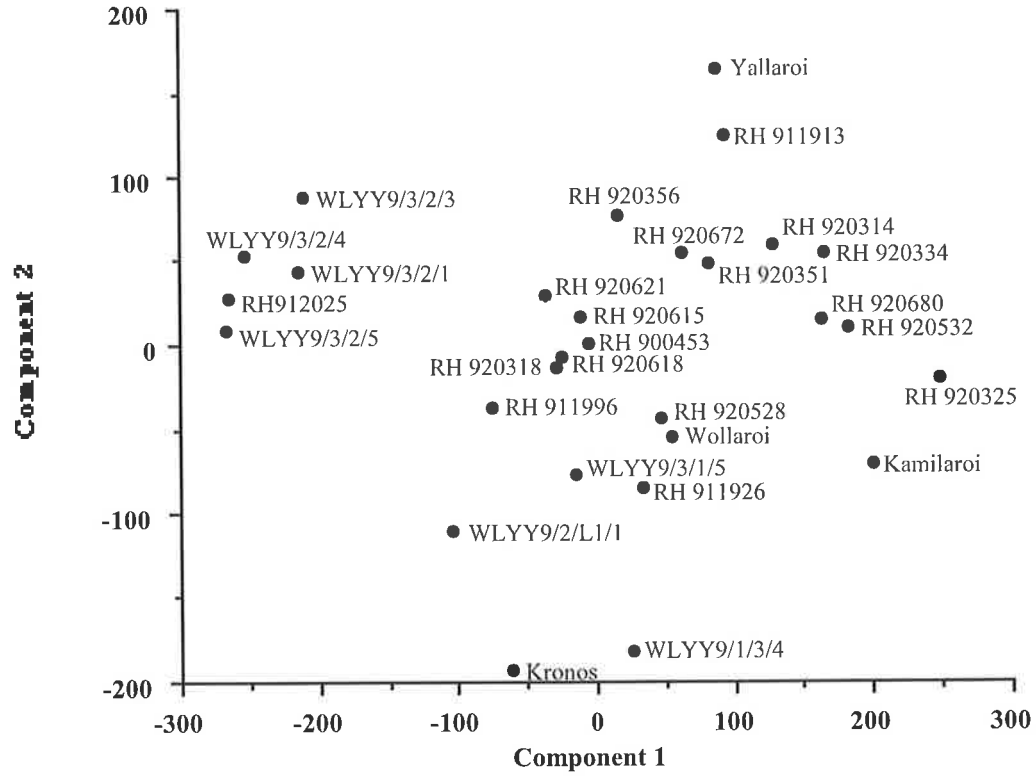


Figure 3.7. Two dimensional biplot of principal components for genotypes in Set 6 (including Trial 10).

a) Grouped.

b) With genotype labels.

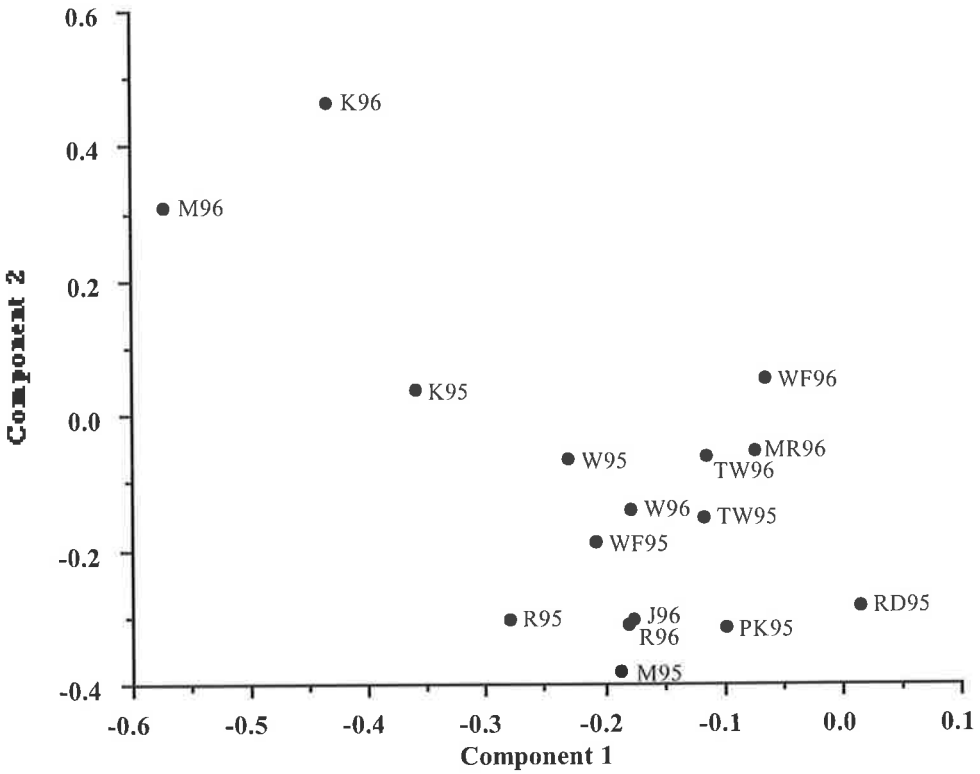
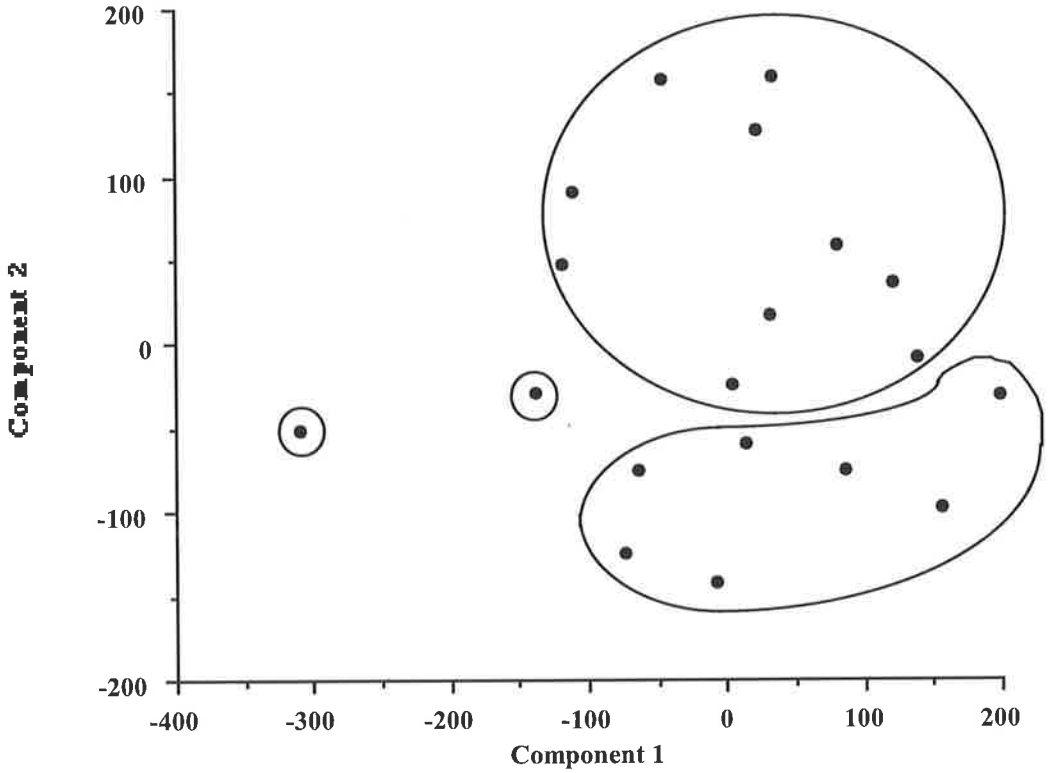


Figure 3.8. Two dimensional biplot of Eigenvectors for environments in Set 6. Location codes as in Appendix C.

The biplot for genotypes in Set 7 (Figure 3.9) discriminated between genotypes similarly as in Set 5 (Figure 3.5). As in Set 6, lines which had non-significant regressions in the adaptation analysis (ie. RH920334, RH920528 and RH920615) had large negative scores on the Genotype Principal Component 2 axis.

Ordination of the 22 environments in Set 7 also produced a biplot (Figure 3.10) similar to that of Set 5 (Figure 3.6). The majority of environments are closely grouped in the centre of the biplot. The largest variation along Environment Principal Component 1 occurred at Kapunda in 1994 and in 1996. For Environment Principal Component 2, the greatest amount of variation between locations occurred at Winulta in 1994 and in 1995, and at Kapunda in 1994 and 1996.

a)



b)

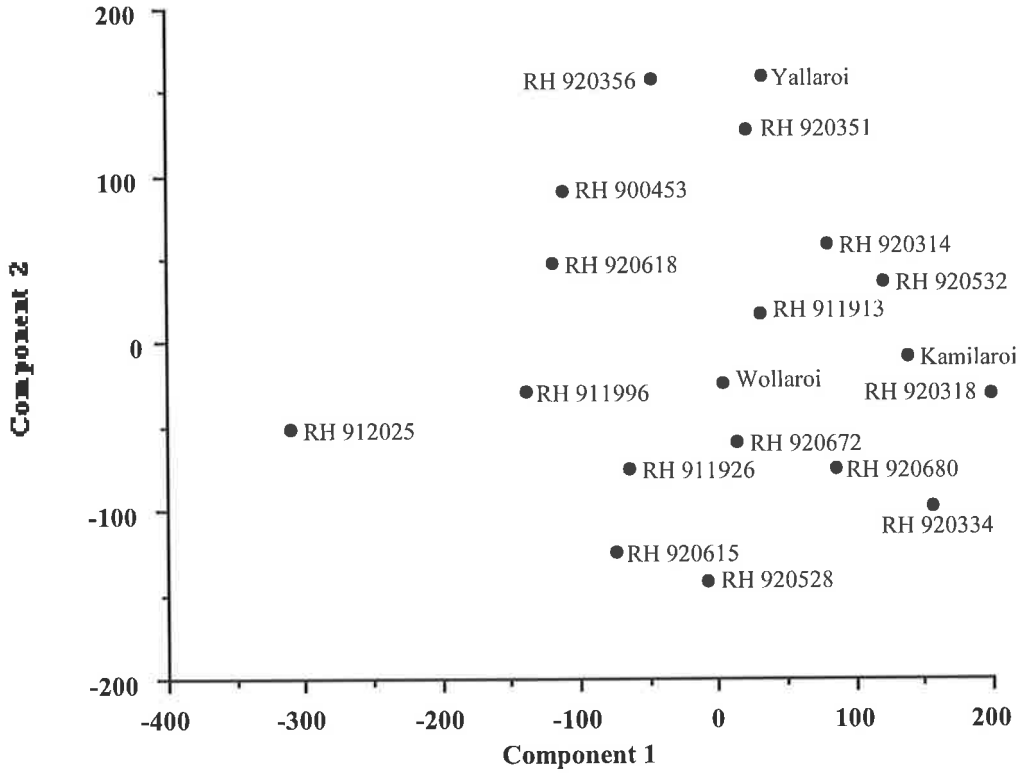


Figure 3.9. Two dimensional biplot of principal components for genotypes in Set 7.

a) Grouped.

b) With genotype labels.

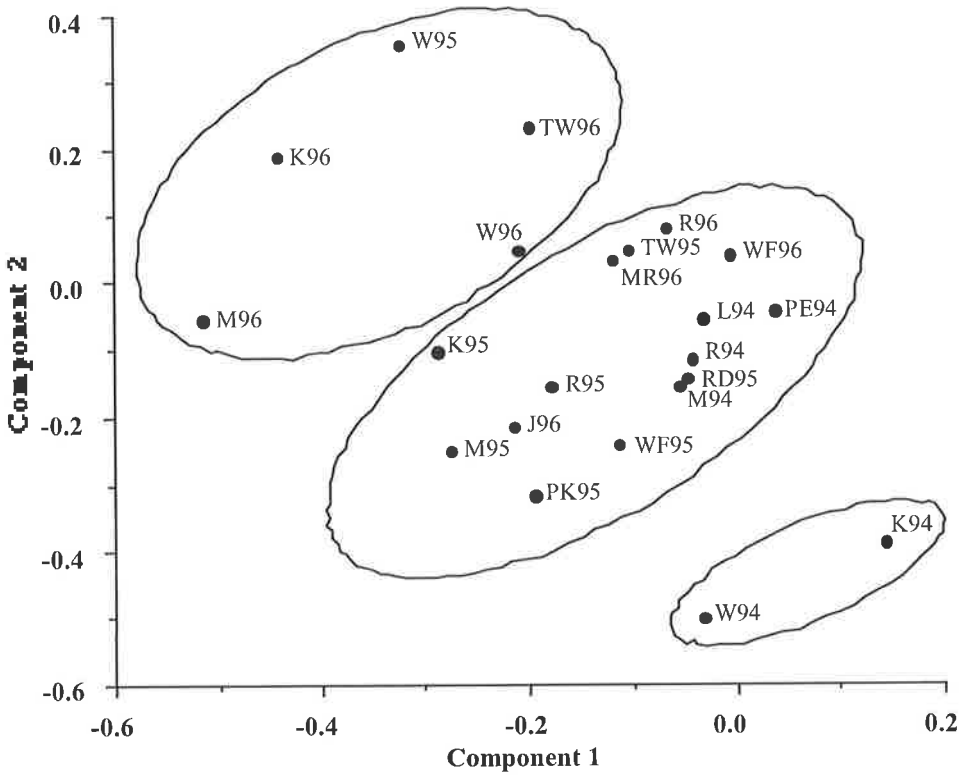


Figure 3.10. Two dimensional biplot of principal components for environments in Set 7. Location codes as in Appendix C.

3.3.3. Regression - adaptation analysis

Set 6

The regression coefficients produced by adaptation analysis for the genotypes in Set 6 are presented in Appendix D5. Regression coefficients for the durums ranged from 0.331 to 1.035, which combined with intercepts on the Y-axis less than Spear, indicates that the durums performed relatively poorer than Spear in unfavourable conditions. The regressions of three genotypes compared to Yallaroi are shown in Figure 3.11, to illustrate the range in adaptation responses. RH920351 had a regression coefficient close to one and mean yield similar to Yallaroi, which indicated RH920351 had no yield improvements on Yallaroi. WLYY9/3/2/1 had a regression coefficient similar to Yallaroi, although mean yield performance was higher than for Yallaroi. This shows WLYY9/3/2/1 was more adapted than Yallaroi to all environments. Although not presented in Figure 3.11, RH912025 (which had

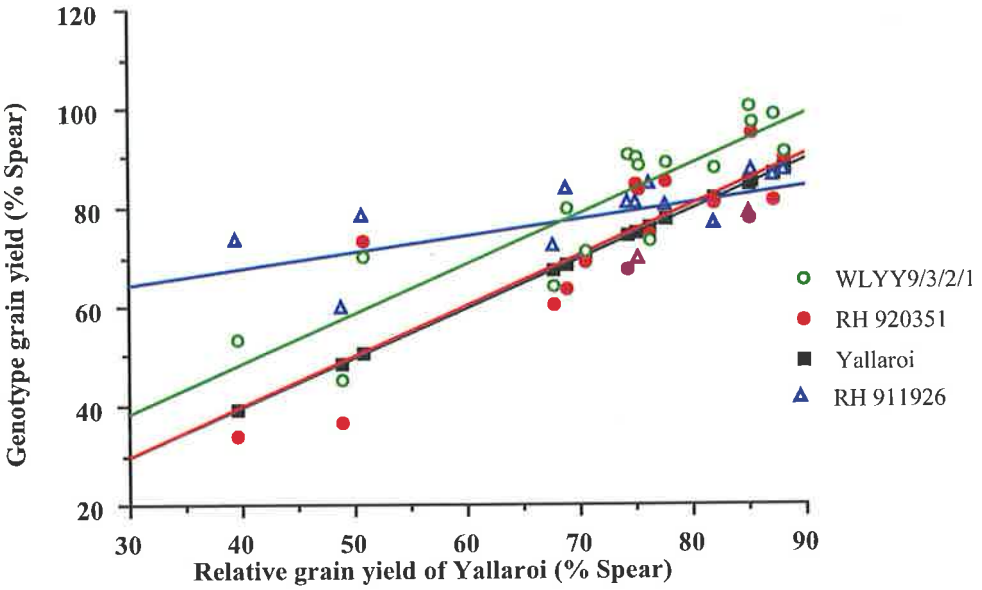
the highest mean yield), had a regression coefficient of 0.855, indicating that this genotype was also better adapted than Yallaroi. RH911926 had the lowest regression value of the durum genotypes, indicating some characteristics of adaptation similar to the bread wheat Spear. This was demonstrated by the ability of RH911926 to produce higher yields in environments where Yallaroi compared less favourably with Spear. The absence of any genotypes with a regression coefficient significantly greater than Yallaroi suggests, of all the genotypes tested, Yallaroi is most suited to favourable growing conditions.

The effect of removing Trial 10 from the analysis tended to decrease the regression coefficient (Figure 3.11b). This was because Trial 10 was infected with crown rot, to which durum wheat is classified as susceptible, and the disease is a major factor causing yield loss. In comparison, resistance to crown rot exists in bread wheat, and subsequently grain yield is not greatly affected. Also in this analysis, the slopes for Kronos and WLYY9/1/3/4 were non-significantly different to 0 (ie. indicating similar to Spear in adaptation, but approximately 100 g/plot lower yielding).

Set 7

The regression coefficients produced by adaptation analysis for the genotypes in Set 7 are presented in Appendix D6. Regression coefficients for the durums ranged from 0.303 to 1.014, with Y-axis intercepts less than Spear, demonstrating that the durums had relatively lower yields than Spear in unfavourable conditions. The adaptation responses of three genotypes compared to Yallaroi are shown in Figure 3.12. The genotype RH912025 had the highest mean yield, combined with a regression coefficient of 0.895. This demonstrated RH912025 was better adapted than Yallaroi, as it was higher yielding in all environments, and may also be useful as a parent in the breeding program. The line RH920351 had a regression coefficient of 1.014 and a mean yield similar to Yallaroi, which shows both lines had the same yield response. The lines RH920528 and RH911926 had low regression values, 0.303 and 0.327, respectively, suggesting adaptation characters more similar to the bread wheat Spear than to Yallaroi.

a)



b)

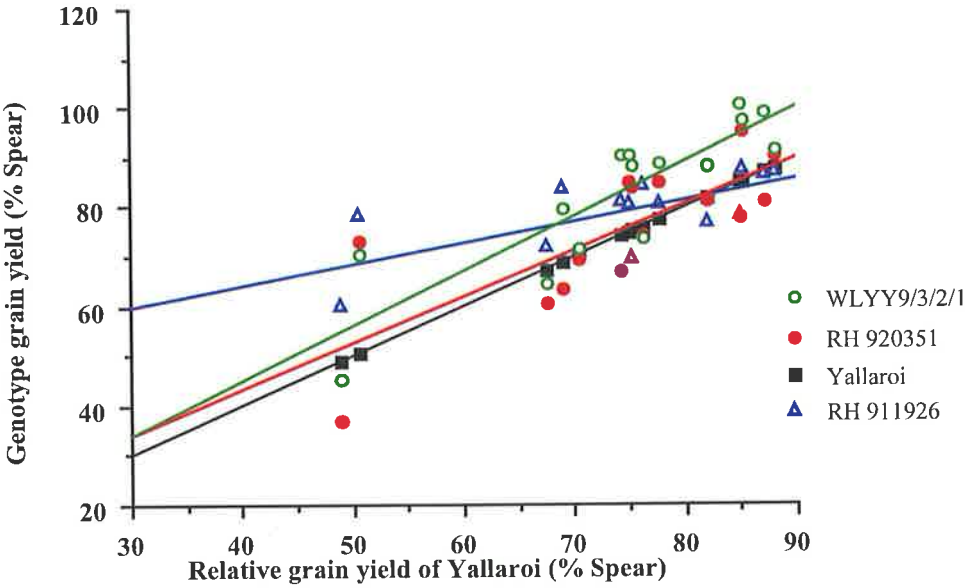
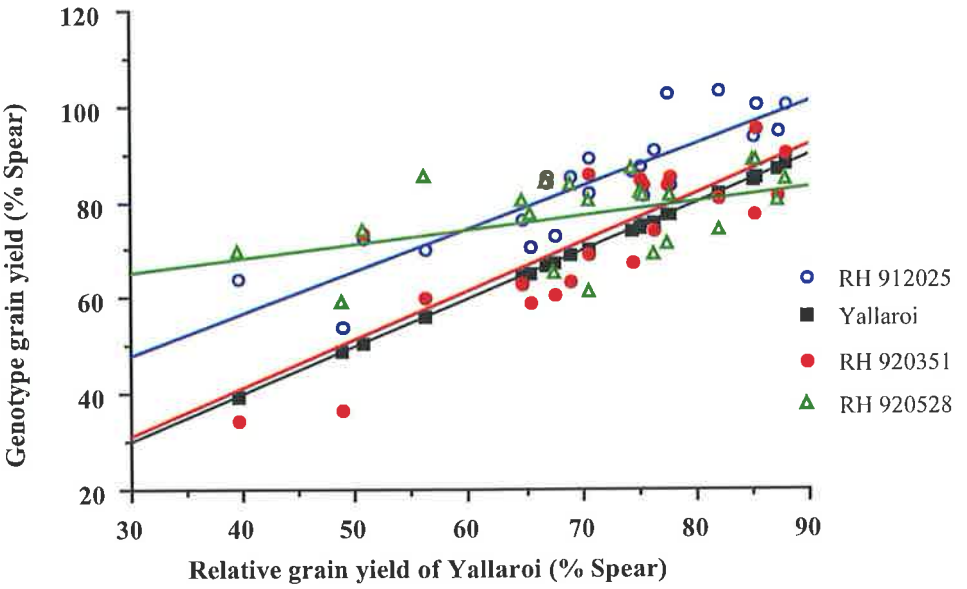


Figure 3.11. Relationship between individual and environment yields relative to Spear for four durum genotypes. Yallaroi is indicated with a slope=1.

a) For environment Set 6 (16 environments).

b) For environment Set 6 with Trial 10 excluded.

a)



b)

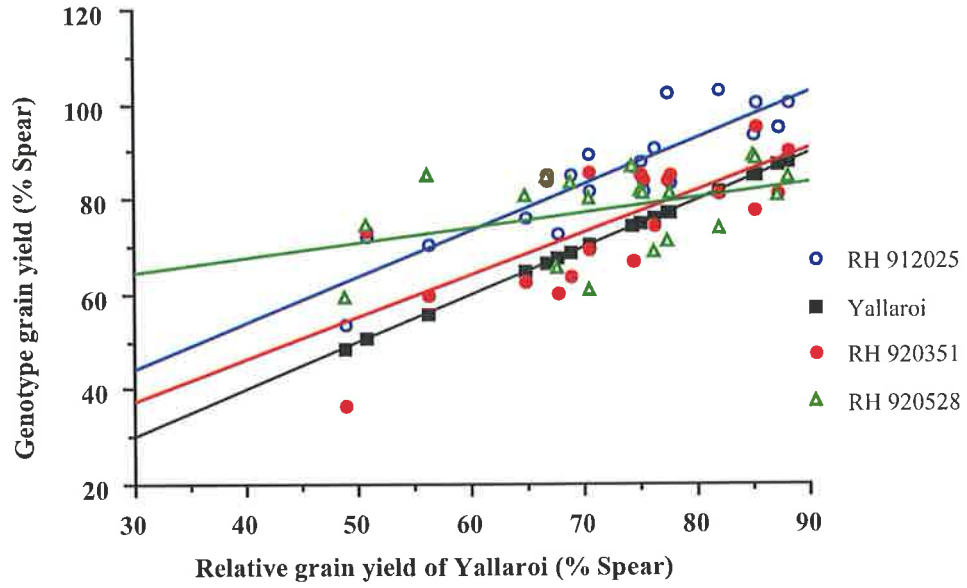


Figure 3.12. Relationship between environment and individual yields relative to Spear for four durum genotypes. Yallaroi is indicated with a slope=1.

a) For environment Set 7 (22 environments).

b) For environment Set 7 with Trials 2 and 10 excluded.

Excluding Trial 2 and Trial 10 from the analysis did not have any significant effect on the regression coefficients (Figure 3.12b). With the exclusion of Trial 10, a decrease in the regression coefficients would have been expected, as in Set 6, but the simultaneous exclusion of Trial 2 reduced this effect. The exclusion of two points reduced the degrees of freedom, reducing the significance of some regressions for the genotypes. Those genotypes which had non-significant regressions included RH920334, RH920528 and RH920615.

3.3.4. Spatial Analysis

The mean yields for each of the 22 trials examined in this analysis are presented in Table 3.12. Mean site yields were highly variable in the seasons 1994 - 1996, ranging from 109 to 536 g/plot (equivalent of 0.36 to 1.79 t/ha), 220 to 935 g/plot (0.73 to 3.12 t/ha), and 256 to 794 g/plot (0.85 to 2.65 t/ha) in 1994, 1995 and 1996, respectively. Generally, the low yields obtained in 1994 were associated with drought conditions, while higher yields occurred in 1995 and 1996.

The correlations with average site for each of the trials are presented in Table 3.12. The correlations indicate that Mallala and Roseworthy performed differently each year, whereas Kapunda and Winulta were relatively stable across years. The trial which performed closest to the average environment for this set of trials was Walker Flat in 1996. This site best represents how each variety would perform on average given the environments present in the analysis. Selection of genotypes for promotion can be based on data from this trial.

Table 3.12. Mean yields (t/ha) and correlations (r) with average site for each of the trial locations in environmental Set 8.

Locality	1994			1995			1996		
	Trial	Yield	r	Trial	Yield	r	Trial	Yield	r
Lowbanks	1	0.36	0.414						
Palmer - Eichler	2	0.60	0.696						
Rudall				9	0.73	0.863			
Palmer - Krause				10	0.79	0.929			
Minnipa							17	0.85	0.832
Jamestown							18	1.69	0.775
Walker Flat				11	1.40	0.858	19	1.62	0.999
Two Wells				12	1.77	0.647	20	1.76	0.734
Kapunda	5	1.27	0.568	13	3.12	0.560	21	2.45	0.475
Mallala	6	0.85	0.591	14	1.80	0.874	22	2.65	0.364
Roseworthy	7	0.91	0.748	15	2.59	0.662	23	2.49	0.399
Winulta	8	1.79	0.858	16	2.59	0.675	24	2.61	0.780

The environmental loadings of the trials are presented in Table 3.13. Environmental loadings must be measured against the correlation with average site. A high correlation with the average site, such as Walker Flat 1996, indicates the site gives average performance and therefore has a low environment loading. Sites with low correlations with the average site have environment loadings that depart from zero in either a negative or positive direction. A very low correlation and a very high environmental loading, such as Mallala 1996, indicates that a particular trial had a strong environmental effect. Results in Chapter 4 suggest Mallala suffered nutrient deficiency in 1996.

Table 3.13. Environment loadings for each of the trials 1994 - 1996.

Locality	1994	1995	1996
Lowbanks	-0.558		
Palmer - Eichler	-0.395		
Rudall		-0.397	
Palmer - Krause		-0.118	
Minnipa			-0.062
Jamestown			0.190
Walker Flat		0.146	-0.053
Two Wells		-0.310	0.120
Kapunda	0.269	0.503	0.671
Mallala	-0.715	-0.146	0.931
Roseworthy	-0.492	0.278	0.212
Winulta	0.193	0.010	0.367

A dendrogram showing how all 22 trials are related was produced (Figure 3.13). Four groups are clearly shown. The trials at Kapunda are close together, but the more unstable locations like Roseworthy and Mallala are spread through all groups. The group containing locations Lowbanks, Palmer – Eichler, Mallala and Roseworthy in 1994 represents the lowest yielding sites in the drought. The grouping of Roseworthy and Walker Flat in 1995, and Two Wells, Jamestown and Winulta in 1996 represents locations where toxic levels of boron were found in Yallaroi (Chapter 4).

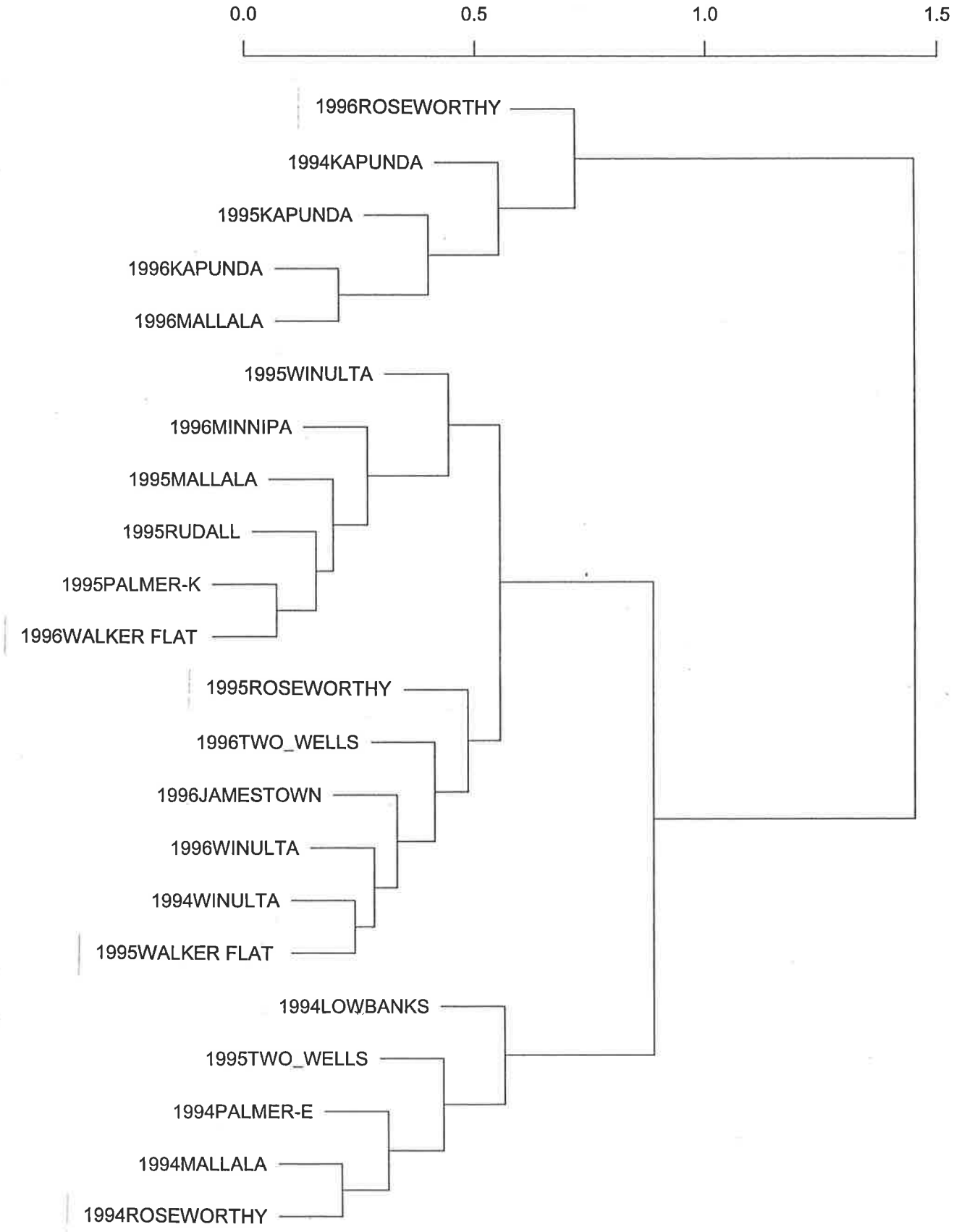


Figure 3.13. Dendrogram for classification of the 22 sites 1994 - 1996 based on correlations between sites.

The average predicted yield of the genotypes is presented in Table 3.14. The predicted yields of durum ranged from 453 g/plot (1.51 t/ha) for RH920325 to 546 g/plot (1.82 t/ha) for WLYY9/2/6/3, compared with 631 g/plot (2.10 t/ha) for the bread wheat Spear. Yallaroi was ranked fifth lowest. The variety common effects were plotted to show the performance of the different varieties across the environments (Figure 3.14). Kronos (var. 5) displayed high yield and stability (ie. minimum environment effect). RH911996 (var. 24), situated in the centre of the plot, had average yield and performs roughly the same at all sites. RH912025 (var. 26) (second ranked durum for predicted yield) was grouped close to RH911996, but displayed less yield stability. The bread wheat Spear (var. 54) had the highest yield, but unexpectedly, was not rated stable.

3.4 Discussion

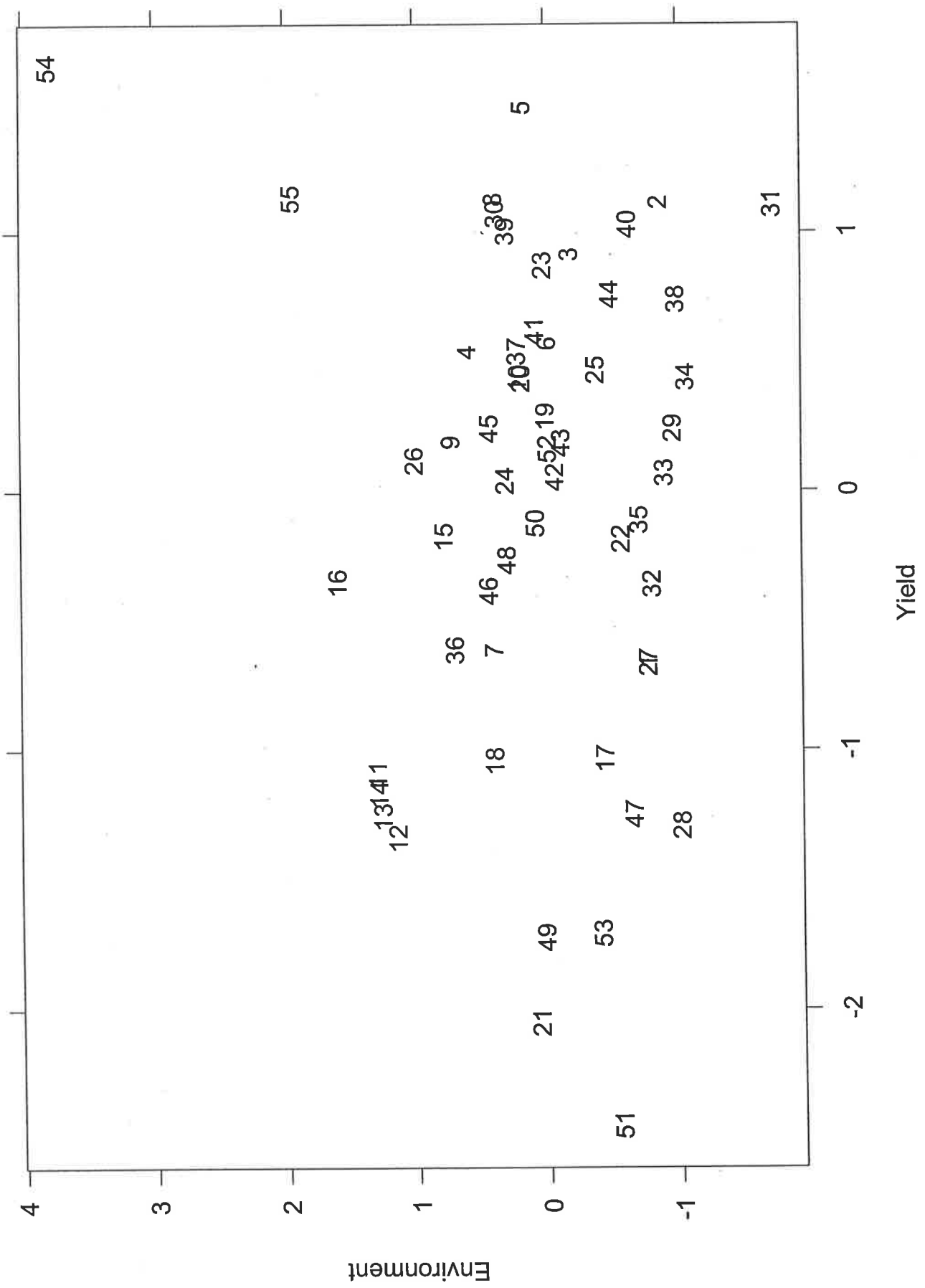
Environmental variation

The rainfall during the months April to October was a major factor influencing the grain yield of durum wheat in South Australia. Durum wheat had lower grain yields for a given amount of rainfall than the bread wheat Spear, however, genotypic variation existed within the durums. The yield response of Spear (3.02 g/plot/mm rainfall) was greater than durum (2.70 g/plot/mm). This suggests durum has a lower water use efficiency, total dry matter or lower harvest index (Shorter *et al.*, 1991) than Spear.

Table 3.14. Average predicted yield for all 55 genotypes grown 1994 - 1996.

Line	Genotype	Predicted Yields (t/ha)
1	Yallaroi	1.56
2	Kamilaroi	1.60
3	Wollaroi	1.67
4	Altar 84	1.74
5	Kronos	1.72
6	Simeto	1.67
7	WLYY9/1/2/2/1	1.69
8	WLYY9/1/3/4	1.73
9	WLYY9/2/L1/1	1.74
10	WLYY9/3/1/5	1.69
11	WLYY9/3/2/1	1.77
12	WLYY9/3/2/3	1.74
13	WLYY9/3/2/4	1.76
14	WLYY9/3/2/5	1.76
15	WLYY9/1/4/4/S	1.74
16	WLYY9/2/6/3	1.82
17	BB 94059/-aL1	1.59
18	(LYY9/-1a*Yal)/4	1.67
19	RH900218	1.67
20	RH900453	1.69
21	RH911840	1.61
22	RH911913	1.60
23	RH911926	1.69
24	RH911996	1.69
25	RH912023	1.63
26	RH912025	1.77
27	RH920274	1.56
28	RH920276	1.52
29	RH920314	1.57
30	RH920318	1.73
31	RH920325	1.51
32	RH920326	1.57
33	RH920334	1.57
34	RH920351	1.56
35	RH920356	1.58
36	RH920405	1.72
37	RH920528	1.70
38	RH920532	1.58
39	RH920615	1.72
40	RH920616	1.62
41	RH920618	1.68
42	RH920621	1.65
43	RH920672	1.65
44	RH920680	1.63
45	RH920777	1.71
46	AUS 19764	1.70
47	Yavaros 'S'	1.56
48	Cormorant	1.68
49	EDYT18-10	1.62
50	EDYT18-19	1.67
51	EDYT19-14	1.54
52	Daki=Cyn	1.66
53	Hagla	1.57
54	Spear	2.10
55	Molineux	1.89

Figure 3.14. The common variety effects of environment against common effects of yield for the 55 genotypes grown 1994 - 1996. Numbers for each genotype are given in Table 3.1.



The influence of rainfall on yield has been analysed previously for other cereal crops in southern Australia (Cornish, 1950; Sparrow, 1972). Goodchild and Boyd (1975) showed that variation in wheat yields from year to year was determined more from the variation in rainfall between seasons than from variation within the growing season. Although total rainfall for locations within a season may be different, each location would tend to receive rainfall at approximately the same time because of the nature of the weather systems. The timing of rainfall between seasons is more variable, and is thus more likely to exert a greater influence on the performance of genotypes. This was demonstrated by significant year effects and non-significant location effects in the ANOVA of Set 5 (four locations over three years). In South Australia, a rapid increase in temperature and evaporation during late spring can result in severe moisture stress, having a marked effect on yield of wheat if it coincides with the start of flowering and grain filling. The remaining variability in yield is due to factors such as physical and chemical properties of the soils and severity of diseases.

In dryland environments, matching time of flowering and maturity with rainfall supply is important for the adaptation of genotypes (Loss and Siddique, 1994). However, in South Australia the climate is not predictable enough to fit varieties to rainfall distribution. The majority of genotypes tested had minimum photoperiod sensitivity (Hare, 1994) and were earlier than the bread wheats. Therefore lower yields of durum compared to bread wheat in environments with terminal water stress was unlikely to be a function of phenology. This could be tested by looking at a group of semi-dwarf genotypes with a range of flowering times. Ceccarelli (1997) has reported that the superiority of barley landraces under stress (low rainfall) conditions was not associated with escape mechanisms such as heading date. Other factors must be contributing to the lower water use efficiency of durum wheat compared with bread wheat in South Australia. Limitations which influence bread wheat yields in this state are boron toxicity, sodicity, trace element deficiencies, and root diseases including cereal cyst nematode and root lesion nematode (Hollamby, 1996). Further research on the effects of these factors on durum yields and assessment for tolerance/resistance is warranted.

Genotype × environment interaction

Genotype × location × year (G×L×Y) interaction accounted for most of the observed variation. Most of the variation was associated with L×Y effects, which indicates that yield evaluation over a number of locations and years is necessary in South Australia. Nyquist (1991) recommended the use of the cross classification model (partitioning environment into year and location components) where possible, unless there is *a priori* information to suggest that the G×Y and G×L interactions are unimportant. This was the case in the present study, where G×L and G×Y were relatively small, suggesting some consistency in the performance of genotypes over locations and years. Analysis of G×L×Y data also becomes complicated by imbalance, due to the way genotypes change over time, and to the loss of whole experiments at some locations in some years, highlighted by the loss of Trial 3 at Two Wells in 1994 due to crown rot. This can now be overcome by spatial analysis.

Analysis of variance for environment Sets 4 and 5 showed G×L×Y terms were predominant which, according to Romagosa and Fox (1993), indicates no simplification involving spatial subdivision of breeding regions is available. However, when spatial analysis was performed, four groups of environments where shown. The durum breeding program for South Australia needs to aim at producing durum genotypes which are widely adapted. Specific adaptation can only be exploited if consistent biotic and/or edaphic stresses are identified. Spatial analysis grouped sites with low rainfall and boron toxicity. Testing needs to be conducted over a representative range of environments, and results in this study show this should include a low rainfall and boron toxic site. This strategy would prevent the gene loss that could occur if testing was done only in non-target environments. To expand durum production into marginal areas testing must be conducted in these areas. Sometimes a breeder's selection environments in one year may have little in common to those experienced in the next (Fox *et al.*, 1985). The sampling problem associated with yearly variation reaffirms the need to identify the specific attributes of the environment that contributes to this variation.

Explanations for G×E interactions

To be able to understand the G×E interaction and utilize it effectively in a breeding program, as much information as possible is needed on the factors responsible for the differential response of genotypes to variable environments. Despite the large number of publications on the subject of G×E interactions, achieving physiological analysis of patterns of genotypic performance has been more difficult than analytical methodology. Principal Component Analysis for the durum wheat genotypes examined in environment Set 4 differentiated groups of genotypes into region of selection origin. It was more difficult to determine natural groupings on the biplot of genotypes in Set 5. This was due to the genotypes being crosses from a pool of three parents used in the NDWIP. This suggests a need for increasing genetic diversity. The inclusion of germplasm from CIMMYT in the spatial analysis demonstrated it was more widely adapted than material from the NDWIP, and therefore is a potential source for improving diversity. Genotypic classification is not possible in spatial analysis as it is assumed varieties come from a single population. However, site classification produced four distinct groups, one of which could be attributed to drought in 1994, and another to boron toxic locations (Chapter 4). Genotypes which differed in genetic or selection origin have also been identified using pattern analysis studies by Mungomery *et al.* (1974) in soybean, Byth *et al.* (1976) and Crossa *et al.* (1991) in wheat, and Fox *et al.* (1990) in triticale. Other studies by Imrie *et al.* (1981) and Imrie and Shanmugasundaram (1987) in mungbean, Cooper *et al.* (1994a, 1994b) in wheat, Jackson *et al.* (1993, 1994) in barley, and Chapman *et al.* (1997a, 1997b) in maize provided physiological explanations for some of the G×E interactions observed in multi-environment trials.

Zubaidi (1996) found the major factors affecting the yield of durum relative to bread wheat in South Australia were that durum tillered less and produced fewer ears/m^2, had poor early vigour and reduced root growth in nutritionally poor soil. These factors are major contributors to low water use efficiency. Other physiological explanations include the

inefficiency of durum in obtaining trace elements from deficient soils (Graham, 1988), and high accumulation of sodium in plant tissue (Brooks, 1991). Genotypic differences exist for these traits, which indicates that improvements in durum varieties can be directed through breeding.

Further research is necessary to identify genotypic variation to pathogens (eg. *Fusarium* spp.) and pests (eg. *Pratylenchus* spp.). Infection with crown rot (*Fusarium* spp.) resulted in death of the experiment at Two Wells in 1994, and reduced yields to less than 55% of Spear at Palmer in 1995. Crop losses due to crown rot are likely to have severe economic implications for farmers. Finding resistance to crown rot (*Fusarium* spp.) in durum wheat is a breeding objective for durum breeders around the world, but no source of resistance in durum has yet been identified. The root lesion nematode (*Pratylenchus neglectus*) was found to cause a yield loss in bread wheat trials at Minnipa in 1996 (Vanstone *et al.*, 1998). Trials are conducted at Minnipa as excessive concentrations of B in the subsoil have been reported (Moody *et al.*, 1993), but no yield advantage for tolerance to B in durum was found at this site in 1996 (Chapter 4). Low water use efficiency by durum occurred at Minnipa in 1996, which may have been caused by root lesion nematode damaging the root system, and also contributed to the unexpected low concentrations of B found in the grain (see Chapter 4).

Principal Component Analysis

For environment Sets 6 and 7 Genotype Principal Component 1 was correlated with variety mean yield, and Genotype Principal Component 2 was correlated with regression coefficients from the adaptation analysis. Plotting genotypic regression coefficients against genotype mean yield is the basis of Finlay and Wilkinson's (1963) interpretation of adaptation. The correlations between the indices used by Finlay and Wilkinson (1963) and the principal components indicates that the biplot of Genotype Principal Component 2 vs Genotype Principal Component 1 produces a scatter diagram representing adaptation. Similarly for

Spatial Analysis (Set 8), this interpretation is represented by plotting common effects due to site (or environment) vs variety common effects.

To summarize, genotypes with Genotype Principal Component 2 scores approximating 0 indicate average stability. When this is combined with a large negative Genotype Principal Component 1 score, genotypes are well adapted; when combined with a large positive Genotype Principal Component 1 score, genotypes are poorly adapted to all the environments. Genotype Principal Component 2 scores above 0 describe genotypes with increasing sensitivity to environmental change (below average stability), and greater specificity of adaptability to high yielding environments. Genotype Principal Component 2 scores below 0 provide a measure of greater resistance to environmental change (above average stability), and therefore increasing specificity of adaptability to low yielding environments.

Performance of the genotypes may be predicted from their relative position on the generalized biplot (Figure 3.15). Interpretation of adaptation applied to the data in Set 7 plotted in Figure 3.9 indicates RH912025, for example, was well adapted to all environments; Yallaroi was specifically adapted to favourable environments; and RH920528 was specifically adapted to unfavourable environments. Similarly, these conclusions were reached for Set 7 in the regression – adaptation analysis.

The concept of stability measurement using PCA has been expounded by Lin *et al.* (1986) whereby the stability of a genotype is related to that of a well known genotype near where it falls in a biplot derived from ordination. This form of stability is useful where experience with previous genotypes, against which stability is measured, provides a frame of reference from which the stability and adaptation of new genotypes can be assessed. For example, in the future, the advanced line RH912025 will provide a reference point on the biplot for genotypes which are well adapted.

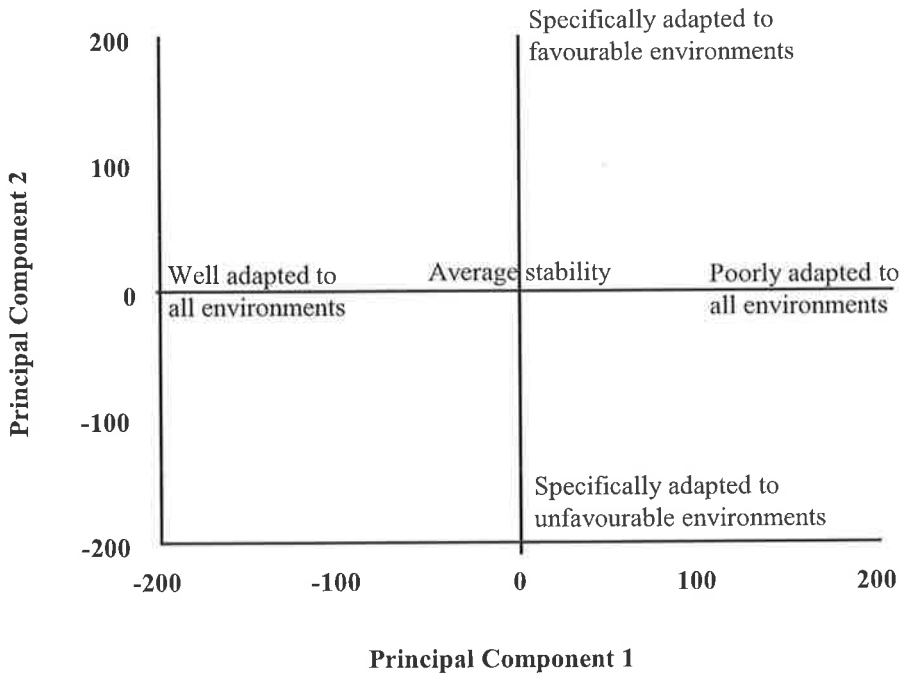


Figure 3.15. A generalized interpretation of the genotype pattern obtained for the biplot of principal components.

Regression - Adaptation analysis

The use of regression analysis provided a measure of yield stability and, hence, adaptation. The variates used for adaptation analysis produced a response similar to the relationship between the yield of durum relative to Spear compared to April - October rainfall. Since rainfall was a major determinant of grain yield of durum, this approach is likely to be advantageous compared to using relative yield of Spear as the independent variable in adaptation analysis. Seif and Pederson (1978) reported a similar finding for spring rainfall in comparison to site mean yield.

The regression analyses of the durum wheats indicated their yields were less stable than that of bread wheat Spear, and that compared to Spear they yielded poorly under unfavourable conditions. The low yields of durum under such conditions provides an opportunity to investigate the causal factors, which will assist in the definition of future breeding objectives.

The genotypes RH920528 (Figure 3.12), RH920334 and RH920615 in Set 7 had highly variable yields such that, after removing environments with a high coefficient of variation from the analysis, the lines of regression were non-significant. Non-significant b-values suggest they are non-significantly different to zero, and hence have adaptation similar to Spear. Locations where outliers occurred suggest specific attributes of adaptation which may be exploited. High yielding outliers may be indicative of resistance and/or tolerance to the constraint limiting yield, and these lines could be used as parents to further improve yield. Research is necessary to identify the traits conferring higher yields to such outliers, and to implement a screening method to select desirable progeny.

Spatial Analysis

Classification of environments produced four distinct groups, compared to the lack of discrimination displayed in PCA biplots. This is likely to be due to the larger size of the genotype data set in the spatial analysis. The trials at Kapunda were close together, indicating relative stability across years. This is in contrast to the results obtained in PCA (Set 7) where the greatest amount of variation between locations occurred at Kapunda in 1994 and 1996. Set 7 of PCA had a subset of common genotypes, predominantly advanced lines from the NDWIP, to achieve a balanced data set. Set 8 for Spatial Analysis additionally included CIMMYT germplasm in 1994, and in 1996 a set of sister lines with different responses to B toxicity. Comparison of the two analyses suggests that there was a change in rankings in the NDWIP advanced lines, but the additional CIMMYT and local genotypes had more consistent grain yields and were better adapted.

A biplot of common effects for Spatial Analysis demonstrated the variety Kronos had high yield and stability, whereas for PCA and regression analysis it was specifically adapted to unfavourable environments. The hexaploid wheats were included in the spatial and regression analyses, but excluded from the PCA. Despite Spear having high predicted yield, it was rated the most unstable genotype, which is in contrast to its generally recognized broad

adaptation and role as the control variety in hexaploid yield evaluation trials. This interpretation of results measured Spear against the average stability of the durum, and not vice versa which is the desired comparison if looking at economics of replacing bread wheat with durum.

Conclusion

The complementary use of interpretations from the analyses assisted in finding agronomically important and statistically significant genotypic patterns in the G×E interactions. ANOVA provided genotypic ranks by mean yields and the sources of variation observed in the interactions. The commercial durum variety in South Australia, Yallaroi, had the lowest overall mean performance in the period 1994 to 1996, whilst the advanced line RH912025 was highest ranked. Genotype × environment interaction was highly significant and L×Y accounted for most of the variation observed. Regression analysis indicated genotypic stability over environments, and demonstrated that Yallaroi was more suited to favourable conditions, while RH912025 and the WLYY9/3/2 sister lines were high yielding and widely adapted and, therefore, suitable replacements for Yallaroi.

Despite the success of biplot graphs of principal components in displaying genotypic domains (relationships between genotypes), and Spatial Analysis for classifying environments, they fail to identify interaction as a source, or make evident which genotype has the highest yield in various domains. PCA will be of more benefit for measuring stability in the future, when greater genetic diversity and more reference genotypes with specific traits exist in the program, against which new genotypes can be assessed. With more experience in evaluating multi-environment trials by Spatial Analysis, particularly after excluding hexaploid wheat from experiments, it is likely to be of more benefit than PCA to examine stability for unbalanced data. Spatial Analysis is useful for promoting genotypes, but, the assumption of a single population with a single genetic variance is a hindrance as classification of genotypes would be a useful tool in selecting parents.

This study demonstrated that rainfall was a major factor influencing durum grain yield, boron (B) toxicity was a major factor in grouping site performance and that a large G×E (G×L×Y) interaction existed for durum wheat in South Australia. Genotypic effects accounted for little of the variation observed in the G×E interaction. This suggests the need to increase genetic diversity in the program to overcome constraints affecting yields in South Australia. The strategies to identify and overcome these constraints can be transferred from knowledge gained in the bread wheat breeding programs for the region. Breeding for resistance and/or tolerance to major stress factors (edaphic, abiotic and biotic), such as B toxicity for example, is the best approach to widen the adaptation of new durum varieties.

Chapter 4

Genetic variation of F_2-derived progeny selected for tolerance to high concentrations of boron

4.1 Introduction

Many regions of the South Australian wheatbelt contain high levels of boron (B) in the subsoil (Cartwright *et al.*, 1984; 1986). The B restricts root growth of wheat (Holloway and Alston, 1992) and results in reduced grain yields of barley (Cartwright *et al.*, 1984) and wheat (Rathjen *et al.*, 1987). As amelioration of excess B from soil is not economically feasible in southern Australia, the use of tolerant varieties is the best option to overcome this constraint (Rathjen *et al.*, 1987). A wide range in genetic variation for response to high concentrations of B has been reported for bread wheat, barley and peas, and genetic variation for B tolerance was found among the commercial varieties of these crops cultivated in South Australia (Paull *et al.*, 1992a; Jenkin, 1993; Bagheri *et al.*, 1992). Significant increases (up to 11%) in grain yield of bread wheat have been achieved by incorporating B tolerance genes into sensitive varieties (Moody *et al.*, 1993), resulting in the deliberate release of moderately tolerant varieties such as BT-Schomburgk (Rathjen *et al.*, 1993).

A previous study found that when a collection of durum wheats, including Yallaroi and advanced Australian lines, was grown at a high B site, all were classified as very sensitive to moderately sensitive to B (Brooks, 1991). A landrace from China, Lingzhi Baimong Baidamai (AUS 14010), with a moderate level of B tolerance was identified when a collection of durum wheat genotypes was screened in soil with a high concentration of B (A.J. Rathjen, pers. comm.). This was confirmed in subsequent studies, which also identified genotypes (AUS 10105 and AUS 10110, originating from India) which were more tolerant than AUS 14010 (Jamjod, 1996; Jamjod *et al.*, 1997). Response to B in durum wheat is under the control of three partially dominant major genes (Jamjod, 1996). Incorporating B tolerance

into sensitive local varieties should therefore be readily achieved through backcrossing. However, the success of backcrossing, with respect to release of adapted varieties, will be determined by linkage of deleterious genes to the donor parent for B tolerance and pleiotropic effects of the gene for B tolerance on other characteristics.

This chapter examines the effects of increasing the level of tolerance to high soil B of Australian durum wheat varieties by transferring gene(s) conferring tolerance to B using the backcross breeding method. The objectives of this study were to use simple screening techniques to identify variation in response to B within a population, and then develop and investigate relationships between B response (root growth in filter paper bioassy), accumulation and grain yield when the population was grown in the field.

4.2 Materials and methods

4.2.1 Plant material

Parental material

A collection of durum accessions from the Australian Winter Cereals Collections (AWCC), Tamworth, was screened for tolerance to high levels of soil B as described by Moody *et al.* (1988). A moderately tolerant line, Lingzhi Baimong Baidamai (AUS 14010), was identified and used as the donor parent. Moderately sensitive Australian varieties and an advanced breeding line from the National Durum Wheat Improvement Program (NDWIP) were used as the recurrent parents in the backcrossing program. The most B tolerant F_1 plants were selected for backcrossing. Seeds of the Australian parental lines were kindly provided by Dr. R.A. Hare, NSW Agriculture, Tamworth. The name, pedigree, origin and response to high concentrations of B for each of the genotypes is shown in Table 4.1. The genotypes were crossed in the following combination:

Wol/3/AUS 14010/2*Yal//RH880009.

Due to the relatively high proportion of common ancestry from Kamilaroi and/or Yallaroi in the pedigree of RH880009 and Wollaroi (Figure 4.1), the progeny can be considered as BC_3.

Control materials

A B tolerant durum wheat landrace, AUS 10105, which was identified by Jamjod (1996) after the backcrossing had been completed, was also included as a tolerant control in the filter paper experiments. Seed was supplied by the AWCC. The bread wheat variety Halberd was used as the moderately B tolerant control in the glasshouse experiment, with seed provided by Mr D.B. Moody, University of Adelaide.

Table 4.1. Pedigree, country of origin, boron response and description of durum wheat genotypes used as parents, and durum and bread wheat controls.

Genotypes	Pedigree	Origin	B response	Description
Durum wheat				
AUS 14010	Lingzhi Baimong Baidamai	China	MT[a]	Tall, facultative
Yallaroi	Guillemot selection. No.3/Kamilaroi sib	Australia	MS[a]	Semi-dwarf variety, spring
Wollaroi	TAM1B-17/Kamilaroi sib//Rokel Sel./ Kamilaroi sib	Australia	MS[a]	Semi-dwarf variety, spring
RH880009	Yallaroi//TAM1B-17/Kamilaroi sib	Australia	MS[a]	NSW advanced line, spring
AUS 10105	AUS 10105	India	T[a]	Tall, late maturity
Bread wheat				
Halberd	Scimitar/Kenya C6042//Bobin/3/Insignia 49	Australia	MT[b]	Tall variety

[a], [b] Jamjod (1996) and Paull (1990), respectively.

Boron response at B100; MS = moderately sensitive, MT = moderately tolerant and T = tolerant.

Figure 4.1. Pedigree chart of Wol/3/AUS 14010/2*Yal//RH880009 (designated WLYY9) demonstrating the high proportion of common ancestry between recurrent parents, Kamilaroi, Yallaroi, Wollaroi and RH880009.
* represents the use of F_1 progeny as parent.

The BC_3F_1 plants were grown in a glasshouse in normal potting mix to produce the BC_3F_2 generation. The BC_3F_2 population was tested in a glasshouse experiment (Section 4.2.2) for response to B and 22 single F_2 plants with the least severe visual leaf symptoms (chlorosis and necrosis) were selected for multiplication and further testing. BC_3F_2-derived BC_3F_3 families were grown in potting mix for multiplication, and a sub-sample of BC_3F_4 seeds from each line was tested in filter paper experiments (Section 4.2.3) for response to B. The filter paper method was adopted when Chantachume (1995) found it more appropriate than soil screening to discriminate between the more tolerant genotypes. The F_4 plants were grown in a bird-proof enclosure at Waite Campus, and up to five lines within each family were selected for appropriate (early to mid-season) maturity in the South Australian growing season. The F_5 seed of 62 selected F_4 lines were sown as individual plots at both Two Wells and Mallala in 1994. However, the plots at Two Wells died due to crown rot (*Fusarium* spp.) infection. Grain harvested from the plots located at Mallala in 1994 was sown as replicated F_6 yield plots at two sites in 1995 (Section 4.2.4). Yield testing of F_7 and F_8 generations was also conducted in 1995 and 1996 (Section 4.2.4).

4.2.2 Glasshouse experiment

Screening the segregating BC_3F_2 population for response to B consisted of applying a high concentration of B (100 mg B kg^{-1} soil) to fertile surface soil and placing the soil in a large box (2m x 1m x 0.25m deep) in a glasshouse (Moody *et al.*, 1988). The soil used was a bulk sample with silty clay loam texture from the surface (0-10 cm) of a red-brown earth (Typic Haploxeralf) obtained from CSIRO Glenthorne Research Farm, O'Halloran Hill, South Australia (Paull *et al.*, 1988b). The concentration of extractable B in hot $CaCl_2$ in this soil was 58 mg kg^{-1} (Spouncer *et al.*, 1992). Seeds were allowed to imbibe at 4°C for four days and then incubated at 25°C for 24 hours to ensure uniform germination. Seeds were sown with spacing of 3 cm x 5 cm, and a grid consisting of Yallaroi (moderately B sensitive durum

parent) and Halberd (moderately B tolerant bread wheat) were sown for reference every 6 rows. One hundred and fifty F_2 seeds were sown. Six weeks after sowing expression of leaf symptoms was rated as the length of necrotic symptoms relative to total leaf length for the youngest expanded blade (YEB), second youngest (YEB+1) and third youngest (YEB+2). Twenty-two individual plants with symptoms similar to Halberd were retained.

4.2.3 Filter paper experiment

The filter paper bioassay described by Chantachume *et al.* (1995) was used to examine sensitivity of root growth in response to B of the BC_3F_4 generation. A solution containing 500 µM $Ca(NO_3).4H_2O$, 2.5 µM $ZnSO_4.4H_2O$ and boric acid at the rate 100 mg B L^{-1} (designated as B100) was prepared. Sheets of filter paper (Ekwip® grade R6 size 36 x 42 cm) were soaked in the solution for two minutes and allowed to drain for two minutes. Fifteen germinated seeds were placed in a single row along the centre of each paper with a spacing of 2 cm between each seed. The paper was then rolled up, covered with aluminium foil and stored vertically at 18°C.

The genotypes were arranged as a randomised complete block design with two replicates. After twelve days, root growth was assessed by measuring the length of the longest seminal root.

4.2.4 Yield evaluation

F_6 lines

The 62 BC_3F_6 lines selected at F_4 (based on appropriate maturity) were sown in 1995 at Two Wells (a site with soil B concentrations in excess of 80 mg/kg below 30 cm depth (Moody *et al.*, 1993), a potentially toxic level in the subsoil and described by Cartwright *et al.* (1987)) and at Mallala (soil B concentration of 13 mg/kg below 30 cm depth (D. Maschmedt, pers.

comm.)), as a control site where low levels occur in the subsoil. The plots were 6 m long x 4 drill rows, and seed was sown at a rate of 40 g/plot (equivalent to 90 kg/ha). Prior to harvest, the plot length was reduced to 4.2 m. Environment and plot descriptions are presented in Chapter 3 (Section 3.2). The sowing dates were 18th May at Two Wells and 1st June at Mallala. Monthly rainfall data for the locations is presented in Appendix A.

Seed for the F_6 experiment was obtained from the F_5 plots grown at Mallala in 1994. The experiment was designed as a randomized complete block with two replicates, with the same randomization at both locations. Each replicate was sixteen plots wide x 8 bays deep. Border plots were sown on both outside rows of the trial. The parental varieties Wollaroi and Yallaroi were alternately sown throughout the experiment on a grid pattern. Wollaroi was sown every sixth and Yallaroi every seventh plot for a total of 38 and 37 plots, respectively. The donor parent (AUS 14010) was not sown, because of its winter habit, as this would have resulted in failure to mature satisfactorily for yield evaluation when grown in South Australia.

At heading, whole shoots of Yallaroi and nine lines were sampled at Two Wells for nutrient analysis. The lines sampled were selected on the basis of a representative range of root lengths observed at B100 in the filter paper experiment, combined with high yield at Mallala in 1994. Samples consisted of six main culms (whole shoots) per plot. Zadok's growth scale was used to assess the stage of development of all plots at Two Wells on 17th October 1995 (152 days after sowing). All experiments were harvested at maturity and grain yields measured. Grain of Yallaroi and the nine selected lines from both Two Wells and Mallala was analysed by ICP spectrometry.

F_7 and F_8 lines

Seven high yielding F_6 progeny lines from Mallala in 1994 were multiplied under irrigation in the summer of 1994/1995 at Nildottie. In 1995 the BC_3F_7 seed of these lines was included in

the genotype × environment (G×E) experiments (described in Chapter 3) for extensive yield evaluation.

In 1996, three BC_3F_7 lines previously found to have the shortest or longest roots in the filter paper experiment were also included in the G×E experiments (Chapter 3). Seed for the F_8 lines was obtained from the F_7 plots grown at Roseworthy in 1995, while seed for the F_7 lines was bulked from the plots grown at Mallala and Two Wells in 1995.

Trials were grown at eight locations across the South Australian wheatbelt, which included Rudall, Minnipa, Palmer, Walker Flat, Two Wells, Kapunda, Jamestown, Mallala, Roseworthy and Winulta (Plate 3.1). Monthly rainfall data for the locations are presented in Appendix A. Environments, trial descriptions and sowing dates are presented in Chapter 3 (Section 3.2). Trials were designed as a randomized complete block with five replicates, and Yallaroi was included as a standard every seventh plot. The trial dimensions were 14 plots x 15 bays. All trials were harvested at maturity and grain yields measured.

Grain samples from five plots of the Yallaroi standard from all sites from the 1995 (except Palmer) and 1996 harvests were taken and analysed by ICP spectrometry. Grain samples were also taken at the 1996 harvest from the ten $BC_3F_{7/8}$ lines in the trials at Two Wells, Roseworthy, Jamestown and Mallala and analysed.

4.2.5 Chemical analyses of plant material

Plant tissues and grain were digested in nitric acid and analysed using inductively coupled plasma (ICP) spectrometry, using the method of Zarcinas *et al.* (1987), to provide the concentrations of eleven elements, including B. Whole shoot samples harvested from field experiments were placed in paper bags and oven-dried at 80°C for 48 hours. Plant tissue samples greater than 700 mg were ground in a stainless steel mill to pass through a 1 mm screen. Zarcinas *et al.* (1987) found no variation in analytical values of B between ground

and whole wheat grain, so samples of grain were digested without grinding. Analytical standards (grain with pre-determined elemental concentrations) were included in each set of digests to allow detection of systematic variation within each set, and to allow comparison between sets.

Immediately prior to weighing for digestion, samples were oven-dried for two hours at 60-80°C. Seven hundred milligram subsamples, or entire samples if less than 700 mg, were placed in aged 75 mL pyrex tubes. Ten millilitres of 70% HNO_3 (A.R. grade) was added and the tubes were allowed to stand overnight at room temperature in a Tecator Digestion System 40. The temperature was raised to 120°C for two hours, and then to 140°C, and maintained at 140°C until about 1 mL of acid remained. After cooling, the samples were diluted to 20 mL with 1% (v/v) nitric acid. The digests of plant samples were filtered through Whatman No. 54 filter papers to remove amorphous siliceous residue, and the grain digests were decanted directly into 15 mL polycarbonate tubes. Samples were stored at 1-2°C until analysis by ICP spectrometry. The concentrations of eleven elements (B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn) were simultaneously determined by a Labtest V-25 Inductively Coupled Plasma - Optical Emission Spectrometer (Zarcinas and Cartwright, 1983).

4.3 Results

4.3.1 Glasshouse experiment

Genotypes in the BC_3F_2 population were visually assessed for severity of B toxicity symptoms (Plate 4.1). The leaf symptoms in durum wheat, which are similar to those in bread wheat described by Paull *et al.* (1990), consisted of chlorotic and/or necrotic lesions which developed from the leaf tips, and along the leaf margin towards the base. Yallaroi developed more severe symptoms than Halberd, with the BC_3F_2 population within the range of the controls (Figure 4.2). Twenty-two individual F_2 plants with symptom levels similar to Halberd were retained. Symptoms were most severe on the oldest leaf, YEB+2 (Figure 4.2).

Plate 4.1. Comparison of the response of genotypes in the BC_3F_2 population grown in soil with 100 mg B kg^{-1}.

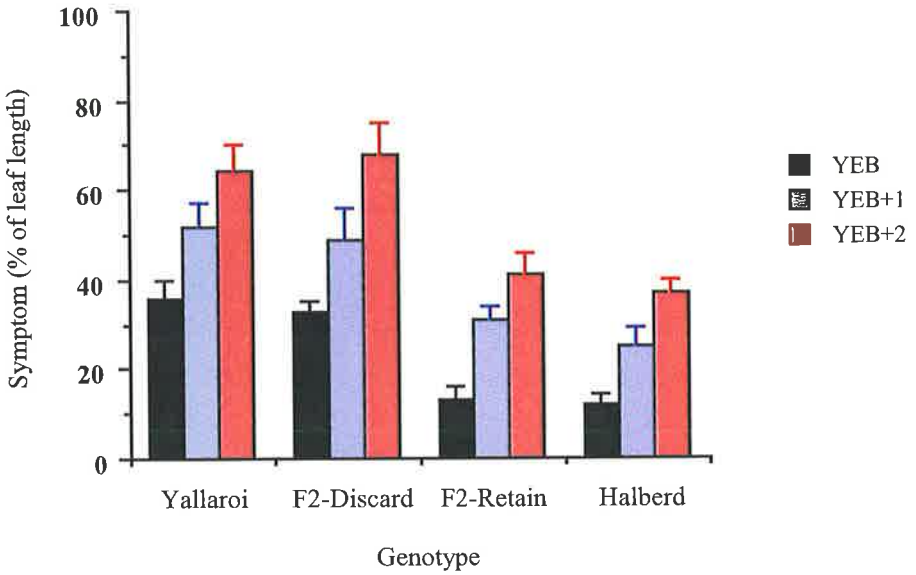


Figure 4.2. Severity of symptoms of B toxicity expressed as a percentage of total leaf length for the first three leaves of Yallaroi, the BC_3F_2 progeny (those discarded {n=128} versus retained {n=22}) and Halberd grown in soil with 100 mg B kg^{-1} applied.
YEB = Youngest expanded leaf blade, YEB+1 = second youngest, YEB+2 = third youngest.

4.3.2 Filter paper experiment

The mean root length of the F_4 lines grown at B100 differed significantly among families. The minimum, maximum, mean and standard deviations for root length of the 22 families, together with results of three parents and control are presented in Table 4.2. The root lengths of the families ranged from 69 - 146 mm. The magnitude of the standard deviation for root length varied considerably among families. This can be attributed to families derived from both heterozygous (large standard deviation) and homozygous (small standard deviation) F_2 plants.

An example comparsion between root lengths of BC_3F_4 progeny lines and parents is shown in Plate 4.2.

Frequency distribution

Response of the BC_3F_4 progeny to a high concentration of B showed continuous variation around a mean of 105 mm (Figure 4.3). As F_2 plants with tolerance to B (on the basis of having the least visual symptoms in high B soil) were selected, it would be expected that the derived families would have root lengths in a range similar to the tolerant parent, AUS 14010. The distribution of the selected derived lines was within the range of root lengths for AUS 14010, however, the mean root length of the progeny distribution was significantly greater than that of AUS 14010 ($t=14.24$, $n=99$).

Due to the continuous distribution it was impossible to classify the plants into discrete categories. Despite this, to examine if there was any variation for grain yield between the lower and upper range of tolerance, the progeny lines were classified into three groups on the basis of root length relative to that of the moderately B tolerant parent mean: root length >93 mm, root length <93 mm and heterogeneous (high standard deviation, s.d.) (Table 4.3).

Table 4.2. Response of F_4 families of Wol/3/AUS 14010/2*Yal//RH880009 to a high concentration of B (100 mg B kg^{-1}) in the filter paper experiment. The minimum and maximum root lengths of lines within families and means and standard deviations of families are compared with three parents, Yallaroi, Wollaroi and AUS 14010, and the control, AUS 10105.

Family	No. of lines	Root length (mm) at B100			
		Min	Max	Mean	s.d.
1	2	129	160	145	16
2	1			69	
3	3	83	109	96	11
4	3	67	123	89	22
5	5	84	121	98	14
6	1			117	
7	3	95	118	108	9
8	1			94	
9	2	76	77	76	0
10	3	130	148	137	8
11	3	99	101	100	1
12	1			129	
13	2	107	125	116	9
14	3	77	96	83	9
15	2	68	105	86	18
16	5	79	137	104	25
17	4	91	153	122	25
18	3	83	112	95	13
19	4	112	160	128	19
20	3	60	79	72	8
21	4	142	150	146	3
22	4	56	98	72	16
LSD (P=0.05) family means				23	
Parent/Control					
Yallaroi		21	101	61	16
Wollaroi		30	112	76	16
AUS 14010		47	151	93	22
AUS 10105		115	211	183	22

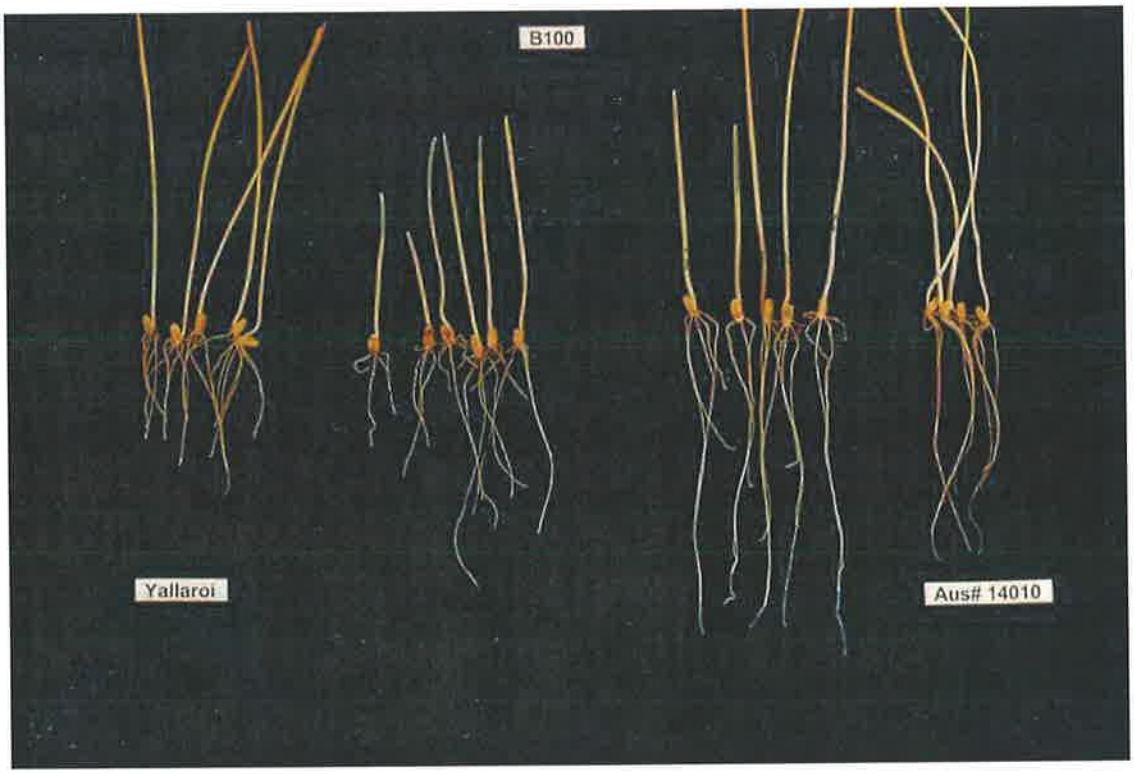


Plate 4.2. Comparison of root length among durum wheat parents and F_4 progeny lines grown in filter paper containing 100 mg B L^{-1}.

From left to right: Yallaroi, F_4 plants that represent response at the lower and upper range of moderate tolerance, and AUS 14010.

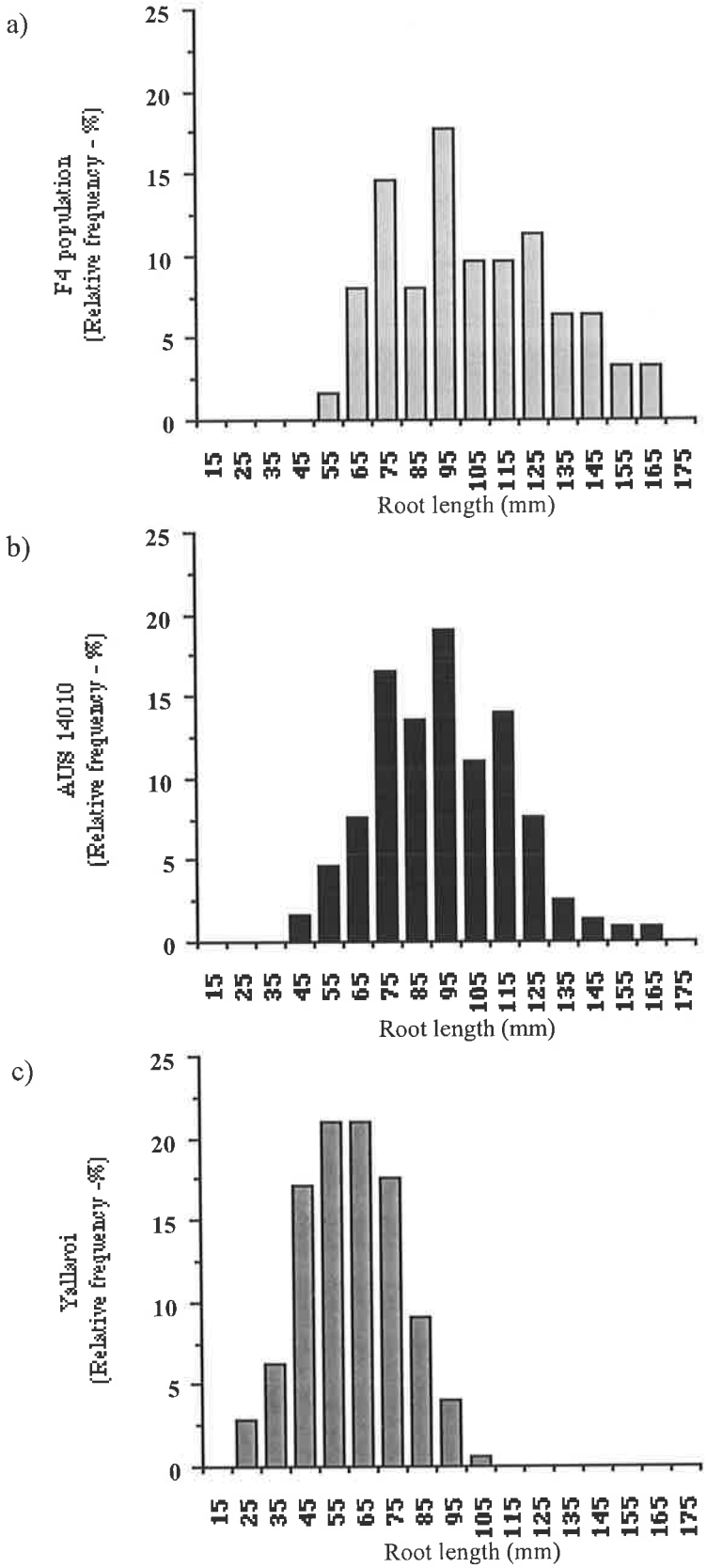


Figure 4.3. Distribution of response to B based on root length in filter papers at B100.
(a) BC_3F_4 population (62 lines), mean was 105.
(b) AUS 14010 (n=237), mean (s.d. in parentheses) was 93 (22).
(c) Yallaroi (n=176), mean (s.d. in parentheses) was 61 (16).

Table 4.3. Classification of F_4 lines in response to a high B concentration, based on root length and standard deviation (s.d.) at B100.

B Response Group	No. of lines	Mean root length (mm) at B100	s.d. range of lines within group	
			Min	Max
RL<93	26	79	12	51
Heterogeneous	12	103	32	54
RL>93	24	133	24	52

RL = root length (mm).

4.3.3 Yield evaluation

F_6 lines

Significant differences among the 62 F_6 lines for grain yield were measured at Mallala in 1995, but there was no significant difference in yield among lines at Two Wells (Table 4.4). There was no visual expression of B toxicity symptoms at Two Wells in 1995. Lack of significance at Two Wells was due to minor differences in yield among the response groups. At Mallala, although there were significant differences between genotypes, there were no significant differences among the different B response groups. The lack of response at Mallala was expected since no symptoms of B toxicity have previously been reported at this site. The standard deviation for grain yield at both locations was high, and this was equally high among all response groups, which suggests other adaptative traits (selective pressures) were influencing yield to a greater extent than the B response.

Table 4.4. Range and mean yields from F_6 lines of Wol/3/AUS 14010/2*Yal//RH880009, classified according to B response at B100, grown at Two Wells and Mallala in 1995.

B Response Group	No.	Yield (t/ha) Two Wells Min	Max	Mean	s.d.	Mallala Min	Max	Mean	s.d.
RL<93	26	1.20	1.74	1.47	0.14	1.24	1.97	1.67	0.18
Heterogeneous	12	1.53	1.93	1.71	0.11	1.36	1.87	1.58	0.14
RL>93	24	1.29	1.94	1.63	0.17	1.33	2.04	1.58	0.16
LSD (P=0.05)		ns				0.37			
c.v. (%)		15				11			

RL = root length (mm).
ns P>0.05
No. = number of lines in response group.

F_7 and F_8 lines

Significant differences among progeny lines for grain yield occurred at only two sites (Kapunda and Rudall) in 1995 (Table 4.5). Nevertheless, the highest yielding progeny line at Walker Flat and Palmer was significantly greater than Yallaroi, and at Roseworthy, Kapunda and Rudall both Yallaroi and Wollaroi. At Mallala, Yallaroi yielded significantly less than the lowest yielding progeny line and Wollaroi. There was also a significant difference in yield between Yallaroi and Wollaroi at Palmer.

In 1996 there was a significant difference among grain yields of progeny lines at all sites except Walker Flat (Table 4.6). The progeny lines had significantly higher grain yields than their recurrent parents, Yallaroi and Wollaroi, at Two Wells, Roseworthy, Mallala, Kapunda and Jamestown, but only Yallaroi at Walker Flat and Winulta. Significant yield differences between Yallaroi and Wollaroi occurred at Two Wells, Roseworthy and Winulta.

Table 4.5. Grain yields (t/ha) and B response (root length at B100) for F_7 lines of Wol/3/AUS 14010/2*Yal//RH880009 (designated as WLYY9), and Yallaroi and Wollaroi at eight locations in 1995.

Line	RL B100	Grain yield (t/ha)							
		Two Wells	Rose-worthy	Mallala	Walker Flat	Winulta	Kapunda	Palmer	Rudall
WLYY9/1/3/4	74	1.95	2.84	1.96	1.65	2.55	2.94	0.92	0.98
WLYY9/2/L1/1	118 (H)	1.85	2.98	1.95	1.44	2.39	3.27	0.79	0.75
WLYY9/3/1/5	149	1.79	2.72	1.76	1.57	2.74	3.07	0.77	0.81
WLYY9/3/2/1	73	1.83	2.75	1.81	1.61	2.63	3.49	0.78	0.63
WLYY9/3/2/3	57	1.84	2.97	1.86	1.63	2.37	3.44	0.80	0.55
WLYY9/3/2/4	61	1.79	2.91	1.92	1.68	2.80	3.40	0.71	0.58
WLYY9/3/2/5	98 (H)	1.81	2.92	2.04	1.47	2.84	3.70	0.89	0.62
Mean		1.83	2.88	1.90	1.57	2.61	3.32	0.81	0.70
Yallaroi	61	1.76	2.28[b]	1.30[c]	1.32[a]	2.59	3.08[a]	0.57[b]	0.68[a]
Wollaroi	76	1.87	2.47[b]	1.89	1.42	2.37	3.10[a]	0.83	0.73[a]
LSD (P=0.05)		0.23	0.29	0.31	0.30	0.53	0.40	0.23	0.16

RL B100 = root length (mm) at B100. H = heterogeneous progeny.

[a] significantly different from highest yielding progeny line at $P<0.05$

[b] significantly different from mean yield of progeny lines at $P<0.05$

[c] significantly different from lowest yielding progeny line at $P<0.05$

Table 4.6. Grain yields (t/ha) and B response (root length at B100) for F_7 and F_8 lines of Wol/3/AUS 14010/2*Yal//RH880009 (designated as WLYY9), and Yallaroi and Wollaroi at eight locations in 1996.

Line	RL B100	Grain yield (t/ha)							
		Two Wells	Rose-worthy	Mallala	Walker Flat	Winulta	Kapunda	Minnipa	James-town
WLYY9/1/2/2/1	83	1.77	2.52	2.88	1.58	2.65	2.38	0.89	1.48
WLYY9/1/3/4	74	1.80	2.54	2.55	1.68	2.73	1.93	0.95	1.78
WLYY9/1/4/4/S	121	2.09	2.66	2.77	1.67	2.72	2.67	0.79	1.76
WLYY9/2/6/3	153	2.09	3.23	2.99	1.60	2.93	2.77	0.85	2.00
WLYY9/2/L1/1	118 (H)	1.78	2.92	2.82	1.60	2.60	2.28	0.92	1.96
WLYY9/3/1/5	149	1.90	2.71	2.40	1.70	2.73	2.41	0.82	1.83
WLYY9/3/2/1	73	1.67	2.63	3.16	1.81	2.82	2.62	0.81	1.80
WLYY9/3/2/3	57	1.65	2.49	3.10	1.75	2.62	2.86	0.85	1.64
WLYY9/3/2/4	61	1.65	2.59	3.28	1.76	2.76	2.66	0.91	1.68
WLYY9/3/2/5	98 (H)	1.71	2.62	3.02	1.69	2.81	2.76	0.86	1.76
Mean		1.81	2.69	2.90	1.68	2.74	2.54	0.86	1.77
Yallaroi	61	1.72[a]	2.30[b]	2.67[a]	1.54[a]	2.46[b]	2.43[a]	0.85	1.55[a]
Wollaroi	76	1.45[a]	2.76	2.33[b]	1.74	2.73	2.39[a]	0.82	1.76[a]
LSD (P=0.05)		0.25	0.35	0.45	0.26	0.27	0.36	0.15	0.23

RL B100 = root length (mm) at B100. H = heterogeneous progeny.

[a] significantly different from highest yielding progeny line at $P<0.05$

[b] significantly different from mean yield of progeny lines at $P<0.05$

There were yield differences among the three B response groups, RL<93, heterogeneous and RL>93, of F_7 and F_8 lines, but this was only significant at Two Wells in 1996 (Table 4.7). However, the yield advantage of the group with RL>93 was not uniform across all sites in 1996. The performance of the heterogeneous group in 1996 was generally intermediate between the RL>93 and RL<93 groups. Over all sites in 1996, the mean yield advantages of the RL>93 group and the heterogeneous group in comparison with the RL<93 group were 3.8% (range -9 to +19%) and 2.1% (range -4% to +11%), respectively.

Table 4.7. Grain yields for RL>93 and heterogeneous (H) groups of Wol/3/AUS 14010/2*Yal//RH880009 progeny, Yallaroi and Wollaroi in replicated trials at eight locations during 1995 and 1996. Grain yields are expressed as a percentage of the RL<93 group.

Site	1995				1996			
	RL>93	H	Yallaroi	Wollaroi	RL>93	H	Yallaroi	Wollaroi
Two Wells	97	99	94	101	119*	102	101	85*
Roseworthy	95	103	80*	86*	112	108	90	108
Mallala	93	106	69*	99	91	98	89	78*
Walker Flat	95	88	81*	86	96	96	90	101
Winulta	106	101	100	92	103	100	91	100
Kapunda	92	105	93	94	105	101	97	96
Palmer (Krause)	97	105	72*	103				
Rudall	118	100	99	106				
Minnipa					93	101	97	93
Jamestown					111	111	92	105

* significantly different from RL<93 types at P<0.05.

Effect of time of flowering upon response to yield

Zadoks scores were used to determine the effect of time of flowering upon grain yield. On 17th October 1995 the stage of development of individual lines varied between Zadok's growth stages 51 (first spikelet of ear just visible) and 69 (flowering complete). The standards, Yallaroi and Wollaroi, were at Zadoks 59 and 69, respectively. These scores were used to group the lines into three categories: late, Zadoks 51-55 (n=15), mid, Zadoks 57-59 (n=38) and early, Zadoks 61-69 (n=9). The mean yields (±SEM) of the three categories at Two Wells in 1995 were 1.52±0.04, 1.59±0.03 and 1.62±0.06 t/ha, respectively, which were not significantly different and therefore not associated with flowering time.

There was no yield advantage at Mallala in 1995 between the three flowering time categories (1.67±0.05, 1.59±0.03 and 1.68±0.06 t/ha, respectively). Mallala was sown later and, consequently, smaller differences in flowering time would have been expressed at this site. In the G×E experiments (Chapter 3) at Mallala in 1995, Yallaroi was the lowest yielding genotype, with a mean grain yield of 1.30 t/ha, while Wollaroi was ranked 17th (n=34) with a

yield of 1.89 t/ha (45% advantage). Therefore, yield at Mallala was not associated with either B tolerance or flowering time, but was associated with other (unidentified) traits.

4.3.4 Chemical analyses of plant material

Boron

F_6 lines

There was no significant difference among the selected progeny lines for concentration of B in whole shoots or in grain when grown at Two Wells, or for grain at Mallala in 1995 (Table 4.8). At Two Wells the level of B in the shoots was within the range reported by Paull (1990) as non-toxic (no critical value defined), but grain concentrations can be considered toxic (greater than 2.0 mg kg^{-1}). The B levels in the grain at Mallala were low. Despite the lack of significant differences among genotypes, at Two Wells in 1995 the concentration of B in shoots was significantly correlated with grain B ($r=0.662$, $P<0.05$) (Figure 4.4).

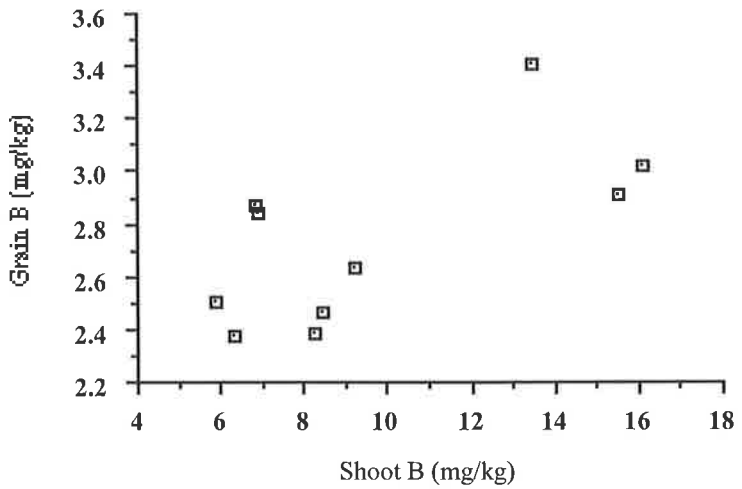


Figure 4.4. The correlation ($r=0.662$, $P<0.05$) between concentration of B in shoots and grain for the selected F_6 lines of Wol/3/AUS 14010/2*Yal//RH880009 at Two Wells in 1995.

Table 4.8. B concentrations (mg kg^{-1}) in shoots and grain for F_6 lines of Wol/3/AUS 14010/2*Yal//RH880009 (designated as WLYY9) and Yallaroi grown at Two Wells and Mallala in 1995.

Genotype	RL B100	B concentration (mg kg^{-1})		
		Two Wells		Mallala
		Shoots	Grain	Grain
WLYY9/1/2/2/1	83	6.8	2.9	1.3
WLYY9/1/3/4	74	9.2	2.6	1.4
WLYY9/2/6/3	153	8.4	2.5	1.7
WLYY9/2/L1/1	118	5.8	2.5	1.7
WLYY9/3/1/5	149	6.3	2.4	1.7
WLYY9/3/2/1	73	8.2	2.4	1.6
WLYY9/3/2/3	57	6.9	2.9	1.7
WLYY9/3/2/4	61	15.5	2.9	1.6
WLYY9/3/2/5	98	16.1	3.0	1.4
Yallaroi	61	13.4	3.4	1.6
LSD (P=0.05)		ns	ns	ns

RL B100 = root length (mm) at B100.
ns P>0.05

F_7 and F_8 lines

In contrast to the 1995 results, in 1996 there were signifcant differences in the concentration of B in grain of the F_7 and F_8 progeny lines grown at Two Wells, Roseworthy, Jamestown and Mallala (Table 4.9). Toxic levels of B (greater than 2.0 mg kg^{-1}) in the grain of the progeny lines occurred at Two Wells, Roseworthy and Jamestown. However, at Mallala, a toxic concentration of B resulted in the grain for the Yallaroi standard only. At Two Wells and Mallala, the B concentration for Yallaroi was higher than the progeny lines, while at Roseworthy and Jamestown it was within the range of the progeny.

Table 4.9. B concentration (mg kg^{-1}) in grain for F_7 and F_8 lines of Wol/3/AUS 14010/2*Yal//RH880009 (designated as WLYY9) and Yallaroi grown at four sites in 1996.

Line	RL	B concentration (mg kg^{-1})			
	B100	Two Wells	Roseworthy	Jamestown	Mallala
WLYY9/1/2/2/1	83	3.6	3.1	2.6	1.6
WLYY9/1/3/4	74	5.2	2.6	2.1	1.6
WLYY9/1/4/4/S	121	5.0	3.3	1.7	1.9
WLYY9/2/6/3	153	6.2	3.1	1.7	1.1
WLYY9/2/L1/1	118	4.8	2.6	2.2	1.9
WLYY9/3/1/5	149	4.5	2.3	2.1	1.9
WLYY9/3/2/1	73	4.9	3.7	2.1	1.8
WLYY9/3/2/3	57	6.4	4.0	2.0	1.0
WLYY9/3/2/4	61	7.9	4.2	1.9	1.3
WLYY9/3/2/5	98	6.7	4.1	1.9	0.9
LSD (P=0.05)		1.4	0.8	0.4	0.5
Yallaroi	61	10.4	3.6	2.0	2.4

RL B100 = root length (mm) at B100.

The concentration of B in grain for individual progeny lines was significantly correlated between sites (except Jamestown) in 1996. There was a significant positive correlation between lines at Two Wells and Roseworthy (Figure 4.5), but the lines at Mallala were negatively correlated to Two Wells and Roseworthy. The unexpected negative correlations associated with grain B concentrations between sites may be attributed to the level at Mallala being non-toxic. There was a highly significant genotype × location interaction ($P<0.001$) for grain B concentration.

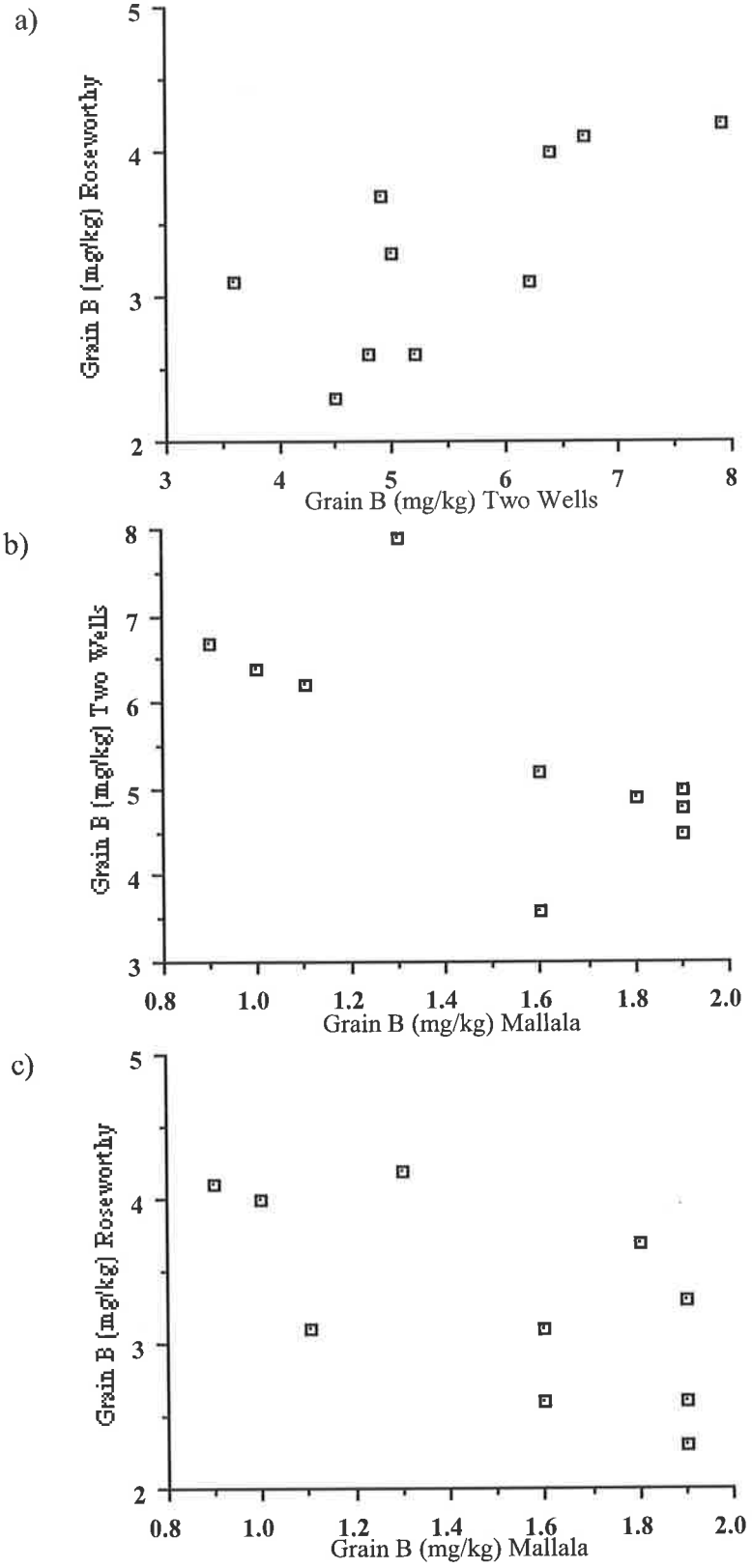


Figure 4.5. The correlation between concentration of B in grain between sites in 1996 for the Wol/3/AUS 14010/2*Yal//RH880009 lines.

(a) concentration of B in the grain at Roseworthy v Two Wells ($r=0.70$, $P<0.05$).

(b) concentration of B in the grain at Two Wells v Mallala ($r=-0.72$, $P<0.05$).

(c) concentration of B in the grain at Roseworthy v Mallala ($r=-0.65$, $P<0.05$).

Parental standard Yallaroi

In 1995 the concentration of B in the grain of the Yallaroi standard was significantly different between sites (Table 4.10). Chemical analysis of grain from Palmer was not performed, because the plants were infected with *Fusarium* crown rot, which may have affected the partitioning of nutrients to the grain which was shrivelled. A toxic concentration (more than 2.0 mg kg^{-1}) of B in the grain of the standard occurred at Two Wells, Roseworthy, Walker Flat and Rudall. The remaining three sites had B concentrations considered non-toxic.

Significant differences between sites for the concentration of B in grain occurred in the experiments conducted in 1996 (Table 4.10). The grain B concentration for Yallaroi was greatest at Two Wells, and high grain B concentrations also occurred at Walker Flat. Concentrations were intermediate (although still toxic) at Roseworthy, Winulta, Mallala and Jamestown but low at Kapunda and Minnipa.

Although there were significant differences for grain B concentration of Yallaroi between sites for the individual seasons, the grain B concentration was much greater in 1996, and there was a highly significant location × year interaction ($P<0.001$). This may reflect different paddocks within locations over years or the underlying level of B.

Table 4.10. B concentration (mg kg^{-1}) in the grain of Yallaroi which was included as the standard in the G×E experiments grown at seven sites in 1995 and at eight sites in 1996. Values are the means of five plots.

Site	B concentration (mg kg^{-1})	
	1995	1996
Two Wells	2.4	10.4
Roseworthy	2.1	3.6
Mallala	1.8	2.4
Walker Flat	2.5	6.2
Winulta	1.3	3.9
Kapunda	1.4	1.1
Rudall	3.4	
Minnipa		1.0
Jamestown		2.0
LSD (P=0.05) site	0.7	1.5
(P=0.05) interaction[a]	1.2	

[a] Interaction between the sites Two Wells, Roseworthy, Mallala, Walker Flat, Winulta and Kapunda in 1995 and 1996.

Correlations between yield and response to B

F_6 lines

Root length at B100 was weakly correlated with grain yield of the 62 F_6 lines in 1995 at Two Wells ($r=0.361$, $P<0.01$) and at Mallala ($r=-0.251$, $P<0.05$) (Figure 4.6). However, the concentration of B in whole shoots and in grain at Two Wells in 1995 of the F_6 lines sampled were not significantly correlated with grain yield, nor was the concentration of B in grain at Mallala in 1995 significantly correlated with grain yield (data not shown). Concentrations of B in whole shoots and in grain of plants grown in the field were not related to root length at

B100 in the filter paper test (data not shown). The absence of strong relationships between these parameters may be expected since, generally, the levels of B were non-toxic in 1995.

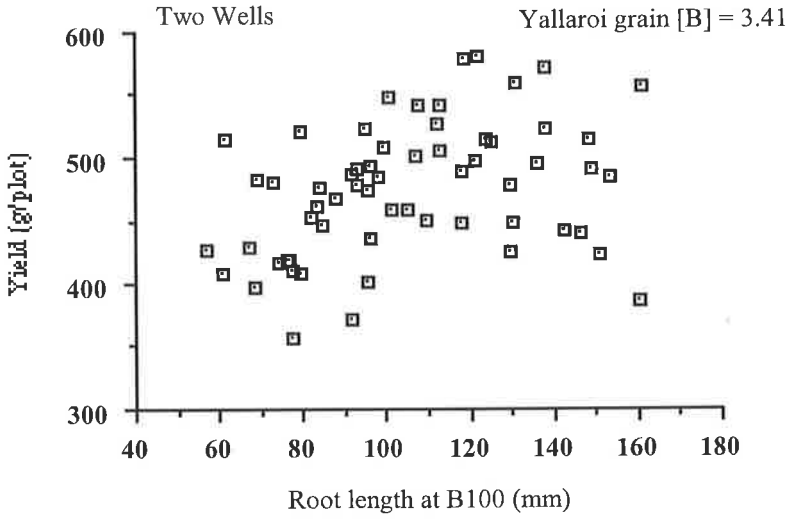
When the 62 individual F_6 lines were grouped into their respective families, root length at B100 remained correlated with grain yield at Mallala ($r=-0.442$, $P<0.05$), but not at Two Wells ($r=0.235$, n.s.) (Figure 4.7). The lack of correlation at Two Wells may have been contributed by the long root lengths associated with families 1 and 21 having a deleterious effect on yield, due to the roots growing through B toxic soil and being exposed to sodicity. When these two families were excluded from the analysis, the correlation was significant ($r=0.586$, $P<0.01$).

F_7 lines

The correlations between root length at B100 in filter paper and grain yield at each of the eight locations in 1995 for the seven F_7 lines were not significant (data not shown). At Rudall and Kapunda, the only sites where significant differences in grain yield between the F_7 lines occurred, root length at B100 was related to grain yield, but not significantly ($r=0.396$ and -0.311, respectively, Figure 4.8). The line WLYY9/1/3/4 was the outlier at both Rudall and Kapunda, which contributed to the correlations not being statistically significant.

The earliness of WLYY9/1/3/4 is likely to have contributed to the extreme differences in yield rankings between the two localities rather than its response to B. When WLYY9/1/3/4 was excluded from the regressions, the correlation became highly significant for Rudall ($r=0.960$, $P<0.01$), but not for Kapunda ($r=0.613$, n.s.).

a)



b)

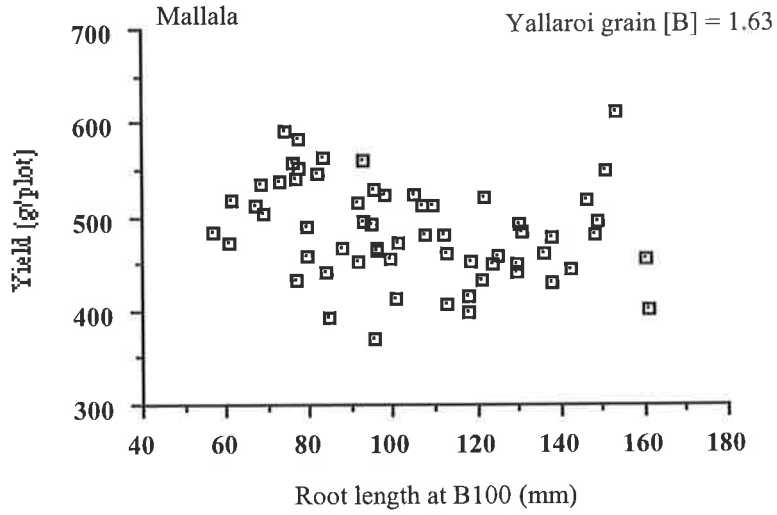
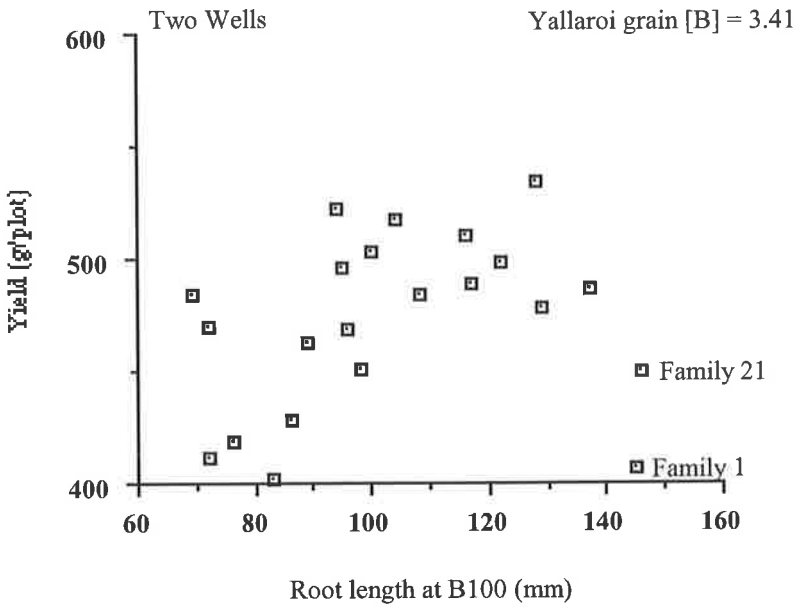


Figure 4.6. The correlation between root length at B100 and grain yield in 1995 for the F_6 lines of Wol/3/AUS 14010/2*Yal//RH880009.

(a) grain yield at Two Wells vs root length at B100 ($r=0.361$, $P<0.01$).

(b) grain yield at Mallala vs root length at B100 ($r=-0.251$, $P<0.05$).

a)



b)

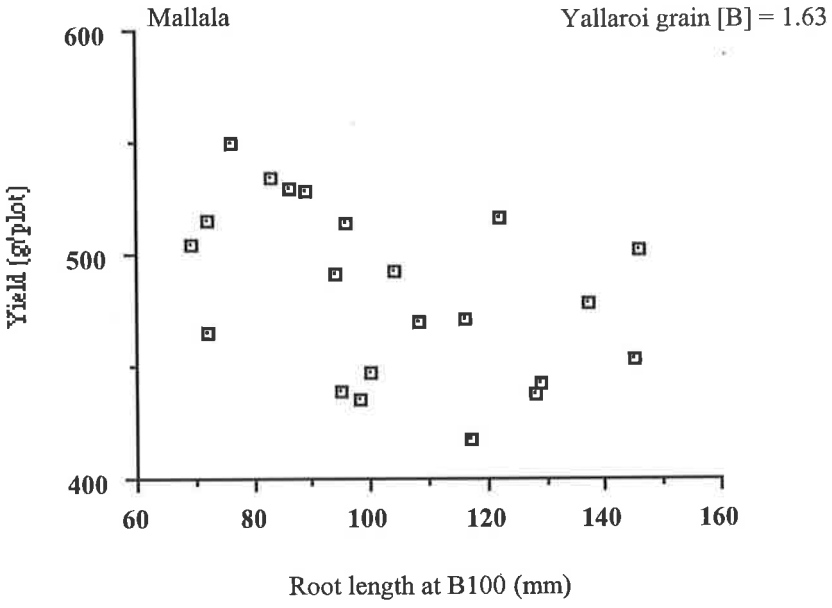
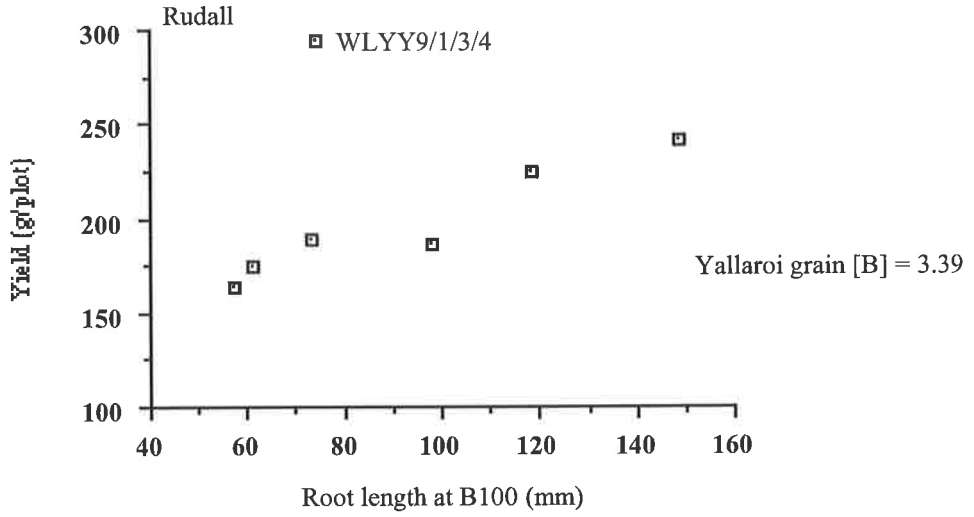


Figure 4.7. The correlation between root length at B100 and grain yield in 1995 for the F_6 families of Wol/3/AUS 14010/2*Yal//RH880009.

(a) grain yield at Two Wells vs root length at B100 ($r=0.235$, n.s.).

(b) grain yield at Mallala vs root length at B100 ($r=-0.442$, $P<0.05$).

a)



b)

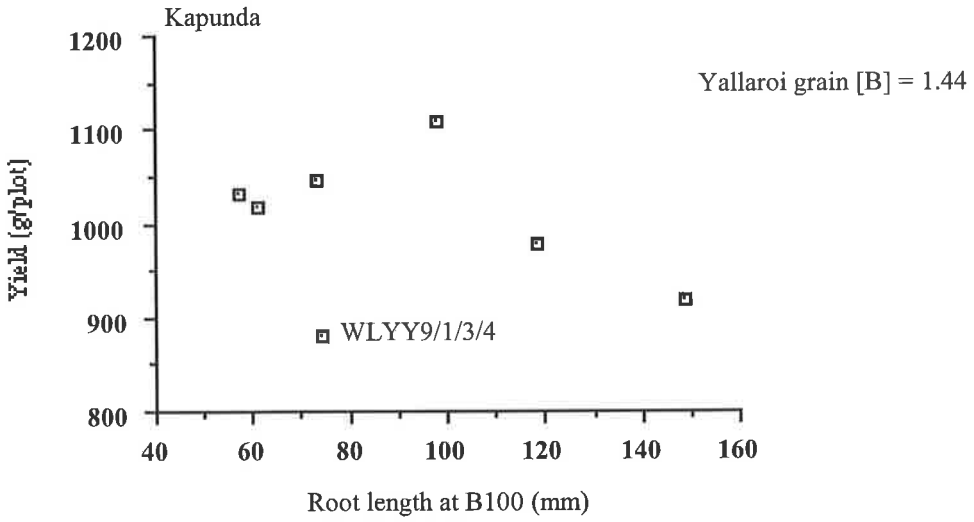


Figure 4.8. Relationship between root length at B100 and grain yield in 1995 for the F_7 lines of Wol/3/AUS 14010/2*Yal//RH880009.

(a) grain yield at Rudall vs root length at B100 (r=0.396, n.s.).

(b) grain yield at Kapunda vs root length at B100 (r=-0.311, n.s.).

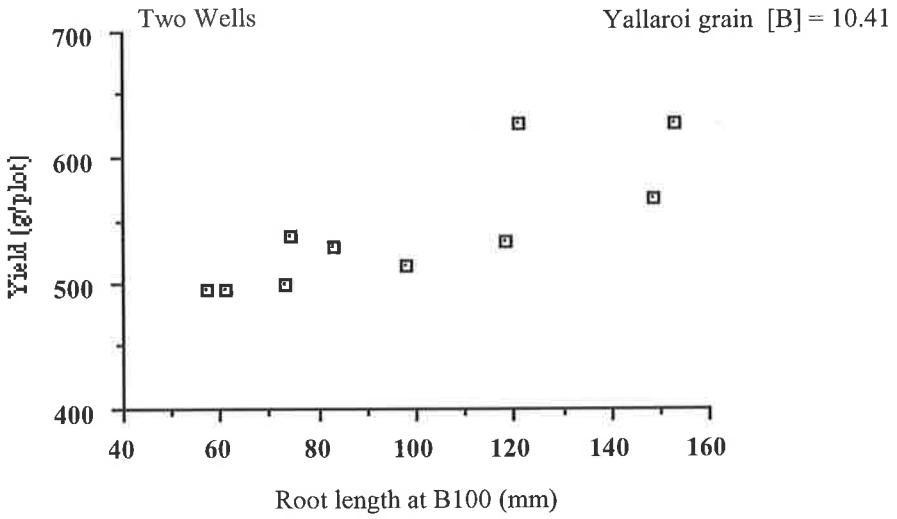
F_7 and F_8 lines

In 1996 a significant and positive correlation between mean line root length at B100 in the filter paper experiment and grain yield occurred at Two Wells ($r=0.815$, $P<0.01$), Roseworthy ($r=0.761$, $P<0.05$) and Jamestown ($r=0.644$, $P<0.05$) (Figure 4.9), locations with toxic concentrations of B in the grain (Table 4.10). However, no correlation was found at Mallala ($r=-0.521$, n.s.), Winulta ($r=0.339$, n.s.) or Walker Flat ($r=-0.557$, n.s.) (Figure 4.9), other sites where toxic grain B levels occurred in Yallaroi. At Mallala, the B concentrations in the grain of the individual lines were lower than the parental standard Yallaroi (Table 4.10), so the absence of a significant correlation between B response and yield is not unexpected. At Winulta and Walker Flat, which had B concentrations higher than Mallala, other environmental factors must have been more important than B in determining yield. There were no significant correlations observed between mean grain B concentration and grain yield for the four sites at which grain was analysed in 1996 (Figure 4.10). However, there was a noticeable contrast in the yield performances of WLLY9/3/2/4 and WLLY9/3/2/5 at Two Wells and Roseworthy compared to Mallala, where high and low grain B concentrations occurred, respectively.

Correlations between locations and seasons

Grain yields were significantly correlated between locations and seasons (Table 4.11). A significant positive correlation occurred between grain yields at Rudall in 1995 and Two Wells in 1996. This supports the notion that a B effect did occur at Rudall in 1995, despite not being significantly correlated with root length at B100 (due to the outlier). A significant negative correlation between Kapunda and Rudall in 1995 indicates that, at Kapunda, tolerance to B may have had a deleterious effect on grain yield, although other factors could be involved, such as maturity. In 1996 there was a highly significant correlation between grain yields at Roseworthy and Jamestown.

a)



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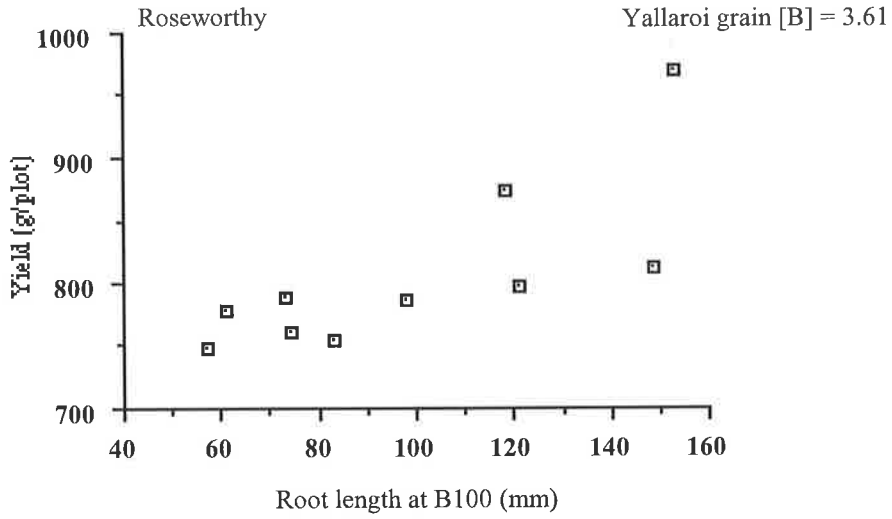
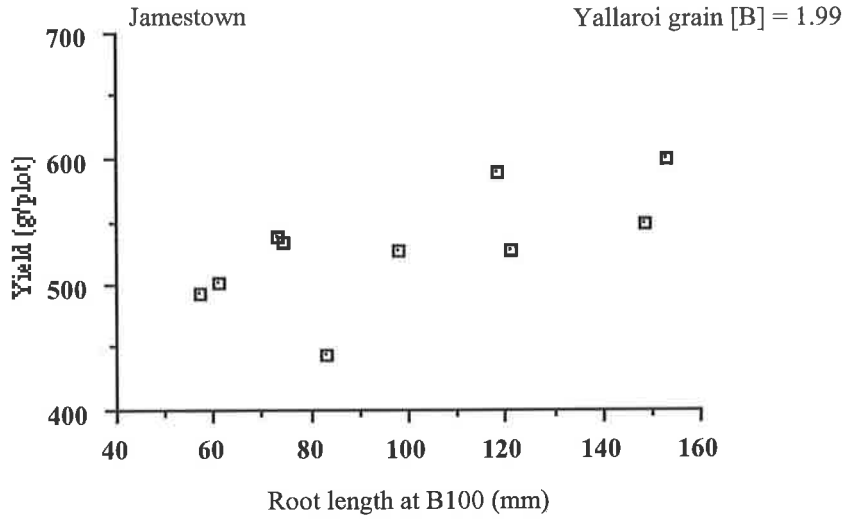


Figure 4.9. Relationship between root length at B100 and grain yield in 1996 for the F_7 and F_8 lines of Wol/3/AUS 14010/2*Yal//RH880009.

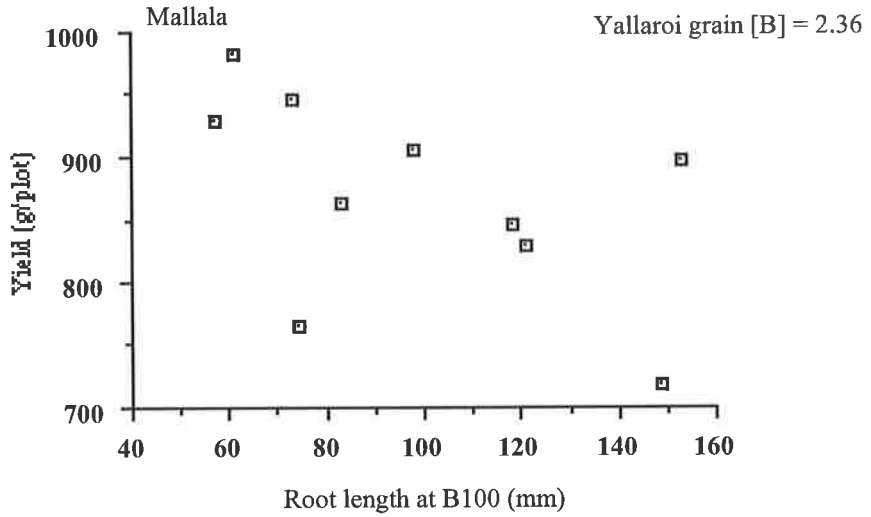
(a) grain yield at Two Wells vs root length at B100 ($r=0.815$, $P<0.01$).

(b) grain yield at Roseworthy vs root length at B100 ($r=0.761$, $P<0.05$).

c)



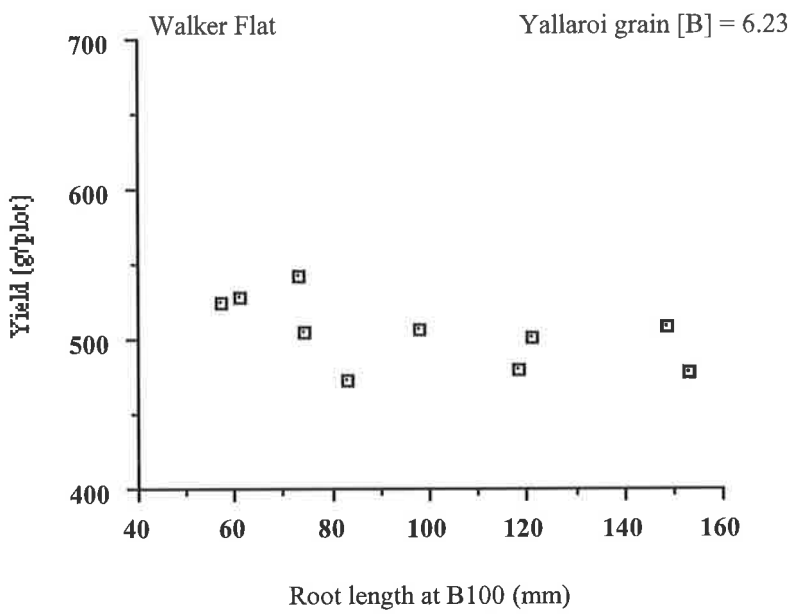
d)



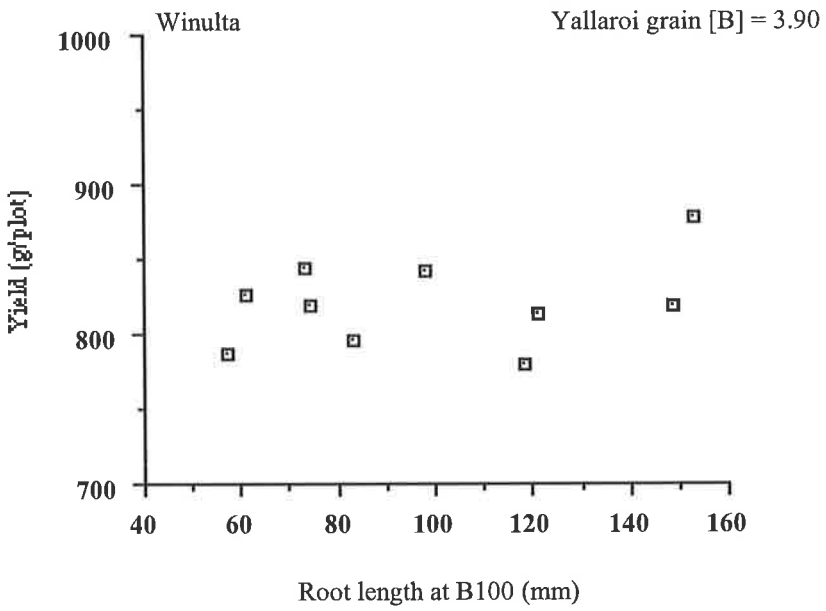
(c) grain yield at Jamestown vs root length at B100 ($r=0.644$, $P<0.05$).

(d) grain yield at Mallala vs root length at B100 ($r=-0.521$, n.s.).

e)



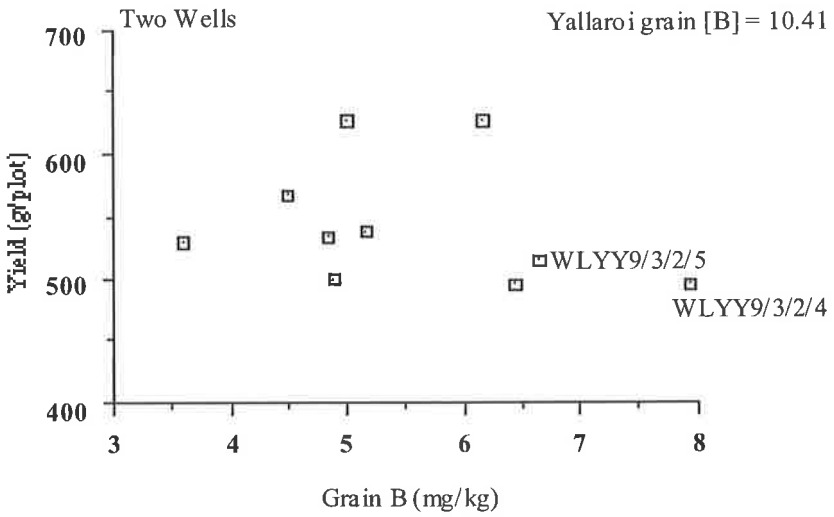
f)



(e) grain yield at Walker Flat vs root length at B100 (r=-0.557, n.s.).

(f) grain yield at Winulta vs root length at B100 (r=0.339, n.s.).

a)



b)

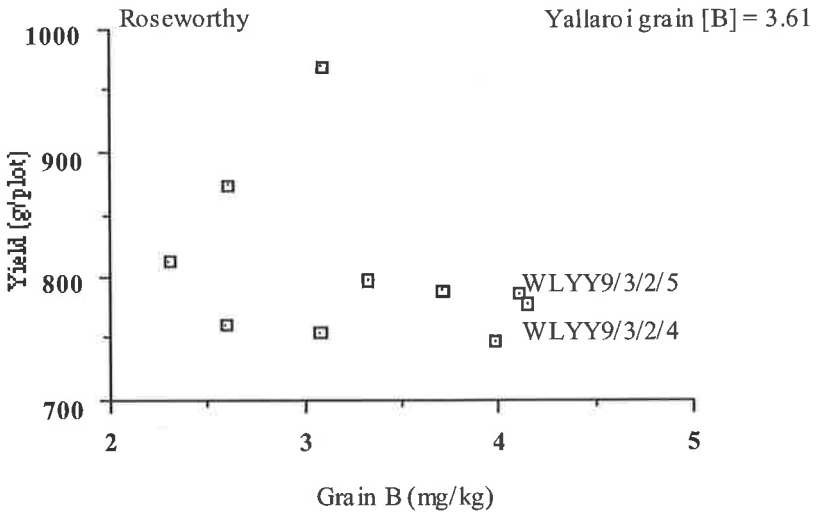
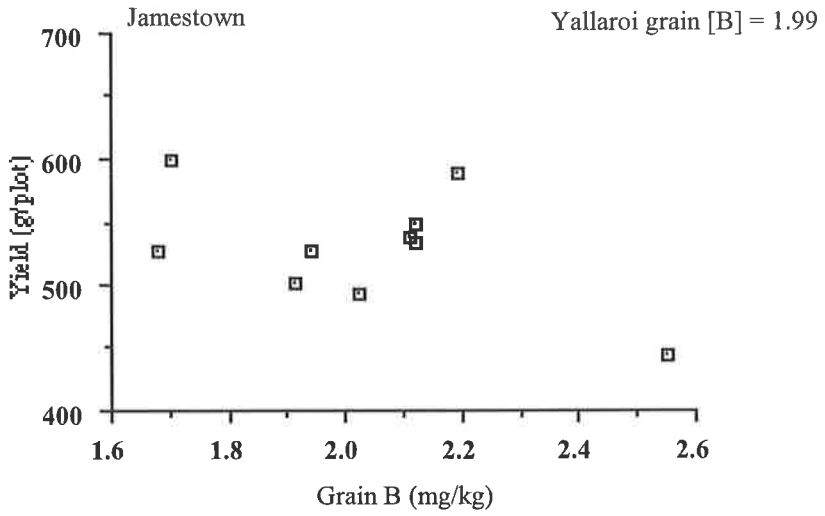


Figure 4.10. Relationship between grain B concentration and grain yield in 1996 for the F_7 and F_8 lines of Wol/3/AUS 14010/2*Yal//RH880009.

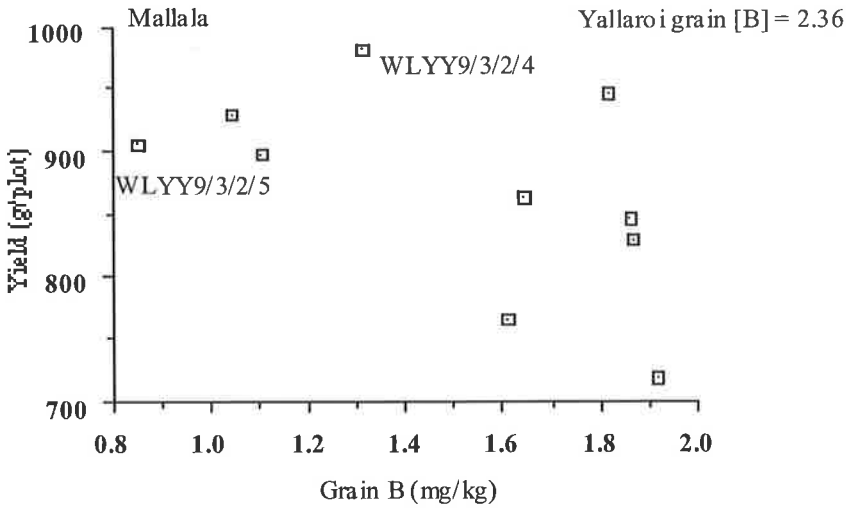
(a) grain yield at Two Wells vs concentration of B in grain at Two Wells (r=-0.259, n.s.).

(b) grain yield at Roseworthy vs concentration of B in grain at Roseworthy (r=-0.321, n.s.).

c)



d)



(c) grain yield at Jamestown vs concentration of B in grain at Jamestown (r=-0.494, n.s.).

(d) grain yield at Mallala vs concentration of B in grain at Mallala (r=-0.544, n.s.).

Table 4.11 Correlation coefficients (r) for comparisons of grain yields at Kapunda and Rudall in 1995 and Two Wells, Roseworthy and Jamestown in 1996. Comparisons are among seven lines in 1995 and ten lines in 1996.

	Kapunda 1995	Rudall 1995	Two Wells 1996	Roseworthy 1996
Rudall 1995	-0.860*			
Two Wells 1996	-0.720	0.777*		
Roseworthy 1996	-0.105	0.179	0.623	
Jamestown 1996	-0.329	0.510	0.474	0.825**

For 1995 comparisons, n=7 * P<0.05.

For 1996 comparisons, n=10 ** P<0.01 * P<0.05

Other elements

Results of the grain analyses from four locations in the 1996 experiments are summarised in Table 4.12. The concentrations of Fe should be viewed with caution, because of the possibility of contamination from sampling instruments and soil. The nitric acid digestion procedure does not extract all Na from grain (Zarcinas *et al.*, 1987), therefore, the results for Na may not represent the total Na content of grain.

The significance of the differences among lines for all elements, including B, is indicated by the F ratios from analysis of variance for concentrations of elements in the grain at Two Wells, Roseworthy, Jamestown and Mallala in 1996. Significant differences occurred among lines for the concentration of many elements in grain at all four sites. The range in concentrations of elements was greatest for B (more than two-fold) at Two Wells and at Mallala, however, this is to be expected since the parents were specifically chosen for contrasting response to B. The significant differences between lines for the concentration of elements in grain indicates that there was segregation at the F_2 generation for accumulation of all elements.

Table 4.12. The minimum and maximum family mean concentrations (mg kg^{-1}) of elements in grain, mean concentrations over all families and F ratios for comparisons among families for experiments grown at four locations in 1996. Mean concentrations of elements in the recurrent parental standard are also included for comparison.

Site	Element										
	Fe	Mn	B	Cu	Zn	Ca	Mg	Na	K	P	S
	(mg kg^{-1})										
Two Wells											
Minimum	30.0	43.1	3.6	4.8	16.7	287.4	1064	126.4	4825	3171	1332
Maximum	38.6	60.5	7.9	6.3	21.2	354.5	1360	229.6	6226	3928	1663
Mean	34.0	49.5	5.5	5.4	18.6	325.0	1189	197.8	5389	3489	1461
$F_{(9,36)}$	7.17	7.82	6.09	6.55	4.27	3.17	15.28	2.46	8.09	7.26	3.33
Yallaroi	35.0	44.7	10.4	5.4	18.1	311.8	1148	156.2	5398	3587	1558
Roseworthy											
Minimum	30.5	34.6	2.3	4.4	17.2	330.0	1297	132.0	5357	3469	1675
Maximum	39.2	39.9	4.2	5.4	22.4	443.0	1502	325.0	6720	4419	2157
Mean	34.2	38.0	3.3	4.9	19.8	380.7	1371	248.0	6143	3990	1860
$F_{(9,36)}$	4.97	1.24	5.10	6.72	1.57	5.32	4.64	1.84	9.26	6.35	12.50
Yallaroi	37.7	37.5	3.6	5.2	20.6	345.6	1375	181.0	6370	4359	1942
Jamestown											
Minimum	29.3	37.1	1.7	3.7	18.4	301.0	1033	19.4	5259	2768	1493
Maximum	34.5	44.5	2.6	4.4	23.0	375.0	1204	32.2	6648	3441	1681
Mean	32.0	40.9	2.0	4.0	20.4	332.8	1110	25.44	6058	3144	1576
$F_{(9,36)}$	5.78	3.16	3.3	3.58	3.38	2.45	3.43	2.32	5.10	2.49	2.86
Yallaroi	35.5	38.9	1.99	3.8	21.7	273.6	1130	21.8	5944	3449	1697
Mallala											
Minimum	22.5	37.4	0.9	2.6	17.0	286.9	1109	65.7	5153	3220	965
Maximum	30.7	50.8	1.9	4.4	23.4	394.6	1310	144.8	5823	4024	1285
Mean	26.6	44.9	1.5	3.4	19.8	325.9	1224	125.7	5589	3612	1097
$F_{(9,36)}$	13.16	12.91	4.43	17.73	9.42	14.46	11.13	2.49	3.28	16.48	11.94
Yallaroi	27.5	48.2	2.4	3.7	21.2	301.9	1270	154.9	5462	3765	1167
Critical $F_{(9,36)}$	2.15										

The occurrence of significant differences among lines for elements other than B allows two types of comparisons to be made. First, the comparison of concentrations in grain between sites can be examined for each element. Second, comparisons can be made between the concentration of B and other elements to determine whether there are pleiotropic interactions between uptake of B and other elements or dilution effects.

As there is very little information on critical or optimal concentrations of elements for durum wheat, the results are compared with the concentrations published for durum (Zubaidi,1996) and for bread wheat (Schultz and French, 1976; Paull, 1990). The comparison between species may be tentative and somewhat speculative, since a higher critical level in durum than in bread wheat exists for Mn (Saberi *et al.*, 1996), and this may also apply to other elements, eg. Zn (Grewal *et al.*, 1996). Although this method will not positively identify nutritional disorders, it will provide a measure of the nutritional status of each site relative to other South Australian sites.

The mean concentrations of elements in grain at the four sites in 1996 were compared with previously published values. The concentrations of P, K, Mn and Zn at all sites were higher than the mean values reported by Zubaidi (1996), Schultz and French (1976) or Paull (1990), indicating no signs of deficiency for these elements. Fe concentrations at all sites were higher than the values reported by Zubaidi (1996). The concentrations of Ca, Mg, Na and Cu at all sites investigated here were below the mean reported values (0.04%, 0.15%, 0.03% and 6 mg kg^{-1}, respectively) of Schultz and French (1976). However, the concentrations of Mg and Cu for the current study were similar to those reported by Paull (1990), while the concentrations of Ca were higher. The concentration of S at Mallala in 1996 was below the minimum reported value (0.12%) of Schultz and French (1976), but similar to those obtained by Paull (1990) in the 1989 season, which could nevertheless be considered a deficient level.

The concentrations of Na at Two Wells and at Roseworthy were higher than the values reported by Zubaidi (1996), while the concentration from Mallala was similar, but Jamestown had a lower Na level. With the exception of Jamestown, the accumulation of Na in durum grain from the other sites was higher than that found in bread wheat by Paull (1990). Although durum is known to accumulate more Na than bread wheat (Joshi *et al.*, 1982), locations where high levels of sodium uptake were recorded may reflect the presence of Na toxic soils which could limit grain yield.

Overall, there were few significant correlations between sites in 1996 (Table 4.13). B was the only element for which the concentrations in the grain were significantly correlated between a number of sites; positively between Two Wells and Roseworthy, negatively between Two Wells and Mallala, and between Roseworthy and Mallala (previously shown in Figure 4.5). The grain concentrations of Na and K were positively correlated between Two Wells and Mallala, and concentration of Fe in grain from Two Wells was significantly correlated with grain concentration from Jamestown. This shows that variation in some nutrients is consistent across some of the sites.

Correlations between B concentrations and that of other elements were compared for grain in 1996 (Table 4.14). The concentration of B was positively correlated with Fe concentration at Two Wells, but negatively at Roseworthy and at Jamestown; positively correlated with Mn at Two Wells and negatively at Jamestown; positively with Cu at Two Wells and at Mallala, and negatively at Jamestown; positively with Zn at Mallala; positively with Mg at Two Wells and Mallala; positively with K at Two Wells and negatively at Jamestown; positively with P at Two Wells and Mallala; and positively with S at Two Wells and Mallala. The inconsistent results for grain suggest that the significant correlations between B and Fe, Mn, Cu, Zn, Mg, K, P and S are either a secondary effect or, if they are the result of direct interaction with uptake of B, the interaction is subject to environmental influences. The generally positive correlations at Two Wells and generally negative correlations at Jamestown are likely to reflect differing maturity, seasonal or soil effects. The variable correlations for grain nutrient

Table 4.13. Correlation coefficients (r) of the concentration of elements in grain between sites for the ten backcross-derived lines grown at Two Wells, Roseworthy, Jamestown and Mallala in 1996.

Site 1	Site 2	Element										
		Fe	Mn	B	Cu	Zn	Ca	Mg	Na	K	P	S
Two Wells	Roseworthy	-0.58	0.14	0.70*	-0.09	-0.58	0.07	-0.59	0.38	0.09	-0.62	-0.24
Two Wells	Jamestown	0.68*	0.57	-0.60	0.47	0.18	0.39	0.55	0.58	0.57	0.60	0.18
Two Wells	Mallala	-0.21	-0.62	-0.72*	-0.48	-0.28	0.48	-0.27	0.63*	0.68*	-0.52	-0.42
Roseworthy	Jamestown	-0.46	-0.34	-0.31	-0.09	-0.29	0.32	-0.47	0.49	-0.44	-0.38	-0.11
Roseworthy	Mallala	0.62	0.04	-0.65*	0.51	0.10	0.26	0.14	0.05	-0.14	0.32	0.44
Jamestown	Mallala	0.01	-0.39	0.33	0.01	-0.04	0.41	-0.27	0.18	0.51	-0.46	-0.12

* $P<0.05$

Table 4.14. Correlation coefficients (r) for comparisons between the concentration of B and other elements in grain for ten progeny lines grown at Two Wells, Roseworthy, Jamestown and Mallala in 1996.

B *vs* Element	Two Wells	Roseworthy	Jamestown	Mallala
B *vs* Fe	0.71**	-0.78**	-0.64*	0.57
Mn	0.82**	-0.16	-0.76*	0.63
Cu	0.77**	-0.61	-0.65*	0.73*
Zn	0.62	-0.31	-0.54	0.76*
Ca	0.43	-0.45	-0.22	0.21
Mg	0.68*	-0.49	-0.54	0.66*
Na	0.51	0.40	-0.49	-0.11
K	0.90**	-0.30	-0.65*	-0.36
P	0.90**	-0.45	-0.56	0.67*
S	0.75*	-0.56	-0.35	0.72*

** $P<0.01$ * $P<0.05$

concentrations across sites suggest that selection of genotypes with low B accumulation is unlikely to consistently affect the nutritional status with respect to the other elements analysed.

Correlation between yield and other elements

The correlation between grain yields at Two Wells, Roseworthy, Jamestown and Mallala in 1996 and mean concentration of elements in grain of F_7 and F_8 lines (Table 4.15) were determined. Significant positive correlations between the concentration of Fe and Cu in grain and grain yield at Jamestown in 1996 may be the result of a limiting supply of Fe and Cu, and occurrence of sufficient genetic variation within the population for the more efficient genotypes to be at a yield advantage. The grain Cu concentration from the site was higher

Table 4.15. Correlation coefficients (r) between grain yield and elemental concentration in grain of F_7 and F_8 lines grown at four sites in 1996.

Site	Element										
	Fe	Mn	B	Cu	Zn	Ca	Mg	Na	K	P	S
Two Wells	0.40	0.20	-0.26	0.20	0.26	0.20	0.33	0.09	-0.61	-0.04	0.09
Roseworthy	-0.07	0.27	-0.32	-0.01	-0.42	-0.29	-0.17	-0.34	-0.42	-0.36	-0.32
Jamestown	0.66*	0.59	-0.49	0.67*	0.37	-0.39	0.30	-0.19	0.14	0.14	0.34
Mallala	-0.81**	-0.67*	-0.54	-0.79**	-0.71*	-0.18	-0.60	0.38	0.08	-0.72*	-0.88**

** $P<0.01$ * $P<0.05$

than the critical concentration of 2.5 mg kg^{-1} for bread wheat (King and Alston, 1975), although durum may be more sensitive to lower levels. As the concentration of Fe in grain at Jamestown was greater than that at Mallala and similar to Two Wells and Roseworthy (Table 4.12) it would seem unlikely that Fe was a yield limiting factor at Jamestown but not the other sites.

Significant negative correlations between the concentration of elements in the grain and grain yield at Mallala in 1996 resulted for six elements. A negative correlation would arise through either toxicity, as is the case for B, or yield related dilution of an element in limited supply. In view of the generally nutritionally deficient soils of South Australia, the latter would be the more likely.

4.4 Discussion

This series of experiments has utilized a population developed by backcrossing moderately B sensitive Australian durum varieties and an advanced line to a moderately tolerant genotype, AUS 14010, to increase the level of B tolerance and evaluate the grain yield of lines with contrasting response to B at a number of sites of different B status. Segregation in the F_2 generation allowed recombination and reassortment of genetically independent characters which were expressed subsequently in the derived lines. Characters which were found to co-vary with B accumulation would therefore be those which either (i) are genetically linked or interact pleiotropically with genes controlling accumulation of B, or (ii) are environmental variables which interact with the response to B.

The filter paper test at high B demonstrated large significant differences in response to toxic levels of B among the F_4 progeny lines. This indicates that some of the F_2s selected in the glasshouse experiment were heterozygous, and selection for B tolerance on the basis of leaf symptom score may not be desirable. Jamjod (1996) found that as a consequence of the

partial dominance of tolerance to B it was necessary to conduct segregation studies in the F_3 generation when it is possible to distinguish homozygous tolerant and sensitive families.

Boron response at B100 of the F_2-derived lines was significantly correlated with grain yield of the F_6 generation at Two Wells and at Mallala in 1995. There was a positive correlation between root length and grain yield from Two Wells, where high concentrations of B in the grain occurred. At Mallala, where grain B concentrations were low, the correlation with grain yield was negative. Similar findings have been reported for barley (Nable *et al.*, 1990b). This suggests that B tolerant varieties may be more susceptible to B deficiency at low B sites since the range between B deficiency and B toxicity for plants is narrower than any other element (Eaton, 1944; Reisenauer *et al.*, 1973).

A sample of seven lines were selected to use in the G×E experiments conducted in 1995 (Chapter 3) on the basis of mean root length in the filter paper experiment, phenotype and grain yield when grown at Mallala in 1994. During 1995, three additional lines were selected and multiplied, including one with shorter roots at B100 (WLYY9/1/2/2/1) and two with longer roots at B100 (WLYY9/1/4/4/S and WLYY9/2/6/3), for inclusion in the G×E experiments for 1996. These three lines had not been included in the initial experiments conducted in 1994 due to limited seed availability. This means of selection for the G×E experiments was adopted for two reasons: first, to reduce the total number of plant samples to be analysed (to increase replication and the number of locations at which the lines were grown); and second, in 1996, to maximise the range in responses to toxic levels of B and so increase the probability of identifying significant relationships between the results of the filter paper assay and other variables.

The filter paper test was useful in identifying B tolerant lines and, at sites high in B, yield was generally positively correlated with root length in this test, although at some other sites the correlation was not evident and other factors contributed to yield differences between genotypes. The F_7 and F_8 lines with longest seedling root length at B100 were found to have

a yield advantage when grown at Rudall (although there was an outlier) in 1995, and at Two Wells and Roseworthy in 1996, sites where moderately high to very high concentrations of B occur in soil (Paull *et al.*, 1992d). The difference in yield ranking of the outlier, WLYY9/1/3/4, at Rudall was likely due to its earlier flowering compared to other lines. The highest concentration of grain B of the Yallaroi standards occurred at Rudall in 1995 and at Two Wells in 1996 (Table 4.10). At Jamestown, where marginally toxic concentrations of B resulted in the grain, there was also a positive correlation between root length at B100 and grain yield. The data shows that there was sufficient genetic variation for tolerance to B within the current population for more tolerant lines to be selected, which may result in better yields when these are grown under high B conditions.

Considerable discrepancies exist within the literature for critical concentrations of B within plants, particularly for assessing toxicity. Yield losses for barley and bread wheat are likely to occur when the concentration of B in the grain exceeds 3 mg kg^{-1} (Cartwright *et al.*, 1984; Paull, 1990). However, it may be considered that there is no critical value for toxicity, merely increasing loss of yield. Despite marked differences in grain B levels at Two Wells and Jamestown, grain yields were positively correlated to root length at B100 at both sites.

In order to estimate the yield advantage of progeny at sites where toxic levels of B occurred, the lines were divided into three categories based on the mean root length of the F_4 generation in the filter paper test: mean root length (RL) >93 mm; mean root length with high standard deviation (heterozygous); or <93 mm. The yields obtained for the three categories and the yield advantage of genotypes with RL>93 category at Two Wells and Rudall in 1995, and at Two Wells, Roseworthy and Jamestown in 1996, are summarised in Table 4.16. Similar yield advantages of B tolerance compared with sensitivity have been reported for bread wheat in South Australia (Paull, 1990; Moody *et al.*, 1993; Campbell *et al.*, 1994). The yield advantage appears to be associated with the severity of stress as indicated by B levels in Yallaroi grain.

Table 4.16. Yields of progeny lines categorised as RL>93 mm, heterozygous (H) or RL<93 mm at locations where genotypes with RL>93 had a yield advantage (expressed as % of genotypes with RL<93).

Site	Year	Yallaroi Grain [B] ($mg\ kg^{-1}$)	Yield (t/ha)			Yield advantage (%)
			RL>93	H	RL<93	
Two Wells	1995	2.4	1.63	1.71	1.47	11
Rudall	1995	3.4	0.81	0.69	0.69	18
Two Wells	1996	10.4	2.03	1.74	1.71	19
Roseworthy	1996	3.6	2.86	2.76	2.56	12
Jamestown	1996	2.0	1.86	1.86	1.68	11

It is significant that a large effect due to B occurred in 1996 compared with 1995, considering total rainfall in both seasons was similar (Appendix A). The difference in severity of B toxicity between the seasons can be explained by rainfall distribution throughout the growing season. At Two Wells in 1995, sufficient rainfall occurred consistently during the growing period of April to October (Figure 4.11), therefore water stress during grain fill was probably low. However, at Two Wells in 1996, rainfall was concentrated through the months July to September, with little rain after flowering and during grain fill in October and November. The plants in 1996 had to rely more on sub-soil moisture reserves to fill the grain, and it is likely that B tolerant plants had greater root activity in the sub-soil, contributing to higher yields. The dependence on root growth in the sub-soil under low rainfall conditions therefore exposed the plants to high soil B levels.

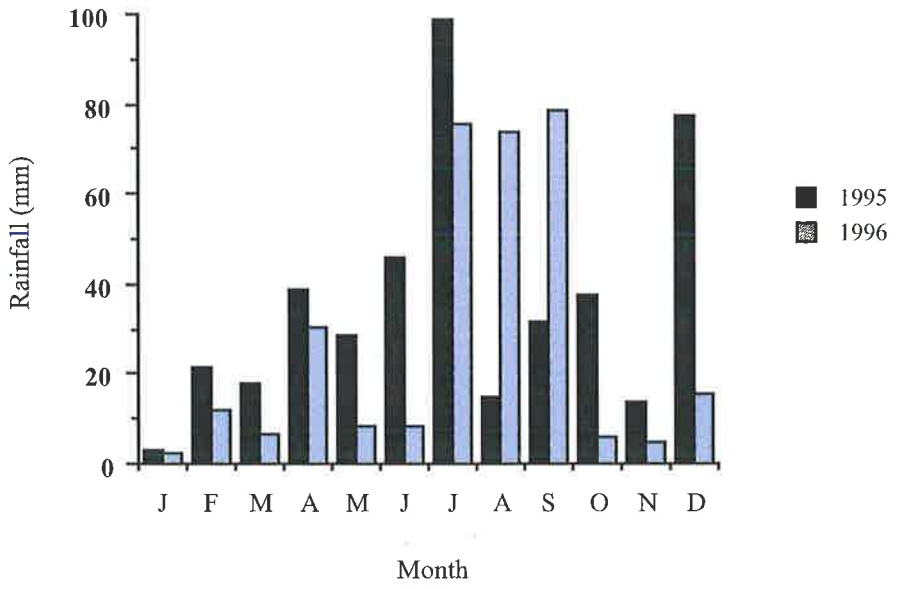


Figure 4.11. Rainfall distribution at Two Wells in 1995 and 1996.

The backcross breeding method was suitable for transferring B tolerance to sensitive Australian durum wheat varieties. A similar success has been achieved in bread wheat by using backcrossing to transfer B tolerance from Halberd to Schomburgk (Rathjen *et al.*, 1993). Since the identification of B toxicity in the soils of southern Australia, there has been a change in the selection strategy rather than parental material in the bread wheat breeding programs. As the sources of tolerance are well adapted to the Australian enivronment, the strategy has been to use only limited backcrossing in the transfer of B tolerance to other local varieties. Lines are selected on the basis of yield at high B sites, rather than for response to B during early generations. The adoption of a high B site for early generation yield testing has resulted in a large increase in the proportion of advanced lines which are moderately tolerant to B (Paull *et al.*, 1992d). This strategy was previously unsuitable for durum wheat, since the donor parents were exotic and poorly adapted to the local environment. It has been necessary to undertake more backcrosses, along with selection for B tolerance at each generation, to produce a population with a minimum level of adaptation. The production and identification of high yielding B tolerant lines in this study, such as WLYY9/2/6/3, will provide more suitable sources to use as donor parents in the breeding program.

Tolerance to B toxicity has been consistently related to B concentrations in various plant tissues (eg. leaf, grain) of crops (Cartwright *et al.*, 1987; Paull *et al.*, 1988a, 1992b) and correlated with grain yield (Rathjen *et al.*, 1987). The results in this study for durum wheat however found no correlations between B concentrations in plant tissue and grain yield. Response to B was rated on root length at B100, and despite being correlated with yield under high B conditions, was not correlated with B accumulation. Nevertheless, the lines WLYY9/3/2/4 and WLYY9/3/2/5 are of interest since their high grain boron concentrations and low yield performance at Two Wells and Roseworthy in 1996 contrasted to that at Mallala (Figure 4.10), and suggests intolerance to B which could have occurred as a result of misclassification in the F_2 generation.

Jamjod (1996) also reported no correlation between root length at B100 and concentration of B in shoots grown in high B soil. Recently, a region on chromosome 3H in barley was found to be associated with variation in root length independent of B accumulation in the control of B tolerance (Jefferies *et al.*, 1999a). It is possible the gene conferring B tolerance derived from AUS 14010 may have a similar mode of action to that on barley chromosome 3H. In hexaploid wheat, Jefferies *et al.* (2000) reported a region on chromosome 7B was associated with the control of boron uptake and with a reduction in the effect of boron toxicity on root growth suppression. Regions on chromosomes 7B and 7D were associated with leaf symptom expression.

Significant differences resulted for the concentrations of most elements in grain. The genetic variation for the other elements allowed comparisons to be made between the accumulation of B and the other elements. The correlations between mean line concentration of B and other elements in grain were inconsistent across sites (Table 4.14), suggesting strong environmental influences acting on the correlations. The accumulation of B in the grain was, therefore, genetically and physiologically independent of other elements measured in these trials. This result is consistent with previous reports on the accumulation of B in the grain for bread

wheat (Paull, 1990). Selection of durum genotypes of low B accumulation should therefore have no consistent effect upon the efficiency of uptake of other nutrients.

The above comments should be qualified and limited to the four genotypes used as parents of the population. Jamjod (1996) demonstrated that response to B in durum wheat is under the control of three independent loci, with Yallaroi differing to AUS 14010 at only one locus. It is therefore possible that other loci controlling response to B may be linked to genes controlling uptake of nutrients or response to environmental factors. As the parents were also chosen specifically on the basis of contrasting response to B, the number of significant differences between lines for the concentrations of other elements was fortunate rather than expected. It is probable that a greater range in efficiency of nutrient uptake exists for all elements than found in this population, especially since Australian durum germplasm is trace element inefficient (Graham, 1988; Saberi *et al.*, 1996; Cakmak *et al.*, 1998). An objective means of testing for genetic interactions between the accumulation of B and other individual elements would be to select parents on the basis of contrasting response to B and accumulation of at least one other element. This method should produce a greater range for the test element among the progeny, and therefore increase the probability of identifying interactions.

The results for B were consistent with the expectation of lines with long roots at high concentrations of B in the filter paper experiment having a yield advantage when grown under high B conditions in the field. The lines with the longest roots at B100 in the filter paper experiment produced the highest yields at Rudall in 1995, and at Two Wells and Roseworthy in 1996, where high concentrations of B occurred in the grain. The results of some other elements will now be discussed in a more speculative manner in an attempt to identify the reasons for anomalous responses in 1996, and to identify nutrients which may be influencing yield.

The Na concentrations in grain were of similar magnitude to those previously reported for durum by Zubaidi (1996), and generally higher than those reported for bread wheat by Paull (1990). Paull (1990) suggested that Na is present in the soil in toxic amounts when site mean concentration of Na in grain is 80 mg kg^{-1}. The Na concentrations in the durum grain from Two Wells, Roseworthy and Mallala were higher than the value considered toxic by Paull (1990). High levels of Na in the soils of South Australia may be in the form of sodicity, transient salinity (Rathjen *et al.*, 1999), or subsoil salinity (D. Maschmedt, pers. comm.).

Durum wheat is known to accumulate high concentrations of Na in plant tissues compared to bread wheat (Joshi *et al.*, 1982; Wyn Jones *et al.*, 1984). This has been attributed to the absence in durum of a gene on chromosome 4D which discriminates between Na and K (Gorham *et al.*, 1987). Bread wheat lines with low Na accumulation in shoots produced relatively greater yields than high Na lines when grown at strongly sodic sites (Paull *et al.*, 1992c). Liu *et al.* (2000) previously claimed the reduction in yield of durum compared to hexaploid wheat at Two Wells in 1994 was due to increased Na shoot concentrations, however, they did not consider the major contributing effect of *Fusarium* spp. infection which was present. Gorham *et al.* (1997) found, by incorporating the *Kna1* gene responsible for K/Na discrimination in bread wheat into durum, that Na accumulation did not increase with exchangeable sodium percentage (ESP) levels, and relative grain yield was greater than in the absence of the gene. Despite the high concentrations of Na in durum grain compared to hexaploid wheat in the current study, there was no relationship between grain yield and grain Na concentration. Since there was no correlation between grain B and Na, this indicates that the accumulation of B and Na are independent. Ultimately, it is likely tolerance to both toxicity traits will need to bred and selected for simultaneously.

Concentration of Na in the grain from Jamestown was lower than that at the other three sites in 1996. The map of saline and sodic soils of Australia (Northcote and Skene, 1972) describes the soil in the region of Jamestown as AS3, or alkaline and strongly sodic (ESP>15). However, at the trial site the soil at 0-20 cm depth had pH 6 increasing to pH 8 at

60 cm. It is likely that the acidity of the topsoil combined with lack of root growth throughout the growing season, due to low April-October rainfall (261.9 mm, Appendix A), contributed to the low Na accumulation at this site.

A contrasting response occurred in 1996 at Mallala compared to Two Wells, Roseworthy and Jamestown for grain elemental concentrations and grain yield. With low B concentrations in the grain, and no yield effect due to B sensitivity, B toxicity was not a yield limiting factor. In the absence of B toxicity at Mallala, high yields were obtained, and the significant negative correlations between grain concentrations of Fe, Mn, Cu, Zn, P and S and grain yield are a response to yield related dilution of limited supplies of nutrients.

The results of these experiments have demonstrated that tolerance to B was successfully transferred to Australian durum wheat germplasm and a yield advantage of up to 19% occurred when grown under high B conditions in the field. The transfer of B tolerance into backcross progeny was simply monitored using a laboratory screening test. Genetic variation for root length at B100 was significantly and positively correlated with grain yield at high B sites. In contrast to bread wheat, no relationship was found between B accumulation in shoots or grain and grain yield. The concentration of B in grain was genetically independent of all other elements, therefore, B accumulation should not directly affect the nutritional status of plants with respect to other elements. The results are encouraging from the point of view of breeding B tolerant varieties for improved yields, and this should see an expansion of durum production into regions of South Australia where B toxicity occurs. However, a greater understanding of other environmental factors and interactions which affect yield in high B environments is required.

Chapter 5

The effect of *Glu-1* loci HMW glutenin subunits on physical dough properties in durum wheat

5.1 Introduction

Until recently, the major area of durum wheat production in Australia has been in northern NSW. The semolina of durum produced in South Australia has been associated with weaker dough strength compared to that from NSW (McKenzie, 1994). This is considered undesirable, as lower dough strength causes complications during the manufacture of pasta, namely, shapes being unable to retain their conformation after extrusion through a die (J. Alvino, pers. comm.). Weak dough strength also contributes to poor pasta cooking performance (Grzybowski and Donnelly, 1979; Damidaux *et al.*, 1980b). To consistently produce good quality pasta products, further research is needed on the parameters of dough strength in durum and their contribution to cooking quality.

Dough strength characteristics of durum have been shown to be affected by the environment (McKenzie, 1994). Maximum daily temperatures during grain filling in South Australia (eg. Roseworthy, 25.4°C; Loxton, 26.7°C) are, on average, lower than those in northern NSW (eg. Tamworth, 28.5°C). The findings in bread wheat (Randall and Moss, 1990) suggest it is highly probable that the cooler temperatures in the southern region contribute to reduced dough strength in locally produced durum. The durum variety Yallaroi originates from the National Durum Wheat Improvement Program (NDWIP) in Tamworth, NSW, and reduced dough strength has been associated with production of this line in South Australia compared to NSW.

When a collection of durum wheat lines from the NDWIP and exotic germplasm was grown in South Australia, the dough strengths of the breeder's lines were stronger compared with a

diverse range of exotic germplasm (Liu and Rathjen, 1994). Therefore, screening modern durum germplasm as a source for varieties with increased strength above the current level will have limited success. An alternative approach, such as genetic manipulation, is more likely to make rapid gains.

Historically, durum wheat was found to have gluten strength too low to manufacture bread with loaf volumes comparative to hexaploid wheat (Kerber and Tipples, 1969), despite the traditional consumption of durum bread in southern Italy. Consequently, the research devoted to quality improvement of durum (pasta- or bread-making) has been minor relative to bread wheat. It has only been since genetic variation for gluten strength in durum wheat has been shown to be associated with the electrophoretic pattern of the *Gli-B1* locus (Damidaux *et al.*, 1978) that numerous studies investigating traits for improved pasta have been conducted. It was later discovered that strong gluten is due to the LMW-2 banding pattern encoded by the *Glu-B3* locus (Pogna *et al.*, 1988), which is highly linked to the *Gli-B1* locus (Payne *et al.*, 1984b) encoding for the γ-gliadin 45 polypeptide (Damidaux *et al.*, 1978).

In bread wheat HMW glutenin subunits are well correlated with the rheological and bread-making qualities of flour (Payne, 1987). Strength can be improved by incorporating desirable HMW alleles at each *Glu-A1* locus. However, interpretation of the findings for HMW studies on dough strength in durum have been less conclusive (du Cros *et al.*, 1982; Autran and Galterio, 1989; Kaan *et al.*, 1993), and confounded by genotypic and phenotypic variation in the same growing environment (eg. Liu, 1994). Despite the importance of cooking quality, the effect of HMW allelic variants on pasta characteristics has not been thoroughly evaluated. This chapter examines the gluten, rheological, and cooked pasta properties of Yallaroi backcross lines incorporating allelic variants of HMW gluten subunits which are desirable for dough strength in bread wheat.

5.2 Materials and methods

5.2.1 Plant material

Parental genotypes

The durum wheat variety Yallaroi was provided by Dr R.A. Hare, NDWIP, Tamworth. Varieties Duramba biotype A (with allelic variant 2* at the *Glu-A1* locus) and Kharkof-5 (subunit 1) were obtained from Drs K.W. Shepherd and C.-Y. Liu, Department of Plant Science, University of Adelaide, Waite Campus; Minieh 72 (AUS 13033) from the AWCC, Tamworth. Dr C.-Y. Liu also very kindly provided seeds of disomic 1D substitution line Langdon 1D(1A) incorporating HMW glutenin alleles *Glu-D1d* (coding for bands 5+10) and a sister line derivative possessing *Glu-B1i* (bands 17+18) which are coded I_{8-2} and N_{4-1}, respectively (see Liu, 1995b).

Donor parents were selected on the basis of allelic variation at the *Glu-1* loci. A backcross program was undertaken using donor parents with the appropriate *Glu-1* allele, and the commercial durum variety Yallaroi as the recurrent parent. The glutenin subunit composition of the parents is presented in Table 5.1. Details of the crossing combinations are presented in Table 5.2. SDS-PAGE was employed to identify glutenin bands of the F_1 progeny after each backcross to Yallaroi. Due to limited availability of time, Duramba A was backcrossed to Yallaroi twice (BC_2), whereas a further backcross (BC_3) was required with Kharkof-5 and I_{8-2} to increase adaptation. N_{4-1} was backcrossed once (BC_1) as when Yallaroi was used as the female parent in the backcross, no progeny possessed subunits 17+18. Then N_{4-1}/Yallaroi was used as the female parent in the cross with Yallaroi, with some progeny having the desired subunits. This suggests there was gametic selection against the subunits 17+18 in the pollen. One F_2-derived line homozygous for bands 17+18 was selected for multiplication. Later, one F_2-derived line from a single cross between Yallaroi and Minieh 72 was also included in the experiments. Minieh 72 has a similar banding pattern to Yallaroi, but in

addition with novel *Glu-A1* subunit V. Due to the late inclusion and time constraint, since Yallaroi and Minieh 72 were agronomically similar, no backcrossing was performed.

Table 5.1. HMW glutenin subunit alleles on homoeologous group 1 and 3 chromosomes (with banding subunits in parenthesis) of donor lines compared to the commercial variety Yallaroi.

Genotype	*Glu-B3*	*Glu-A1*	*Glu-B1*	*Glu-D1*
Yallaroi	2	*c* (0)	*IX* (7+16)	
Kharkof-5	2	*a* (1)	*b* (7+8)	
Duramba A	2	*b* (2*)	*f* (13+16)	
Minieh 72	1	(*V*)	*IX* (7+16)	
$N_{4\text{-}1}$	1	*c* (0)	*i* (17+18)	
$I_{8\text{-}2}$	1		*b* (7+8)	*d* (5+10)

Table 5.2 Backcrossing procedure used to generate progeny lines from donor parents.

Cross	Number of F_2-derived families
4*Yallaroi/Kharkof-5	24
2*Yallaroi//Duramba A/Yallaroi	19
Yallaroi/Minieh 72	1
$N_{4\text{-}1}$/2*Yallaroi	1
3*Yallaroi//$I_{8\text{-}2}$/Yallaroi	9

After the final cross, heterozygous F_1 plants were grown in a glasshouse in potting mix to produce the F_2 generation. For the progeny lines of Kharkof-5, Duramba A and $I_{8\text{-}2}$ up to 24 F_2 plants were grown in pots in the glasshouse. Bulked F_3 seed from a single F_2 plant was sown as a row. The seed obtained from each F_3 row was sown as an individual F_4 plot. The procedure for developing and evaluating the lines is outlined in Figure 5.1.

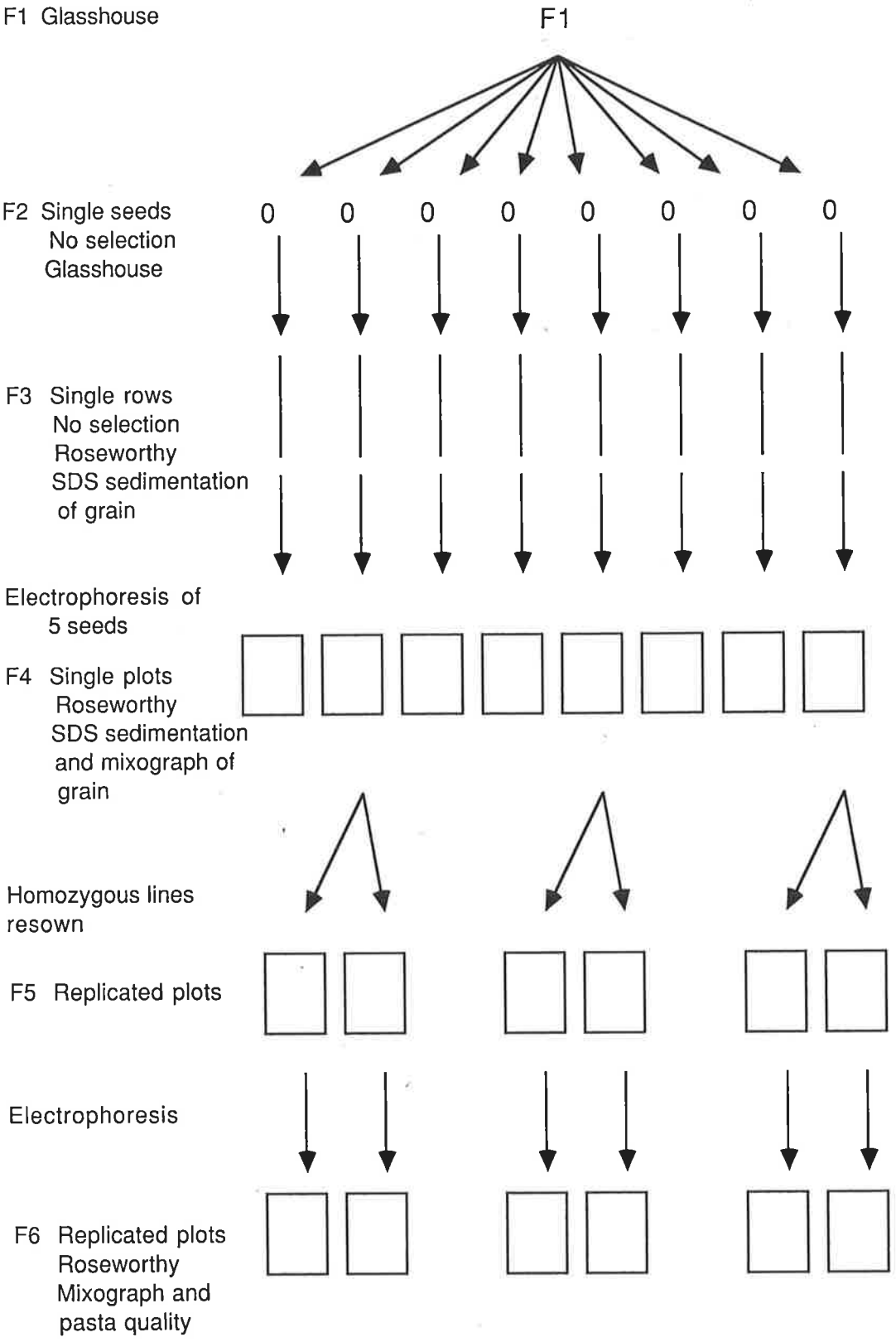


Figure 5.1. Procedure for the development and evaluation of the backcross lines. The F3 and later generation plots were based on individual F2-derived lines.

To test for homogenity after the final backcross, five F_4 seeds of each F_2-derived family were analysed separately for their glutenin composition by electrophoresis. Due to the segregation of alleles in the Duramba A population, four F_4 seeds (two lines from two F_2-derived families) with the possible HMW allelic combinations were selected and two F_5 seeds from each of the four lines were multiplied (see Figure 5.2). To confirm the banding patterns present in all the lines, three F_6 seeds were retested by electrophoresis. The previous findings were confirmed, however, there were no I_{8-2} progeny lines possessing the bands 5+10. More extensive testing was conducted on the I_{8-2} progeny lines, with less than 20% identified as having 5+10.

Control genotypes

The genotypes, country of origin and glutenin subunit composition of the controls are presented in Table 5.3. The durum wheat variety Wollaroi, which is the current benchmark for quality in Australia, and Tamaroi (RH912025), the replacement commercial variety in South Australia, were supplied by Dr R.A. Hare, NDWIP, Tamworth; Dukem 5, an advanced line from CIMMYT, by AWCC; Duramba biotype C, with the allelic variant null at the *Glu-A1* locus and LMW-2 pattern, encoded by the *Glu-B3* locus, by Drs K.W. Shepherd and C.-Y. Liu, Department of Plant Science, University of Adelaide, Waite Campus; and Simeto, an Italian variety with high dough strength, by Dr D. Lafiandra, University of Tuscia, Viterbo, Italy.

The hexaploid wheat varieties Buckley, a soft grain biscuit wheat, and Molineux, a hard grain bread wheat, were provided by Dr A.J. Rathjen, Department of Plant Science, University of Adelaide, Waite Campus; and Warimex, a variety in which the *Glu-A1* allele is absent, by AWCC.

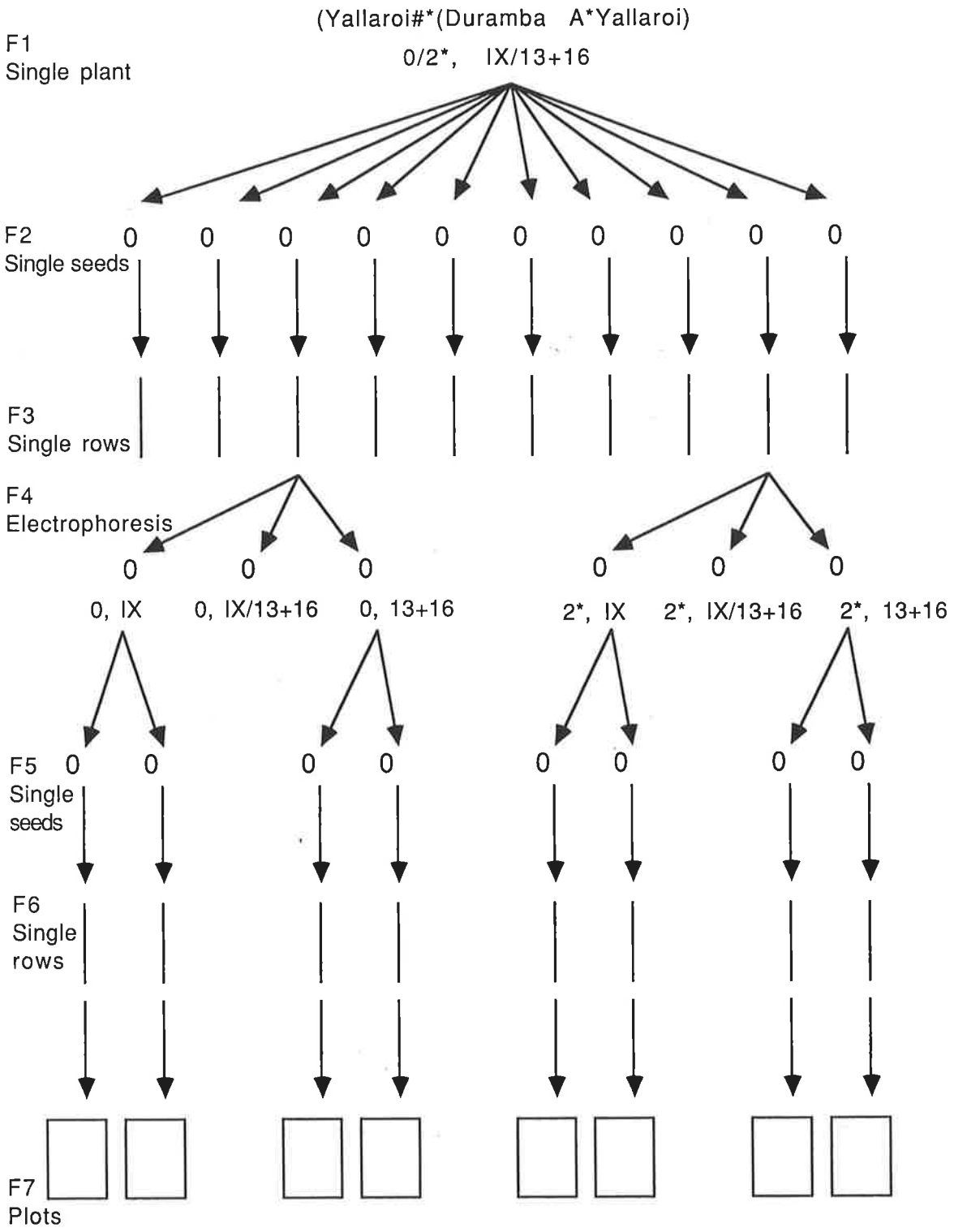


Figure 5.2. Procedure used to select homozygous BC2F7 genotypes with four combinations of HMW glutenin subunits 0, 2*, IX and 13+16, from the backcrossing between Yallaroi and Duramba A.

5.2.2 Field experiments

Assessment of F_3 progeny lines

Up to 50 seeds of 24 BC_3F_3 progeny lines of Kharkof-5, 19 BC_2F_3 progeny lines of Duramba A, and 9 BC_3F_3 progeny lines of $I_{8\text{-}2}$ and control varieties (durums Yallaroi and Dukem 5, and hexaploid wheat Buckley) were sown in single unreplicated rows. The seed in each row was derived from a single F_2 plant. The experiment was mechanically sown in the field at University of Adelaide, Roseworthy Campus, on 7th August 1995 and harvested by hand in January 1996. No artificial nitrogen fertiliser was applied.

Table 5.3. Genotype, AUS No.‡, origin and glutenin allele (with subunit composition in parenthesis) of controls.

Genotype	AUS No.	Origin	*Glu-A1* allele	*Glu-B1* allele	*Glu-D1* allele	*Glu-A3* allele	*Glu-B3* allele	*Glu-D3* allele
Durum								
Yallaroi		Aust	*c* (0)	*IX* (7+16)			2	
Wollaroi	25926	Aust	*c* (0)	*b* (7+8)			2	
Tamaroi	27663	Aust	*c* (0)	*d* (6+8)			2	
Dukem 5	25228	Mexico	*c* (0)	*d* (6+8)			2	
Duramba C		Aust	*c* (0)	*f* (13+16)			2	
Simeto		Italy	*c* (0)	*b* (7+8)			2	
Hexaploid wheat								
Buckley	27202	Aust	*a* (1)	*c* (7+9)	*a* (2+12)	*c*	*b*	*c*
Molineux	24457	Aust	*a* (1)	*b* (7+8)	*d* (5+10)	*c*	*b*	*c*
Warimex	13848	Mexico	*c* (0)	*b* (7+8)	*d* (5+10)	*b*	*b*	*a*

‡ Australian accession number, AWCC, Tamworth.

Aust = Australia.

Assessment of $F_{6:7}$ progeny lines

Homogeneous $F_{6:7}$ progeny lines (5 x Kharkof-5; 14 x Duramba A; 1 x Minieh 72; 1 x N_{4-1}) and controls were grown at Roseworthy Campus. No homozygous 1D(1A) substitution lines were available for inclusion. The experiment was designed as a randomized complete block with three replicates. ANOVA was performed using the GENSTAT for WINDOWS (Release 5.42) 5th Edition software package. Yallaroi was sown throughout the experiment on a grid pattern. Plots consisted of four rows, 4.2 m long, with 15 cm row spacings. Plots and bays were spaced 30 cm and 1.8 m apart, respectively. Seed density for durum wheat was 40 g/plot, equivalent to sowing at 80 kg/ha. Bread wheat varieties were sown at a density of 30 g/plot, equivalent to 60 kg/ha. Trial management and environmental description are presented in Chapter 3 (Section 3.2.2). The experiment was sown on 18th June 1998, and harvested December 1998. Pivot DAP (NPKS : 16-18-0-3 Zn2%) at a rate of 115 kg/ha was drilled with the seed at a depth of 5 cm during the sowing of the experiment

5.2.3 Analytical methods

Extraction and electrophoresis of seed endosperm storage proteins

Single seeds had one third of the endosperm cut off from the brush end. The embryo end was retained for growing the plants to multiply seed. The brush end of the endosperm was crushed with a hammer and glutenins were extracted as a group and separated by sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE).

The glutenins from the protein sample were extracted following the procedure of Lawrence and Shepherd (1980). The protein samples had 100 µl of sample extraction buffer containing 2% (v/v) mercaptoethanol (ME) added. The sample extraction buffer was prepared by mixing 0.125M tris(hydroxymethyl)aminomethane (Tris), 8% (w/v) sodium dodecyl sulphate (SDS), 30% (v/v) glycerol and 0.002% (w/v) bromophenol blue in water adjusted to pH 6.8 with

HCl. The samples were incubated at 60°C for one hour. The extracts were centrifuged in a Beckman Microfuge-11 for two minutes at speed ten, and 10 µl of supernatant loaded onto a slab gel of SDS-PAGE.

The following procedure is based on the discontinuous-buffer system of Laemmli (1970). Electrophoresis by uniform SDS-PAGE was performed using the method of Lawrence and Shepherd (1980). The separating gel was 180 x 160 x 1.0 mm, containing 10% (w/v) acrylamide, 0.1% (w/v) bisacrylamide (Bis), 0.1% SDS and 0.375M Tris adjusted to pH 8.8 with HCl. The stacking gel (20 mm) contained 3.5% (w/v) acrylamide, 0.08% Bis, 0.1% (w/v) SDS and 0.125M Tris adjusted to pH 6.8 with HCl. Both gels were polymerized with N, N, N', N'-tetramethyl-ethylenediamine (TEMED) and ammonium persulphate. The electrode buffer, for both upper (cathodal) and lower (anodal) tanks, contained 0.1% (w/v) SDS and 0.025M Tris adjusted to pH 8.3 with glycine. Ten microlitres of the protein samples were loaded into wells at the cathodal end and were electrophoresed at a constant current of 40 mA per gel.

Gels were stained for at least three hours in a solution consisting of one part 1% (w/v) Coomassie Brilliant Blue R mixed with 40 parts 6% (w/v) trichloroacetic acid in water : methanol : glacial acetic acid (80 : 20 : 7) as described by Lawrence and Shepherd (1980). The stained gel was placed in deionized water for one day with several changes of water for destaining.

Milling

Assessment of F_3 progeny lines

Wholemeal samples (25 g) were prepared by grinding the grain in a hammer mill (Falling Number 3100) equipped with a 0.8 mm screen. No tempering was involved.

Assessment of $F_{6:7}$ progeny lines

For mixograph tests and pasta-making, cleaned sub-samples of grain from each plot (200 g) were tempered to 16.5% moisture content before being milled on a Brabender Quadrumat Junior Mill equipped with 6XXX nylon mesh (by Drs M.J. Sissons and R.A. Hare, Cereal Chemistry Laboratory, NSW Agriculture, Tamworth).

Quality tests

Assessment of F_3 progeny lines

Total protein concentration (%) of the samples was determined by Near-Infrared Reflectance (NIR) Spectroscopy using a Technicon Infra-Alyzer 450 calibrated specifically for durum wheat in collaboration with the Grain Quality Laboratory, South Australian Research and Development Institute. The SDS-sedimentation test was performed to measure the gluten strength following the procedure of Axford *et al.* (1979), with a control sample in every batch of ten. Six grams of the wholemeal flour samples was mixed with 50 ml of water in a 100 ml measuring cylinder. The material was dispersed by rapid shaking for fifteen seconds and again two and four minutes later. Immediately after shaking, 50 ml of SDS-lactic acid reagent [30 g SDS/l and 20 ml/l lactic acid solution (88% AnalaR grade lactic acid : water = 1 : 8 (v/v))] were added and mixed by inverting the cylinder four times. The material was inverted four times again at two, four and six minutes. The contents of the cylinders were allowed to settle for 20 minutes before sedimentation volumes (ml) were read. Specific SDS-sedimentation was calculated by dividing the sedimentation volume by protein concentration to offset protein effect.

Assessment of $F_{6:7}$ progeny lines

Protein concentration was determined as described above for assessment of F_3 progeny lines.

The rheological properties were determined with a 10 g mixograph (by Cereal Chemistry Laboratory, NSW Agriculture, Tamworth) using the method number 54-40A of American Association of Cereal Chemists (1984). The amount of water added in the mixograph was 6.8 ml. The total dough volume therefore remained constant for the mixograph. Ideally a farinograph is necessary to determine water absorption, but since a limited quantity of sample was available, this was not possible. Samples were mixed in the mixograph for 6.00 - 6.25 minutes.

Parameters measured from the mixograph curve were:

i. time (seconds) to reach the maximum height (mix time - MT) - should be moderate to long,
ii. height at peak resistance (PR) in mixograph units - higher the better,
iii. differences of height at PR and 3 minutes later - resistance breakdown (RBD) - lower the better,
iv. differences of bandwidth at PR and 3 minutes later - bandwidth breakdown (BBD) - lower the better,
v. time (seconds) to reach peak bandwidth (TPBW) - related to MT, and
vi. peak bandwidth (PBW) - wider is better, in mixograph units.

Pasta preparation

Pasta was produced for each field plot replicate on a small-scale by Cereal Chemistry Laboratory, NSW Agriculture, Tamworth, according to the method of Sissons *et al.* (2002). Semolina was processed into spaghetti (diameter of the dried pasta 1.88 ± 0.04 mm) using a small-scale procedure. Semolina samples were hydrated to 30% absorption and mixed in a 50 g farinograph bowl heated to 40°C briefly, then for 2 min under a vacuum of 65 kPa. The resulting dry dough was transferred to a stainless steel rest chamber (2.5 cm internal diameter

by 13.5 cm length), threaded at the bottom to take a screw cap. Cylinder temperature was maintained at 50°C by a circulating water bath via a copper tubing wrapped outside the cylinder. A pressure of 7 000 kPa was applied to the dough for 9 min, then the screw cap replaced with a four-hole teflon coated spaghetti die with a threaded collar. The pasta was then extruded at a constant rate and cut to lengths of ca. 48 cm, looped over metal rods and hung in the drying cabinet maintained at 25°C and 85% relative humidity. After the last sample was processed, the drying cycle commenced using a temperature humidity chamber (50°C) for 13 h with a gradual decrease in the relative humidity. Spaghetti samples were stored at 22°C, 55% RH for at least 7 days before analysis.

Pasta quality evaluation

Cooking quality was evaluated with a suite of tests. The optimum cooking time (OCT: the time taken for the white core in the middle of the strand to disappear when squashed between two microscope slides) was determined. The pasta was cooked to OCT and the firmness, resilience and stickiness measured using the TA.XT2 texture analyser (Wood *et al.*, 2001). Firmness and resilience are defined from the texture analyzer force versus time plot. The peak height (maximum cutting force) of the first peak was defined as the firmness and the ratio of the peak height of the second (cutting force) to the first (cutting force) peak as the resilience.

5.3 Results

5.3.1 Assessment of F_3 progeny lines

No data for the Yallaroi control is available as seedlings did not emerge, suggesting there was a problem with seed viability.

Distribution of flour protein concentration, SDS-sedimentation (SDSS) volumes between durums and bread wheat, and correlation among these parameters

There was a wide range in grain protein concentration among the 52 progeny lines (7.2% - 12.8%, mean 10.5%). The durum control, Dukem 5, had a grain protein concentration of 8.3% and the hexaploid wheat, Buckley, 9.7%. The sedimentation values of the progeny lines ranged from 34.0 to 68.0 ml, with a mean of 42.4 ml. Dukem 5 had a sedimentation volume of 39.0 ml, and Buckley 50.0 ml. Grain protein concentration of the progeny lines was weakly correlated with the SDSS values ($r=0.274$, $P<0.05$) (Figure 5.3). However, when the two outliers, (4*Yallaroi/Kharkof-5)/2/2 and (3*Yallaroi//I_{8-2}/Yallaroi)/4/6, were excluded, there was no significant correlation.

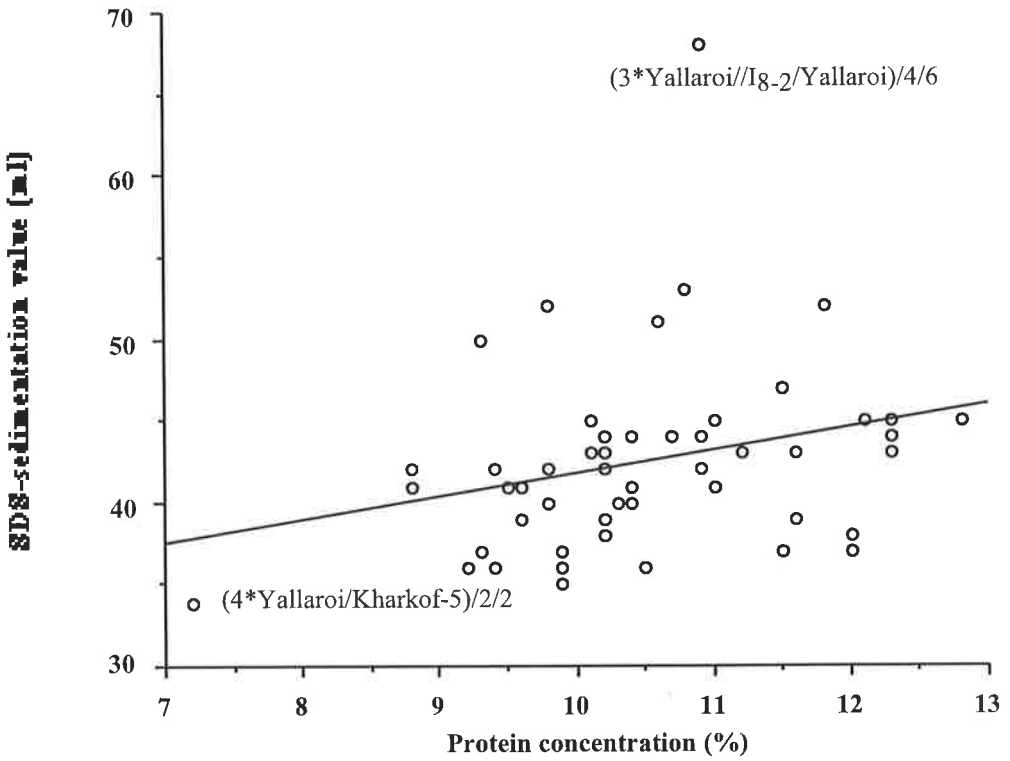


Figure 5.3. The relationship between SDSS values and grain protein concentration of durum progeny lines grown at Roseworthy in 1995.

The I_{8-2} progeny line Selection 4/6 which had the highest sedimentation volume, unfortunately after milling, had insufficient seed for sowing (as a row).

Associations between HMW glutenin alleles and quality properties among the durum progeny lines

The 52 durum progeny lines were analysed for their HMW glutenin composition. The banding patterns for the HMW glutenin subunits are demonstrated in Plate 5.1 and those present summarized in Tables 5.4 and 5.5. Electrophoresis showed that three *Glu-A1* alleles (*Glu-A1a, Glu-A1b* and *Glu-A1c*), two *Glu-B1* alleles (*Glu-B1IX* and *Glu-B1f*) and one *Glu-D1* allele (*Glu-D1d*) occured in the progeny lines.

SDS-sedimentation results for Glu-A1 and Glu-D1 loci

T-tests showed significant differences in SDSS values between HMW alleles. The homozygous Kharfok-5 derived progeny with the allele *Glu-A1a* had significantly higher SDSS values (42.6 ± 1.3 ml) than their null allelic variant (39.0 ± 3.7 ml) at the 0.1% level (Table 5.6). Similarly, homozygous Duramba A derived progeny with the allele *Glu-A1b* had significantly higher SDSS values (43.9 ± 11.3 ml) than the null sibs (38.5 ± 2.6 ml) ($P<0.01$). The SDSS values of the three sets of F_2-derived null progeny were similar to the durum control Dukem 5. Progeny heterozygous for alleles *Glu-A1a* and *Glu-A1c* or *Glu-A1b* and *Glu-A1c* had SDSS values intermediate to the homozygous progeny.

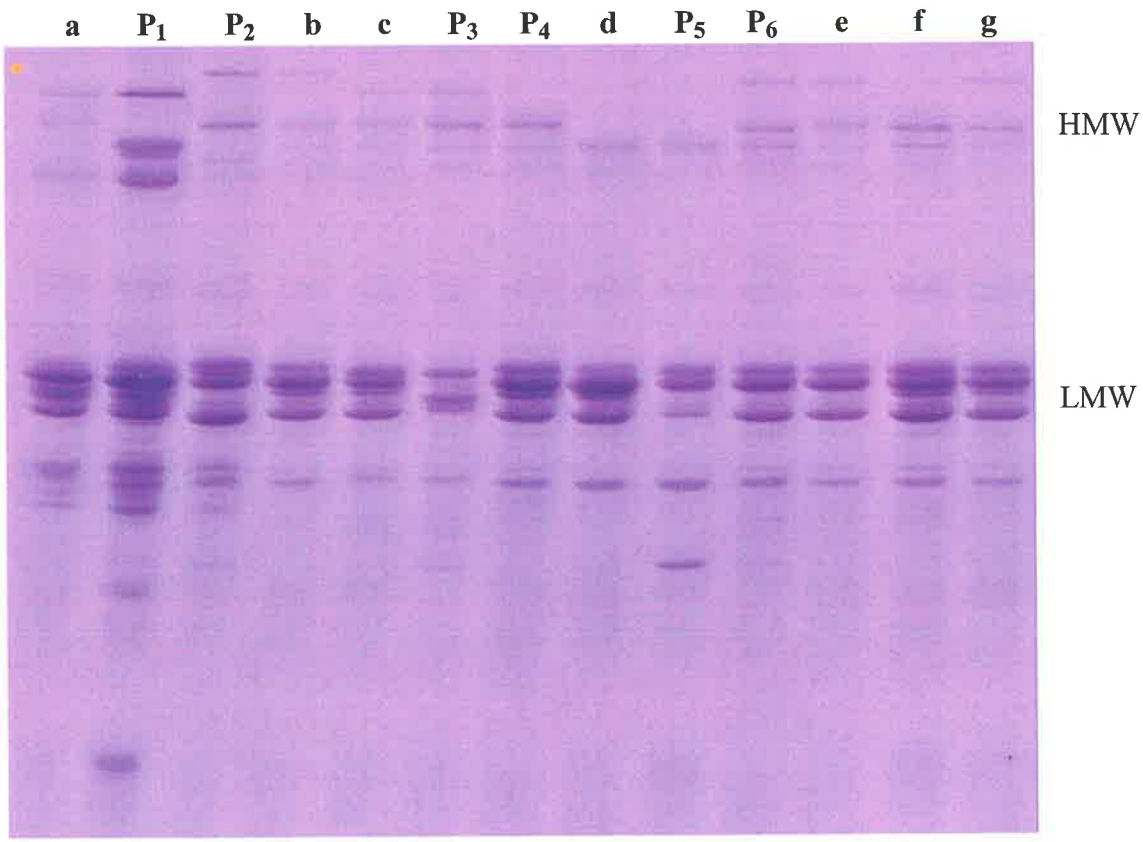


Plate 5.1. SDS-PAGE patterns of glutenin subunits of durum wheat backcross progeny lines with different phenotypes, and the parents.

P_1, I_{8-2}; P_2, Kharkof-5; P_3, Minieh 72; P_4, Yallaroi; P_5, N_{4-1}; P_6, Duramba A

(a) 3*Yallaroi//I_{8-2}/Yallaroi (5+10, 7+16)

(b) 4*Yallaroi/Kharkof-5 (1, 7+16)

(c) Yallaroi/Minieh 72 (*V*, 7+16)

(d) N_{4-1}/2*Yallaroi (0,17+18)

(e) 2*Yallaroi//Duramba A/Yallaroi (2*, 7+16)

(f) 2*Yallaroi//Duramba A/Yallaroi (0, 13+16)

(g) 2*Yallaroi//Duramba A/Yallaroi (2*, 13+16)

Table 5.4. HMW glutenin alleles in durum backcrossed progeny lines grown at Roseworthy in 1995.

Glu-A1		*Glu-B1*	
Alleles	Number	Alleles	Number
Kharkof-5 derived progeny			
a (1)	7	*IX*	24
a (1)/*c* (0) mixture	8		
c (0)	9		
Duramba A derived progeny			
b (2*)	9	*f* (13+16)	3
b (2*)/*c* (0) mixture	4	*f* (13+16)/*IX* mixture	4
c (0)	6	*IX*	12
Glu-D1		*Glu-B1*	
I_{8-2} derived progeny			
d (5+10)	4	*IX*	9
d (5+10)/*Glu-A1c*	1		
Glu-A1c	4		

Table 5.5. Number of combined *Glu-1* alleles in Duramba A derived progeny lines from Table 5.4.

Alleles (*Glu-A1* + *Glu-B1*)	Number
b + *f*	1
b + *f*/*IX*	2
b + *IX*	6
b/*c* + *f*	1
b/*c* + *IX*	3
c + *f*	1
c + *f*/*IX*	2
c + *IX*	3

The chromosome 1D(1A) substitution progeny lines had significantly (P<0.001) higher SDSS values (mean 54.8 ± 6.7 ml) than those with a null *Glu-A1* allele (41.0 ± 2.3 ml) (Table 5.6). The SDSS values of the progeny lines with the 1D(1A) substitution were also significantly higher than the progeny lines with *Glu-A1a* (P<0.001) or *Glu-A1b* (P<0.001). The progeny lines having subunits 5+10 had a mean SDSS value (54.8 ± 6.7 ml), which was similar to the hexaploid wheat control Buckley with subunits 2+12 (50 ml).

Since grain protein concentration of the progeny lines was significantly correlated, although not strongly, with the SDSS values, specific SDSS values were calculated to offset the effect of protein concentration on SDSS. As expected, progeny with 1D substitution (53.0 ± 5.0 ml) had significantly higher specific SDSS values (P<0.001) than sibs with null *Glu-A1* (39.0 ± 4.0 ml), and the other progeny lines with *Glu-A1a* (43 ± 3.0 ml) or *Glu-A1b* (39.0 ± 4.0 ml) (Table 5.6). The progeny group homozygous for the allele *Glu-A1a* had significantly (P<0.001) higher values than the *Glu-A1b* progeny group. However, the specific SDSS values between the null progeny in the Kharkof-5 and Duramba A derived groups also differed significantly at the 5% level. In the Duramba A derived group, no significant difference existed between progeny with *Glu-A1b* or *Glu*-A1c. Within the present population this suggests an overall ranking of subunits contributing to specific SDSS volumes as 5+10 > 1 > 2* ≥0.

SDS-sedimentation results for Glu-B1 locus

The progeny group homozygous for *Glu-B1f* (40.3 ± 3.3 ml) were not significantly different from heterogeneous *Glu-B1f/IX* (42.5 ± 6.6 ml) or homozygous *Glu-B1IX* progeny (42.5 ± 2.2 ml) (Table 5.7).

Table 5.6. Mean SDSS values and specific SDSS values for different *Glu-A1* and *Glu-D1* alleles from wheat grown at Roseworthy in 1995.

Alleles	Frequency	SDSS value (ml) (Mean ± s.d.)	Specific SDSS (Mean ± s.d.)
Kharkof-5 derived progeny			
Glu-A1a (1)	7	42.6 ± 1.3	43.0 ± 3.0
Glu-A1a (1)/*c* (0)	8	40.5 ± 3.0	39.0 ± 3.0
Glu-A1c (0)	9	39.0 ± 3.7	40.0 ± 4.0
Duramba A derived progeny			
Glu-A1b (2*)	9	43.9 ± 11.3	39.0 ± 4.0
Glu-A1b (2*)/*c* (0)	4	42.5 ± 1.7	40.0 ± 4.0
Glu-A1c (0)	6	38.5 ± 2.6	37.0 ± 3.0
I_{8-2} derived progeny			
Glu-D1d (5+10)	4	54.8 ± 6.7	53.0 ± 5.0
Glu-D1d (5+10)/*Glu-A1c*(0)	1	50.0	54.0
Glu-A1c (0)	4	41.0 ± 2.3	39.0 ± 4.0
Controls			
Dukem 5	1	39.0	47.0
Buckley	1	50.0	52.0

Table 5.7. Mean SDSS values and specific SDSS values for different *Glu-B1* alleles in durum wheat grown at Roseworthy in 1995.

Alleles	Frequency	SDSS value (ml) (Mean ± s.d.)	Specific SDSS (Mean ± s.d.)
Duramba A derived progeny			
Glu-B1f (13+16)	3	40.3 ± 3.3	40.0 ± 5.0
Glu-B1f (13+16)/*IX*	4	42.5 ± 6.6	37.0 ± 5.0
Glu-B1IX	12	42.5 ± 2.2	39.0 ± 2.0

5.3.2 Assessment of $F_{6:7}$ progeny lines

Distribution of grain yield and protein concentration between durums and hexaploid wheat

Grain yield varied significantly ($P<0.001$) between lines (Table 5.8). Yallaroi yielded significantly higher than the controls Duramba C, Simeto and Kharkof-5. Yallaroi also had yields significantly higher than several progeny lines with allelic HMW variants. No progeny lines were significantly higher yielding than Yallaroi. Yields were significantly different within the Duramba A introgression population with subunits 0, 7+16. There were no significant differences between the yields of Yallaroi and the two hexaploid wheats. There were no significant differences between lines for protein production (grain yield x protein concentration) (data not shown).

Grain protein concentration ranged from 10.4 % to 13.5 %, and varied significantly ($P<0.01$) between the lines tested. The Duramba biotypes had significantly higher grain protein concentrations than the controls, except Yallaroi and Kharkof-5 (Table 5.8). The protein concentrations also varied significantly within the Duramba A introgression populations with subunits 0, 13+16 and 2*, 7+16.

The variation in grain protein concentration had a significant effect on peak resistance, bandwidth breakdown, peak bandwidth, cooked pasta firmness and stickiness and was used as a co-variate in the analysis of variance for these variates.

Table 5.8. Mean values of major quality parameters[a] for durum progeny lines and other durum and hexaploid wheat controls grown in 1998.

Genotypes	No.	Yield	PC	MT	PR‡	RBD	BBD‡	TPBW	PBW‡
Kharkof-5 derived progeny									
Glu-A1c (0, 7+16)	1	1.28	12.6	245	400	11.7	22.5	145	357
Glu-A1a (1, 7+16)	4	0.87 - 1.09	11.6 - 12.1	196 - 223	356 - 422	7.3 - 13.0	19.9 - 29.9	100 - 125	353 - 426
Duramba A derived progeny									
Glu-A1c (0)/*Glu-B1IX* (7+16)	3	0.80 - 1.18	12.0 - 12.2	189 - 202	368 - 393	7.3 - 11.3	9.9 - 31.9	116 - 125	351 - 496
Glu-A1c (0)/*Glu-B1f* (13+16)	3	0.94 - 1.10	10.4 - 12.4	220 - 278	342 - 377	6.2 - 12.0	3.5 - 35.1	83 - 114	361 - 411
Glu-A1b (2*)/*Glu-B1IX* (7+16)	6	0.96 - 1.18	11.1 - 12.5	200 - 263	297 - 380	8.0 - 40.0	-0.7 - 32.3	58 - 257	313 - 371
Glu-A1b (2*)/*Glu-B1f* (13+16)	2	1.09 - 1.12	11.4 - 11.5	241 - 260	352 - 377	8.7 - 12.0	26.3 - 28.0	97 - 118	329 - 394
N_{4-1} derived progeny									
Glu-B1i (0,17+18)	1	0.91	11.0	230	355	10.3	5.3	89	275
Minieh 72 derived progeny			0.00						
Glu-A1V (V, 7+16)	1	0.80	12.1	225	417	12.7	15.2	151	364
Durum controls									
Yallaroi (0, 7+16)		1.12	12.3	190	306	39.0	34.6	120	231
Wollaroi (0, 7+8)		1.10	12.0	195	349	9.0	22.6	108	276
Duramba A (2*, 13+16)		0.95	13.5	206	457	12.0	13.8	108	440
Duramba C (0, 13+16)		0.81	13.4	134	346	19.8	27.7	75	240
Tamaroi (0, 6+8)		1.12	11.9	320	343	3.0	6.9	82	359
Simeto (0, 7+8)		0.89	11.6	232	411	8.3	-19.6	138	351
Kharkof-5 (1, 7+8)		0.73	12.3	683	253	-1.0	-4.0	364	251
Hexaploid wheat									
Molineux (1, 7+8, 5+10)		1.14	11.2	259	310	6.3	31.4	153	187
Warimex (0, 7+8, 5+10)		1.03	11.4	271	396	11.3	43.6	189	270
LSD (0.05)		0.23	1.3	99	61	17.9	n.s.	101	93

[a] Yield (t/ha); Protein concentration (PC) (%). Mix Time (MT) (in seconds); Peak Resistance (PR); Resistance Breakdown (RBD); Bandwidth Breakdown (BBD) (in mixograph units); Time to Peak Bandwidth (TPBW) (in seconds); Peak Bandwidth (PBW) (in mixograph units). ‡ Adjusted means for grain protein concentration co-variate.

Distribution of HMW glutenin alleles among the durum progeny lines

The HMW glutenin subunit composition of F_6 seed for the progeny lines was retested. The numbers of homozygous lines which were sown are summarized in Table 5.8. Four *Glu-A1* alleles (*Glu-A1a*, *Glu-A1b*, *Glu-A1c* and *Glu-A1V*), three *Glu-B1* alleles (*Glu-B1IX*, *Glu-B1f* and *Glu-B1i*) were present. No homozygous lines possessing chromosome 1D(1A) with *Glu-D1d* (5+10) were present, and therefore not sown.

Associations between HMW glutenin alleles and dough rheology and cooked pasta properties among the durum progeny lines, varieties and hexaploid wheats

Mix time

There were highly significant differences ($P<0.001$) in mixograph mix time between the lines. The donor parent, Kharkof-5, had a significantly longer mix time than the other lines assessed (Table 5.8). Tamaroi had a significantly longer mix time than the durum controls Yallaroi, Wollaroi, Duramba A and Duramba C. Duramba C had a significantly shorter mix time than the progeny with the Duramba A introgression of subunits 2*, 13+16, and five of the six progeny lines with 2*, 7+16.

There were no significant differences between the mix times of Yallaroi and the progeny populations (Table 5.8). However, if Kharkof-5 is dropped from the ANOVA, there are highly significant differences ($P<0.001$) between the lines, with LSD(5%) = 57. This results in three of the eleven progeny lines with either the introgression of 2* or 13+16 having significantly longer mix times than Yallaroi.

Peak resistance

To remove the effect of protein concentration ($P<0.001$), it was included as a co-variate in the ANOVA. There was highly significant variation ($P<0.001$) between the lines for peak resistance. Duramba A had a peak resistance significantly higher than the bread wheats Molineux and Warimex, and all the durum controls (Yallaroi, Kharkof-5, Wollaroi, Duramba C and Tamaroi), except Simeto (Table 5.8). Yallaroi and Kharkof-5 had low peak values.

The progeny line with the introgression of the allele *Glu-A1V* from Minieh 72 had a significantly higher peak resistance than Yallaroi and the progeny line with the allele *Glu-B1i* (subunits 17+18). Duramba A derived progeny lines, with allelic variants of Yallaroi (ie. 0, 7+16), had significantly higher peaks compared to Yallaroi. Half the progeny with the subunits 2*, 7+16, were significantly higher than Yallaroi, but lower than Duramba A. One line (out of two) with subunits 2*, 13+16, and two lines (out of three) with subunits 0, 13+16, were significantly higher than Yallaroi. The Kharkof-5 derived progeny line with subunits 0, 7+16, and three out of four progeny with subunits 1, 7+16, were significantly higher than Yallaroi.

Resistance Breakdown

There was significant variation ($P<0.05$) between the genotypes for resistance breakdown (Table 5.8). Yallaroi and one progeny line with subunits 2*, 7+16, had a significantly high breakdowns, but this is likely to be due these two lines recording high values in one replication. The negative value obtained for Kharkof-5 indicates it had not reached PR after 6 minutes.

Bandwidth Breakdown

Grain protein concentration had a significant ($P<0.05$) effect on bandwidth breakdown. Grain protein concentration was used as a co-variate, but there were no significant differences observed between the genotypes for bandwidth breakdown (Table 5.8). Kharkof-5 had a negative value as PR had not been reached. The result for Simeto indicates that the bandwidth continued to increase after PR.

Time to Peak Bandwidth

Time to peak bandwidth varied highly significantly ($P<0.001$) between the lines. Kharkof-5 had a significantly longer time to peak bandwidth than all the lines (Table 5.8). Duramba C and Tamaroi had significantly shorter times than the hexaploid wheat Warimex.

The progeny lines, except one with subunits 2*, 7+16, were not siginificantly different to Yallaroi. The exception with 2*, 7+16, recorded a high value in one replication, and one replication did not have sufficient grain for testing, suggesting this result should be viewed with caution.

Peak Bandwidth

Grain protein concentration contributed significantly ($P<0.05$) to peak bandwidth and was used as a co-variate. Peak bandwidth was highly significant ($P<0.001$) between genotypes. Duramba A, Tamaroi and Simeto had significantly wider peak bands than Yallaroi, Kharkof-5, Duramba C and the bread wheat Molineux (Table 5.8).

The progeny lines, except with the introgression of subunits 17+18 and two lines with 2*, 7+16, had significantly wider peak bands than Yallaroi. The range of peak bandwidths in the Duramba A derived population with subunits 0 or 2*, 7+16 were significantly different.

Cooked Pasta Firmness

Grain protein concentration had a highly significant ($P<0.001$) effect on cooked pasta firmness. When protein concentration was used a co-variate, there was no significant difference for firmness between the lines (Table 5.9).

Resilience of Cooked Pasta

Significant ($P<0.01$) genetic variation for resilience of cooked pasta occurred. The bread wheat Molineux produced the most resilient cooked pasta, significantly greater than Yallaroi and Tamaroi (Table 5.9).

Significant differences occurred within the Kharkof-5 introgression population with subunits 1, 7+16 and the Duramba A introgression populations with subunits 0, 13+16 and 2*, 7+16. No progeny lines were significantly different from Yallaroi.

Stickiness of Cooked Pasta

Grain protein concentration had a significant ($P<0.01$) effect on stickiness. Grain protein concentration was used as a co-variate. Highly significant differences ($P<0.001$) for stickiness of cooked pasta occurred between lines. Kharkof-5 produced the most sticky pasta (Table 5.9). The hexaploid wheats, Molineux and Warimex, also produced sticky pasta. Tamaroi and Duramba A produced the least sticky pastas, and were significantly less sticky than Yallaroi. Progeny with the introgression of subunit 2* from Duramba A with either subunits 7+16 or 13+16 had significantly different values for stickiness within the populations, but this did not occur for the 0, 13+16 or 0, 7+16 lines.

Table 5.9. Mean values of cooked pasta quality parameters[a] for durum progeny lines and other durum and hexaploid wheat controls grown in 1998.

Genotypes	No.	PC	Cooked pasta		
			Firmness‡	Resilience	Stickiness‡
Kharkof-5 derived progeny					
Glu-A1c (0, 7+16)	1	12.6	421	0.52	28.5
Glu-A1a (1, 7+16)	4	11.6 - 12.1	414 - 425	0.48 - 0.67	28.7 - 31.5
Duramba A derived progeny					
Glu-A1c (0)/*Glu-B1X* (7+16)	3	12.0 - 12.2	399 - 437	0.50 - 0.61	30.0 - 33.3
Glu-A1c (0)/*Glu-B1f* (13+16)	3	10.4 - 12.4	408 - 437	0.41 - 0.57	27.0 - 30.8
Glu-A1b (2*)/*Glu-B1X* (7+16)	6	11.1 - 12.5	381 - 425	0.44 - 0.65	25.2 - 33.7
Glu-A1b (2*)/*Glu-B1f* (13+16)	2	11.4 - 11.5	427 - 431	0.41 - 0.44	27.5 - 33.5
N_{4-1} derived progeny					
Glu-B1i (0,17+18)	1	11.0	400	0.55	30.3
Minieh 72 derived progeny					
Glu-A1V (V, 7+16)	1	12.1	408	0.51	32.3
Durum controls					
Yallaroi (0, 7+16)		12.3	405	0.53	31.5
Wollaroi (0, 7+8)		12.0	458	0.63	34.5
Duramba A (2*, 13+16)		13.5	423	0.59	26.4
Duramba C (0, 13+16)		13.4	437	0.60	29.8
Tamaroi (0, 6+8)		11.9	449	0.58	25.6
Simeto (0, 7+8)		11.6	413	0.62	32.3
Kharkof-5 (1, 7+8)		12.3	401	0.64	43.2
Hexaploid wheat					
Molineux (1, 7+8, 5+10)		11.2	358	0.73	34.8
Warimex (0, 7+8, 5+10)		11.4	393	0.69	36.2
LSD (0.05)		1.3		0.15	4.8

[a] Grain protein concnetration (PC) (%); Firmness (g); Stickiness (g/sec).
‡ Adjusted means for grain protein concentration co-variate.

Correlations between protein concentration, mixograph parameters and cooked pasta traits

Significant correlations existed between grain protein, mixograph parameters and cooking characteristics among the durum lines (Table 5.10). Grain protein concentration was significantly and positively correlated with peak resistance, bandwidth breakdown and cooked pasta firmness. Mix time was significantly and positively correlated with cooked pasta stickiness. Peak resistance was significantly and postively correlated with cooked pasta firmness, and negatively correlated with cooked pasta stickiness. There was a significant negative correlation between cooked pasta firmness and stickiness.

Table 5.10. Correlations between quality parameters[a] of the durum progeny lines and durum wheat controls in 1998 (n=31).

	PC	MT	PR	RBD	BBD	TPBW	PBW	FIRM	RESIL
MT	-.073								
PR	.530**	-.621***							
RBD	.314	-.375*	.101						
BBD	.365*	-.388*	.448**	.202					
TPBW	.174	.775***	-.455*	.045	-.241				
PBW	.183	-.406*	.675***	-.248	.117	-.487**			
FIRM	.663***	-.280	.569***	.256	.335	-.207	.306		
RESIL	.063	.142	-.095	-.222	-.040	.140	-.349	-.029	
STICK	-.322	.486**	-.574***	-.265	-.256	.469**	-.517**	-.454*	.326

*** P<0.001 ** P<0.01 * P<0.05

[a] Protein Concentration (PC) (in %); Mix Time (MT) (in seconds); Peak Resistance (PR); Resistance Breakdown (RBD); Bandwidth Breakdown (BBD) (in mixograph units); Time to Peak Bandwidth (TPBW) (in seconds); Peak Bandwidth (PBW) (in mixograph units); Firmness (FIRM) of cooked pasta; Resilience (RESIL) of cooked pasta; Stickiness (STICK) of cooked pasta.

5.4 Discussion

5.4.1 Yield and quality differences between durum and hexaploid wheats

The present study showed that although differences in grain protein concentrations and yields occurred, both durum and hexaploid wheats synthesized similar quantities of protein (yield x grain protein concentration). This demonstrates manipulation of the grain protein composition did not affect protein accumulation in the grain. A negative relationship is often associated with grain protein concentrations and yield (Cox *et al.*, 1985; Day *et al.*, 1985). Since protein content has a major influence on quality characteristics, on-farm N management practices need monitoring for grain to meet manufacturers' minimum specifications.

The SDSS test is commonly used as an indicator of gluten quality (strength) for durum and hexaploid wheat (Axford *et al.*, 1979; Dexter and Matsuo, 1980; Preston *et al.*, 1982). Good pasta and good bread-making quality are associated with a high sedimentation volume, although its use may be limited by a positive correlation with protein content (Kovacs *et al.*, 1995a). In this study, the durum lines (with the exception of 1D(1A) substitution) had lower mean SDSS values than a hexaploid control, which is consistent with previous reports (Dexter *et al.*, 1981; Liu, 1994).

The test using mixograph curve parameters is another indicator of dough strength (Kovacs *et al.*, 1997). The National Durum Wheat Improvement Program (NDWIP) have found a long mix time (at least 3.5 minutes), good peak height, and a wide width of the curve at peak mixing are all indicators of strong gluten (M. Sissons, pers. comm.). Yallaroi and Wollaroi are currently utilized in the NDWIP as relevant checks for greater strength. Significant differences occurred between the durum controls for the mixograph parameters MT, PR, TPBW and PBW. Although protein content was a confounding factor for PR and PBW, at similar high protein concentrations, Duramba A with subunit 2* was significantly stronger

than its null biotype, Duramba C, for these two parameters. The values for the hexaploid wheats were within the range found for the durum controls.

Pasta samples were made from each control genotype to evaluate cooking performance. The cooked pasta produced from hexaploid wheat had higher resilience than Yallaroi, which was the least resilient durum control. Pasta made from hexaploid wheat was more sticky when cooked than durum pasta.

5.4.2 Quality associations with particular glutenin alleles within durum progeny lines

It is important to note in this study, that since the progeny analysed were the result of backcrossing with the commercial durum variety Yallaroi as the recurrent parent, the genetic basis of the major effects of quality differences are likely to be ascribed to the segregation of genes on homoeologous group 1 chromosomes. This allowed the association of HMW glutenin subunit alleles with quality attributes to be more precisely measured.

Previous studies of relationships between *Glu-A1* alleles and quality of durum products have reported no association, or associations which were weak or not consistent. No relationship was found between *Glu-A1* subunits and SDSS volumes by Carrillo *et al.* (1990) and Liu and Rathjen (1994). While examining Duramba biotypes, du Cros *et al.* (1982) earlier found no relationship between the *Glu-A1* HMW subunits and mixograph dough strength. Later, du Cros (1987) found HMW glutenin subunit 2* had a positive effect on gluten strength as measured by mixograph height. Kaan *et al.* (1993) noted that the subunits 1, 2* and 2** were associated with higher SDSS values than the *Glu-A1* null phenotype. Similarly, Galterio *et al.* (1998) found subunit 1 exerted a minor but positive effect on SDSS volume. Conversely, Autran and Galterio (1989) showed subunit 2** was associated with poor condition of the spaghetti surface when overcooked. In the present study, incorporation of functional allelic variants (subunit 1 or 2*) at the *Glu-A1* locus or substitution 1D(1A) into durum variety Yallaroi significantly improved SDSS. Similar improvements were found in specific SDSS

for *Glu-D1d* and *Glu-A1a,* but not *Glu-A1b*. Payne *et al.* (1987) ranked the subunits in hexaploid as 5+10 > 1 = 2* > 0, but the present results for durum suggest 5+10 > 1 > 2* ≥ 0.

The substitution of chromosome 1D had a major effect on durum wheat quality. The F_3 1D(1A) population had higher SDSS values than Yallaroi, and similar to the hexaploid wheat Buckley (with subunits 2+12). This is consistent with previous findings that chromosome 1D in a durum background improves dough strength (Liu *et al.*, 1994a, 1994b, 1995). The 1D(1A) genotype which had the highest SDSS value yielded poorly, and sufficient seed was not available for resowing. This genotype is worthy of further evaluation in the future, particularly assessing pasta cooking quality, when sufficient seed is available for testing.

Genetic compositions in the future may be determined by whether there is yield loss associated with substitution of an entire D chromosome pair for the respective homoeologous counterpart (Joppa and Williams, 1988). Currently an effort is being made to multiply progeny lines of Yallaroi homozygous for the 1D chromosome pair for inclusion in field trials. The deleterious effect of complete chromosome substitution may be reduced by translocation with the appropriate segment of chromosome 1D into a durum wheat background (Kaltsikes *et al.*, 1968). Durum lines possessing translocations of subunits 5+10 have recently been reported having higher dough strength (Ammar *et al.*, 1997; Lafiandra *et al.*, 2000), however, yield data was not presented. Alternatively, transformation is another option, with the recent incorporation of subunit 5 from chromosome 1D into durum (He *et al.*, 1999).

Mixograph parameters are used as a measure of durum gluten strength (Kovacs *et al.*, 1997). Mix time increased with the incorporation of the subunits 2* or 13+16 in the Duramba A introgression population. The progeny line with the introgression of the allele *Glu-A1V* from Minieh 72 had a significantly higher peak resistance than Yallaroi and the progeny line with subunits 17+18. Progeny lines with subunits 1, 2* or 13+16 had PR significantly higher than Yallaroi. However, the Kharkof-5 and Duramba A introgression populations with the same

allelic composition as Yallaroi (0, 7+16) also had significantly higher PR than Yallaroi but not significantly different to the Minieh 72 introgression. Nearly all progeny lines, with the exception of the one possessing subunits 17+18, had significantly wider peak bandwidth than Yallaroi, indicating superior gluten strength.

The existence of the *Glu-B1* allele encoding 17+18 has not been reported in tetraploid wheat (Vallega and Waines, 1987; Vallega, 1988; Ciaffi *et al.*, 1993; Liu, 1994; Turchetta *et al.*, 1995). In hexaploid wheat, subunits 17+18 at the *Glu-B1* locus was found to have a major positive influence on rheological properties (Payne *et al.*, 1987; Lawrence *et al.*, 1988; Gupta *et al.*, 1994), however, Randall *et al.* (1993) found the allele undesirable. *Glu-B1i* was incorporated into a durum constitution by Liu (1995b), who found the influence of subunits 17+18 on SDSS was not superior to 7+8. In the present study the progeny line with 17+18 had SDSS values and rheological properties similar to Yallaroi. Recently, Eagles *et al.* (2002) examined a large number of samples from southern Australian wheat breeding programs using statistical techniques appropriate for unbalanced data. They reported epistatic interaction between particular loci for dough quality characters. The influence of the *Glu-B1i* allele was dependent on the frequency of alleles at the LMW glutenin loci. The contrast in results obtained for superiority of the *Glu-B1i* allele between bread wheat and durum could be due to variation of alleles at the LMW loci, and needs further investigation.

The progeny results for the remaining mixograph parameters, RBD, BBD and TPBW, were not significant or showed minor differences (which can be explained by one replication for the significantly different genotype recording a high value). These findings agree with the opinion of the NDWIP that MT, PR and PBW are the mixograph parameters which are useful indicators of gluten strength.

The texture of cooked pasta is an important cooking quality attribute which affects consumer acceptance. Previously the properties of texture have been based on 'sensory judgement' (Kovacs *et al.*, 1997) which are subject to personal preference (bias) in taste panels and

scored on a scale. High firmness is preferred from a sensory point of view. The test used in this study was instrument-based, and resembles the first bite in sensory analysis. Cooked pasta firmness was highly significant and positively correlated with grain protein concentration, which agrees with Sissons *et al.* (2000), and mixograph peak resistance. Kovacs *et al.* (1997) previously reported mixograph PR was one of the best predictors of firmness, which agrees with the present results, however, they found no correlation with protein content. When protein content was used as a co-variate in the ANOVA no significant difference for firmness existed between the genotypes. Similarly, Rao *et al.* (2001) found only the very weak gluten category, possessing LMW-1, could be differentiated according to pasta cooking quality (based on firmness). In view of this, compared to the effect of industrial process variables on pasta quality (Debbouz and Doetkott, 1996), breeding may only make a minor contribution to improving firmness.

Resilience can be related to the elasticity of pasta, which can be measured by tests like the alveograph or viscoelastograph. The requirements for fresh, sheeted, long, and short dried pasta goods in terms of elasticity are likely to differ. According to Quaglia (1988), the lack of dough extensibility of durum limits the loaf volume potential of cultivars with strong gluten. In the present study resilience was measured using the TA.XT2, which provided some measure of elasticity. How this relates to pasta end product requirements is uncertain and exacerbated by being independent of rheological parameters and, therefore, unrelated to gluten strength. Resilience did not vary between Yallaroi and its progeny, however, there was significant variation within the populations with the introgression of the HMW subunits 1, 2* or 13+16, but not the combination 2* and 13+16.

It is desirable for cooked pasta to be non-sticky. The majority of progeny lines were not significantly different from Yallaroi. However, there were significant differences for stickiness within the two populations with the subunit 2* introgression. Stickiness was significantly positively correlated with MT and TPBW, and negatively with PR, PBW and firmness. Conversely, previous studies have shown firmness and stickiness are independent

characters (Autran *et al.*, 1986; D'Egidio *et al.*, 1993). Sissons *et al.* (2000) found a weak negative correlation between stickiness and protein content. Although stickiness was not correlated with protein concentration in the present study, variation in protein content had a significant effect on stickiness in the ANOVA and was subsequently used as a co-variate.

The role of HMW glutenin subunits does not appear as important in the texture of cooked pasta as compared with their role in determining bread-making quality. In bread wheat a positive relationship exists between the number of expressed HMW glutenin subunits and the amount of large polymeric glutenin, which contributes to flour processing properties (Lawrence *et al.*, 1988). Similarly, Ciaffi *et al.* (1995) demonstrated that durum wheat lines possessing both x- and y-type subunits at the *Glu-A1* locus had higher dough strength and baking performance than those with only one or neither of the alleles, but they did not assess pasta quality. In this study, the findings showed that increasing the dosage of HMW glutenins by replacing the null *Glu-A1* allele did not improve pasta firmness. An increased proportion of unextractable, large-sized polymers has been reported in durum wheat possessing HMW subunits 5+10 (Ammar *et al.*, 1997; Lafiandra *et al.*, 2000), but further research is still necessary to determine its effect on pasta cooking quality.

Alternatively, LMW glutenin complexes between loci may need greater evaluation and the identification of novel genes may prove beneficial for improving pasta. The LMW glutenin subunits are further divided into B, C and D subunits. Extensive variation for B subunits has been found in a collection of durums from the Mediterranean region and North Africa, and wild and less cultivated tetraploid wheat lines (Liu and Shepherd, 1996). Porceddu *et al.* (1998) reported variation in the LMW glutenin B subunits accounted for 54% of total variation in SDSS values. Good quality durum wheats had a higher absolute amount of LMW glutenin, which was greater in LMW-2, and a relative predominance of LMW-s nucleotide sequence type and LMW-m type subunits which act as polymer chain extenders. The α-type and γ-type glutenin subunits have an odd number of cysteine residues and act as glutenin chain terminators, with negative effects in terms of pasta quality. The authors recommended

breeding for quality should consider selection for LMW glutenin subunits and against α- and γ-type glutenin subunits.

The genetic variation which existed between varieties in the present study was largely due to variation in grain protein concentration. This was demonstrated by correlations between protein concentration and SDSS, mixograph peak resistance, bandwidth breakdown and cooked pasta firmness. Differences in grain protein concentrations among the genotypes affected the ANOVA for peak resistance, bandwidth breakdown, peak bandwidth, cooked pasta firmness and stickiness, and was therefore used as a co-variate. Protein concentration is therefore a major confounding factor in these parameters. This confirms the previous findings of Sissons *et al.* (2000) that variation in protein content had a bigger effect on pasta firmness and stickiness than gluten strength. To remove the protein effect and evaluate the contribution of the HMW glutenins, it would advantageous to extract the gluten fraction and reconstitute semolina with a constant protein concentration (see Sissons *et al.*, 2002).

The factors associated with pasta quality appear to be independent of traits contributing to bread-making. Hence, more emphasis should be placed on establishing the criterion for evaluation of pasta quality as opposed to evaluating how characters which contribute to bread-making influence pasta. Little work has been conducted on the role of starch on pasta quality. Cunin *et al.* (1995) suggested that both the state of starch and the surface structure contribute to the development of the elastic texture and in particular to the stickiness of pasta. The resilience requirements for the different pasta end products also needs further evaluation. In the future it may be found the resilience of particular varieties is more suited to associated products/shapes, and they can be segregated accordingly. Resilience was independent of rheological characters, but appears to be associated with HMW composition. Due to the cost factor, and absence of a population possessing subunits 5+10, replicated evaluation by mixograph and cooked pasta was only conducted on samples from one site for one season. Further testing of the lines, including those with 1D(1A), at a number of sites will enable the investigation of genotype × environment interaction.

In summary, the present study for the first time investigated the effect of certain HMW glutenin subunits on aspects of dough strength and cooked pasta quality in a common commercial durum variety background. It could be concluded that grain protein concentration had a greater effect than HMW glutenin subunits in influencing dough strength parameters of durum wheat and cooked pasta quality. Variation for HMW subunits is most likely to play a beneficial role at lower protein concentrations. In evaluation of the F_3 progeny, where the mean protein concentration was 10.5%, significant increases in SDSS were associated with the incorporation of subunits 1, 2* or 5+10. Replicated evaluation of grain at a constant, low protein level is warranted to confirm this.

5.4.3 Future objectives

Results presented in this chapter demonstrated that variation in grain protein concentration had a greater influence than HMW composition on the quality parameters measured. Nevertheless, HMW allelic variants did contribute to genetic variation at similar protein levels.

Australian commercial and advanced breeder's durum lines have previously been shown to possess higher dough strength than lines from other countries, but lower than that of bread wheats (Liu, 1994). The present results do not suggest that the hexaploids contribute any favourable characteristics upon pasta quality. In fact, they can be considered detrimental as they produced cooked pasta more resilient and sticky than that produced from durum. Nevertheless, it is still necessary to examine the effect of incorporation of the *Glu-D1* subunits 5+10 in a durum background on mixograph parameters and pasta cooking quality. Reselections of Yallaroi progeny with 1D(1A) subsitution have been multiplied for field trials to obtain sufficient grain for replicated evaluation. Later the contribution of HMW alleles in durum for bread-baking may also be assessed to determine the feasibilty of utilizing durum flour for bread.

The variety Wollaroi has replaced Yallaroi as the quality benchmark in Australia (Hare, 1996). Wollaroi possesses the allele *Glu-B1b* (coding for bands 7+8), which has consistently been shown to associate with higher SDSS values than other alleles (Payne *et al.*, 1988; Pogna *et al.*, 1990; Liu, 1994; Peña *et al.*, 1994), and in this study produced firmer pasta (although not significant). Simeto (also possessing subunits 7+8), which is considered as having strong gluten (Marchylo and Dexter, 1996), had a significantly high PR. Therefore on these bases, the subunits 7+8 have been incorporated into Yallaroi, and the resulting progeny lines are entering field trials. Despite Wollaroi and Simeto having the same *Glu-B1* and *Glu-B3* composition, the present results indicate a marginally significant different mixograph PR between the two varieties. Other tests, such as the farinograph, extensograph or alveograph, may reveal further differences. The mixograph strength of Simeto is a partial response to a high water absorption requirement (Marchylo and Dexter, 1996). Also, the cooked pasta firmness and stickiness values for these two varieties did not follow the findings of a negative correlation for other genotypes. Evaluation of a (Wollaroi x Simeto) population, including high performance liquid chromatography (HPLC) to determine the distribution of glutenin polymers and glutenin fraction, is likely to elucidate factors contributing to pasta texture.

In addition, genes with both 1Ax + 1Ay subunits from *T. monococcum, T. urarta* or *T. dicoccoides* (Waines and Payne, 1987; Levy and Feldman, 1989) could be introduced. It has been prosposed that glutenin alleles coding for a higher number of bands (corresponding to synthesis of more aggregative proteins) enhance quality (Lawrence *et al.*, 1988; Singh *et al.*, 1990). Incorporation of both x- and y-type subunits at the *Glu-A1* locus in durum with LMW-2 resulted in dough rheological properties and baking performance as good as bread wheat controls (Ciaffi *et al.*, 1995), however, pasta texture was not evaluated and still requires investigation.

Chapter 6

General Discussion

6.1 Genotype × environment interaction

A collection of durum wheat genotypes, based on material from several breeding programs (mainly the NDWIP, Tamworth), was grown in South Australia to examine the nature of genotype × environment (G×E) interactions in the cereal belt of the State. Most of the observed variation in grain yield was accounted for by G×E interaction, and most of this was associated with the location × year (L×Y) component. Therefore, testing across both these dimensions is important. In the present study genotype × location (G×L) and genotype × year (G×Y) components were relatively small, which suggests some consistency in the performance of genotypes over locations and years. Lack of crossover (quantitative) interaction between genotypes may mean they are genetically homogeneous, which is probably due to the narrow range of genetic diversity amongst commercial durum wheat germplasm (Vallega and Zitelli, 1973; Bianchi and Boggini, 1996). Larger sources of variation are likely to occur if a collection containing landraces was grown. However, when such a collection was grown in South Australia earlier last decade the landraces were late maturing and tall (lacking semi-dwarfing genes), therefore susceptible to lodging, making them poorly adapted (Brooks, 1991). To increase genetic diversity, a crossing program is needed which transfers desirable traits from the landraces into cultivated varieties. A large and rich gene resource for broadening genetic variability of durum can be found in its wild relatives, *T. turgidum* var. *dicoccoides* and var. *dicoccum* (Feldman and Millet, 1993). The hybrids from these crosses will require the breeder to select against undesirable wild characters, such as a brittle rachis and adherent glumes, in addition to tall height and late maturity.

The type of adaptation (broad or specific) indicated by the components of G×E analysis is dependent on the range of genetic material that was examined in the chosen environments. In non-crossover interactions where genotypes are genetically homogeneous, which was likely for the germplasm in this study, the environments are usually heterogeneous. This finding should be expected as the sites in this study were specifically chosen to represent a wide range in growing conditions. The G×L component accounted for little of the observed variation, which indicates specific adaptation can not be exploited (ie. the target areas of production can not be sub-divided into homogeneous regions). In addition, non-repeatable G×E interactions are accomodated by selection for broad adaptation. Therefore, a breeding program for durum wheat in South Australia should aim at producing genotypes with broad adaptation. Similarly, breeding for wide adaptation has been the objective of the bread wheat programs in the state (Hollamby *et al.*, 1994). Wide adaptation is necessary to cover seasonal effects, as the breeding-selection cycle is lengthened by the requirement to test across years (Hollamby, 1999).

A major factor influencing grain yield of durum wheat in South Australia was seasonal rainfall. Durum wheat had a lower grain yield per unit of rainfall than the bread wheat variety Spear. This suggests durum has a lower water use efficiency compared with Spear, and more research is required to define the factors responsible for this yield deficiency compared with bread wheat. A primary factor is likely to be that durum lacks early vigour (B. Brooks, pers. obs.; Zubaidi, 1996), resulting in poor establishment and slower rates of vegetative development. Establishment can be affected by maternal effects, since variation in soil nutrient levels may alter seed quality and seedling survival without necessarily affecting seed mass (Aarssen and Burton, 1990). After emergence, vigorous seedlings which produce a larger leaf canopy can prevent the loss of moisture from the soil via evaporation. Greater early vigour can increase a crop's seasonal water use efficiency by as much as 25%, and thereby increase total crop biomass (Siddique *et al.*, 1990; Regan *et al.*, 1992; López-Castañeda and Richards, 1994). Greater ground cover early in the season should also improve the crop's competitiveness with weeds (Lemerle *et al.*, 1996). A higher rate of biomass

production and greater above ground biomass at maturity in recent durum genotypes from CIMMYT have contributed to the improvements in yield (Waddington *et al.*, 1987).

Edaphic and biotic constraints in the root zone are likely to contribute to the reduced yields of durum compared with tolerant, locally adapted bread wheats. These constraints impede root growth which results in a corresponding reduction in the uptake of nutrients and water (particularly with the onset of post-anthesis drought). B toxicity has been shown to reduce the yields of bread wheat (Paull *et al.*, 1988a), and results from Chapter 4 found durum seedlings with the longest roots in a high concentration B solution had a yield advantage at sites with toxic levels of B in the soil. Other factors that have been found to cause yield loss of bread wheats in the state include trace element deficiencies, cereal cyst nematode (*Heterodera avenae*) and root lesion nematode (*Pratylenchus neglectus*) (Graham, 1988; Rathjen *et al.*, 1998; Vanstone *et al.*, 1998). Genetic variation for each of these traits exists in durum (Saberi *et al.*, 1997; Rathjen *et al.*, 1998; Vanstone, unpublished data), and a breeding program which incorporates the appropriate resistance and/or tolerance for each of these constraints will improve adaptation of durum to South Australia.

The outcome of the yield evaluation trials conducted in this study was identifying a genotype, RH912025, with broad adaptation and increased yield. This line was subsequently released as the variety Tamaroi (Hare, 1998) to replace Yallaroi. RH912025 had a yield per unit rainfall not significantly lower than the bread wheat Spear. Since RH912025 is a progeny line of the CIMMYT release Altar 84, better early vigour (based on visual appraisal of field plots) probably contributed to improved yield. Further research is necessary to confirm this. Results also indicated that RH912025 was a high yielding genotype at sites with B toxic soil, although it performed poorly in the B filter paper test (unpublished data). B toxic and sodic subsoils generally occur together, therefore, the yield advantage of RH912025 may reflect a higher tolerance of sodic subsoils than Yallaroi (C.-Y. Liu, pers. comm.).

The evaluation of genotypes was best summarized by complementing the interpretations from the four statistical models used in this study. However, detailed statistical analyses will have limited application without appropriate attention to understanding the underlying biological basis of the differences highlighted by such analyses. Genotypic effects were a minor source of the observed variation in G×E, which indicates the rankings of genotypes were similar. The commercial durum variety Yallaroi had the lowest overall rank for yield performance, and was specifically adapted to favourable environments. This has contributed to the lack of expansion of durum production into more marginal areas of South Australia.

6.2 Improving B tolerance

To increase the level of tolerance of Australian durum varieties to high concentrations of B in the soil, moderately B sensitive Australian varieties and an advanced line were backcrossed to a moderately tolerant genotype, AUS 14010. Despite selecting B tolerant F_2 lines, in some families the seedling F_4 progeny had significant differences in sensitivity when grown in toxic levels of B in a filter paper assay. Correlations to response in the field were conducted to examine genetic variation. A contrast in response for two genotypes, WLYY9/3/2/4 and WLYY9/3/2/5, at Mallala (a low B site) compared to Two Wells and Roseworthy (sites with high B effects) suggested intolerance to B existed in the progeny population. Jamjod (1996) found segregation for a single gene, *Bo$_T$2*, in the cross (AUS 14010 x Yallaroi). This suggests in the present study there was some contribution from minor genes conferring B tolerance, and the closely related recurrent parents Wollaroi or RH880009 are probable sources.

The use of molecular markers has been identified as a useful selection tool in plant breeding to identify genotypes with specific genes. In bread wheat, a strong association was identified between B tolerance, as measured by the root length assay, and restriction fragment length ploymorphism (RFLP) marker loci *xKsuG10* on chromosome 4A (Paull *et al.*, 1993) and *Xpsr680* on chromosome 7B (Jefferies *et al.*, 2000). A similar response has been found on homeologous chromosome 4H of barley (Jefferies *et al.*, 1999a). Regions on chromosomes

7B and 7D in bread wheat were associated with leaf symptom expression (Jefferies *et al.*, 2000). In durum wheat, the probable location of the B tolerance gene (*Bo$_T$2*) is between markers *PSR121* and *CDO347* on chromosome 7B, but confirmation is necessary (Jamjod, 1996). Marker-assisted selection for B tolerance has been advocated for barley to identify individuals carrying four quantitative trait loci (QTLs) (Jefferies *et al.*, 1999a) and for bread wheat (Jefferies *et al.*, 2000). However, many bread wheat breeding lines carrying the marker alleles for *Bo1* and *Bo2* do not contain the B tolerance genes (F.C. Ogbonnaya and N.C. Subrahmanyan, pers. comm.), which is limiting the use of marker-assisted selection. For durum, until markers are identified and confirmed, the root length bioassay remains the most suitable selection technique as it is simple, reliable and more economic.

Although laboratory screening techniques are effective in the selection of B tolerant genotypes, they must still undergo evaluation in field trials to confirm there is a yield advantage and to identify superior yielding lines. The results for B were consistent with the expectation of families with long roots at high concentrations of B in the filter paper assay having a grain yield advantage of up to 19% when grown under high B conditions in the field. Similar yield advantages of B tolerance compared to B sensitivity have been reported for bread wheat in South Australia (Paull, 1990; Moody *et al.*, 1993; Campbell *et al.*, 1994). There was sufficient genetic variation for tolerance to B within the current backcross population for more tolerant lines to be selected, and this should result in the release of varieties with higher yields when grown at locations with high B. The presence of this material in the evaluation of G×E trials was beneficial, particularly in spatial analysis that classified a group of sites which could be attributed to B toxic locations. This demonstrates the key role tolerance to B toxicity plays in the adaptation of germplasm to the cereal belt of South Australia.

The concentrations of most elements in the grain differed significantly among families, and accumulation of B in the grain was genetically independent of the other elements. The selection of durum genotypes with low B accumulation should therefore have no effect upon

the efficiency of uptake of other elements. It is fortunate that significant differences for other elements exist, since Australian durum germplasm is extremely trace element inefficient (Graham, 1988; Saberi *et al.*, 1996; Cakmak *et al.*, 1998). Therefore, in a breeding program to increase adaptation to South Australia, lines could simultaneously be selected for B tolerance and trace element efficiency.

Concentrations of other elements in grain revealed that levels of Na were generally higher than those reported for bread wheat (Paull, 1990). Durum wheat is known to accumulate high concentrations of Na in tissues compared to bread wheat (Joshi *et al.*, 1982; Wyn Jones *et al.*, 1984). This may lead to increased susceptibility of durum to Na toxicity in the sodic soils of South Australia, which are estimated to cover 63% of the arable land (Northcote and Skene, 1972). Although the current study found no relationship between grain yield and grain Na concentration, relatively higher yields have been associated with low Na accumulation in shoots of bread wheat (Paull *et al.*, 1992c). Since high concentrations of B are generally associated with sodic subsoils, for B tolerant varieties to be well adapted to the soil environment they would also require tolerance to sodicity. It has been suggested by Zubaidi (1996) and R. Munns (pers. comm.) that durum has a greater physiological tolerance to elevated Na concentrations rather than a mechanism reducing uptake. This suggestion is likely to be a reflection of the lack of genetic variation within durum for uptake. Identifying tetrapolid sources of low Na accumulation should be of benefit. When the gene responsible for K/Na discrimination in bread wheat was incorporated into durum, Na accumulation did not increase with sodicity, and relative grain yield was greater (Gorham *et al.*, 1997). Research is required to assess genetic variation for tolerance to sodicity in durum, particularly if sources are identified which accumulate levels of Na as low as bread wheat. If the mechanism of tolerance to sodicity in durum is determined to be reduced accumulation, it can be selected for in the field in tandem with tolerance to B, by selecting for grain yield at a site with a high concentration of B and a sodic subsoil.

6.3 Improving dough strength

The SDSS test has been widely used to evaluate the gluten strength in durum wheat (Dick and Quick, 1983). The sedimentation volume is well correlated with bread-making quality (Axford *et al.*, 1979) as well as with pasta cooking quality (Dexter and Matsuo, 1980). Varieties with strong gluten produce pasta with greater firmness after cooking and increased tolerance to overcooking (Matsuo and Irvine, 1970; Grzybowski and Donnelly, 1979). In this study, the durum controls had lower mean SDSS values than the hexaploid controls, which is consistent with previous findings of Dexter *et al.* (1981) and Liu (1994).

The mixograph test with curve parameters long mix time (MT) (at least 3.5 minutes), good peak height (PR), and a wide width of the curve at peak mixing (PBW) are also used as indicators of strong gluten (M. Sissons, pers. comm.). Significant differences occurred between the durum controls for the mixograph parameters MT, PR, TPBW and PBW. Although protein content was a confounding factor for PR and PBW, at similar high protein concentrations, Duramba A with subunit 2* was significantly stronger than its null biotype, Duramba C, for these two parameters. The values for the hexaploid wheats were within the range found for the durum controls.

Cooking performance is the ultimate quality test of the end product. It is therefore surprising that no standard method of evaluation has been adopted internationally by the pasta industry. In the past, the properties of cooked pasta texture have been based on 'sensory judgement' (Kovacs *et al.*, 1997). In this study, an instrument-based test was used which resembles the first bite in sensory analysis. The cooked pasta produced from hexaploid wheat had higher resilience than Yallaroi, which was the least resilient durum control. Pasta made from hexaploid wheat was more sticky when cooked than durum pasta.

Previous studies examining the effect of allelic variation of grain storage proteins for gluten strength have been confounded by genotypic and phenotypic variation encountered in the

same growing environment (eg. Liu, 1994). In the present study, tetraploid donor lines were selected on the basis of HMW glutenin subunits that are associated with dough strength in hexaploid wheat. The progeny analysed were the result of backcrossing donor lines with the commercial durum variety Yallaroi as the recurrent parent, so that the association of HMW glutenin subunit alleles with quality attributes could be more precisely measured.

In the present study, the incorporation of functional allelic variants at the *Glu-A1* locus into backcross lines of Yallaroi improved SDSS compared with the null. When chromosome 1D (with subunits 5+10) was substituted for chromosome 1A, the progeny lines were associated with higher sedimentation volumes compared with the durums, but similar to the hexaploid Buckley (subunits 2+12), indicating the superiority of chromosome 1D in contributing to gluten strength. The results tended to agree with the ranking of subunits associated with dough strength in hexaploid wheat (Payne *et al.*, 1987), but in durum the subunit 1 was superior to 2* rather than equivalent. Screening F_6 seed revealed that generation of 1D(1A) progeny were heterogeneous, and therefore unable to be assessed by mixograph. Nevertheless, the results for the F_4 seed were consistent with previous findings: chromosome 1D in a durum background improves dough quality (Liu *et al.*, 1994a, 1994b, 1995). In the future, mixograph evaluation and cooking performance of a homozygous 1D(1A) genotype is warranted.

The relationships between the HMW glutenins and mixograph parameters were examined. An increase in mix time was associated with incorporating subunits 2* or 13+16, and a higher peak resistance resulted with the allele *Glu-A1V*, subunits 1, 2* or 13+16, but unexpectedly, no improvements were observed with the subunits 17+18. The subunits 17+18 have a major positive influence on rheological properties in hexaploid wheat (Payne *et al.*, 1987; Lawrence *et al.*, 1988; Gupta *et al.*, 1994). Previously, Liu (1995b) reported that when subunits 17+18 were incorporated into durum, their influence on SDSS was not superior to 7+8. The contrasting findings for the *Glu-B1i* allele probably result from an interaction between the *Glu-B1* locus and the *Glu-A3* and *Glu-B3* loci (Eagles *et al.*, 2002). The effect of HMW

allelic variants on the remaining mixograph parameters, RBD, BBD and TPBW were not significant.

The cooking quality of pasta samples produced from the different genotypes was evaluated. The present study agreed with the previous findings that cooked pasta firmness was highly significant and positively correlated with grain protein concentration (Sissons *et al.*, 2000), and mixograph peak resistance (Kovacs *et al.*, 1997). When the effect of protein content was removed, no differences for firmness occurred between genotypes, indicating no influence of allelic HMW glutenins. The resilience of cooked pasta was independent of rheological parameters, however, there was significant variation within the progeny populations with the introgression of subunits 1, 2* or 13+16. This variation of elasticity may used to evaluate the suitability of specific alleles for producing the various conformations of pasta. Cooked pasta stickiness was significantly negatively correlated with firmness. Previous studies have shown firmness and stickiness are independent characters (Autran *et al.*, 1986; D'Egidio *et al.*, 1993). Sissons *et al.* (2000) found a weak negative correlation between stickiness and protein content. Although stickiness was not correlated with protein concentration in this study, variation in protein content had a significant effect on stickiness in the ANOVA. The role of HMW glutenin subunits do not therefore appear as important in the texture of cooked pasta as compared with their role in determining bread-making quality.

6.4 Future breeding objectives

At the commencement of this study, very little was known on the level of adaptation of durum wheat in South Australia. Therefore, the breeding objectives for increasing adaptation were based on the knowledge of constraints to growing other crops in the region, with specific reference to bread wheat. Due to the closely related genomes of durum and bread wheat, the hexaploid gave a valuable insight into breeding methodologies. For example, adaptative traits under control of major genes can be quickly and readily incorporated through backcrossing. During the period of study, the relative importance of traits known to effect bread wheat to a

minor degree have been identified to have a larger impact on durum production (eg. Mn and Zn deficiency, and susceptibility to *Fusarium* crown rot). Other projects have identified genetic variation for some of these traits, eg. Mn (Saberi *et al.*, 1997). Therefore, incorporating Mn efficiency into the more adapted B tolerant lines, described in Chapter 4, has become an additional breeding objective.

The present study agrees with the earlier findings that a narrow range of genetic diversity exists among commercial durum wheat germplasm (Vallega and Zitelli, 1973; Bianchi and Boggini, 1996). Therefore the probability of identifying adaptive traits among this germplasm to an environment where these lines have not been historically selected and grown will be low. Thus, there is awareness of the need to screen a wide range of landraces and related tetraploids when searching for desirable traits. The success of locating suitable germplasm is, however, dependent on having identified the adaptive trait, and determined a suitable screening technique. Screening a diverse range of tetraploids has led to the identification of genotypes which have improved tolerance to salinity through Na exclusion (Richards *et al.*, 1999). These lines are likely to provide a valuable source of tolerance to the widespread sodic soils of South Australia.

An important process leading to the release of adapted varieties is the transfer of desirable genes into local breeding material. As donor genes are incorporated into recurrent parents by backcrossing, when progeny with the desired gene have been selected they can replace the older recurrent parent in the crossing program. This is considered as parent building (or gene pyramiding) and enables desirable traits to be transferred to a higher frequency of progeny relatively early in the breeding program. This study showed Tamaroi (syn. RH912025) was widely adapted and high yielding, and its improved agronomic performance makes it a more suitable recurrent parent in the breeding program than Yallaroi. For parent building, Tamaroi has been backcrossed to a B tolerant line (Chapter 4) and a line with the HMW glutenin allele *Glu-A1b* (Chapter 5). The two backcross derived lines with B tolerance and *Glu-A1b*,

respectively, are then crossed together. With both genes present in a single Tamaroi background, a new recurrent parent is developed.

When endeavouring to increase the level of tolerance to B in Australian durum varieties, for their successful adoption, a level of tolerance at least equivalent to local bread wheats is necessary. In the present study, a moderately B tolerant line was used as the donor parent, and therefore it would be expected that the moderately B tolerant backcross progeny would have a level of tolerance similar to the bread wheat variety Halberd. To further increase the level of B tolerance in Australian durum varieties the genotype AUS 10105, identified by Jamjod (1996) as B tolerant, has been used as a donor parent. This should raise the level of B tolerance equivalent to that of the exotic bread wheat G61450. However, as there is a narrow range between B deficiency and B toxicity (Eaton, 1944; Reisenauer *et al.*, 1973), an improvement in stress tolerance without increased flexibility of adaptation may lead to an increase in susceptibility to deficiency (Rathjen *et al.*, 1987). It may therefore be necessary to breed a range of varieties with a level of tolerance appropriate to the B status of the soils in the region where they are intended to be cultivated.

Backcrossing is a suitable method of transferring the major genes conferring tolerance to B into local varieties. As the sources of B tolerance in bread wheat are well adapted to the South Australian environment, there has been only limited backcrossing in the transfer of B tolerance to other local varieties. In contrast, the B tolerant durum genotypes are exotic landraces, and are unadapted and genetically remote from the local varieties. Therefore, more backcrosses are required to fully recover a larger portion of the recurrent parent's genotype. After three backcrosses the derivatives still contain a substantial contribution from the donor parent. For bread wheat, when Halberd was used as the donor parent, it was after six backcrosses that superior lines to both the recurrent parent and BC_3-derivative were produced (Rathjen *et al.*, 1993). Therefore, when transferring a single gene from an exotic source, more complete backcrossing or intensive selection will be required than is undertaken conventionally.

The effect of subunits 5+10 from chromosome 1D on durum rheological and pasta parameters still requires evaluation. The finding in this study that chromosome 1D in a durum background increases SDSS volume is consistent with previous findings (Liu *et al.*, 1994a, 1994b, 1995), however, more extensive testing is necessary. If the 1D(1A) substitution is demonstrated to be beneficial, the commercial viability of such lines will be determined by whether there is a yield loss associated with substitution of an entire D chromosome pair for the 1A pair. The breeding program is currently establishing a homozygous population of 1D(1A) families derived from an F_6 $I_{8\text{-}2}$ progeny line to conduct further investigations on yield response and the effects on dough properties and pasta-making. In the long term, the recent successful translocation of the appropriate segment of the 1D chromosome with subunits 5+10 into durum (Ammar *et al.*, 1997; Lafiandra *et al.*, 2000) is likely to overcome the potential detrimental effect of substitution on yield.

Grain with higher protein concentration has been found to improve mixogram scores and cooking quality (Bhatt and Derera, 1975; Joppa *et al.*, 1991). Genetic improvement in protein concentration has been restricted by the negative correlation between productivity and seed protein concentration found in segregating populations in all cereals (Cox *et al.*, 1985; Day *et al.*, 1985). Therefore, in addition to incorporating desirable HMW glutenins to improve dough strength, the simultaneous objective to increase protein concentrations may be achieved by incorporating the high protein gene located on chromosome 6B of a *T. dicoccoides* accession (Joppa *et al.*, 1991) to offset the dilution effect associated with increased yields.

Finally, durum wheat is highly susceptible to the disease crown rot (Wildermuth *et al.*, 1999), caused by the fungus *Fusarium* spp. No sources of resistance have been identified in durum wheat. Screening for resistance within tetraploid species identified *T. turgidum* ssp. *carthlicum* accessions with partial resistance to crown rot (Wildermuth *et al.*, 1999), but these lines have subsequently been discovered to have the D genome (G.B. Wildermuth, pers.

comm.). Crown rot has been linked to Zn deficiency (Sparrow and Graham, 1988), whereby application of Zn can decrease infection by *F. graminearum* Group 1 at the basal part of the wheat culm (Grewal *et al.*, 1996). Zn inefficient durum varieties (intolerant to Zn deficiency) require higher rates of Zn fertilization to reduce disease levels compared with the more efficient bread wheat varieties such as Excalibur. Similarly, an inverse correlation between plant Zn concentration and the severity of Rhizoctonia root rot of wheat has been reported by Thongbai *et al.* (1993). Therefore, durum varieties with improved Zn efficiency should be selected to reduce susceptibility to diseases, and raise productivity in areas with Zn deficiency.

6.5 Summary

The Green Revolution saw the development of semi-dwarf durum wheat varieties which were able to produce yields higher than bread wheat under high input conditions (irrigation and fertilizer) (Breth, 1975; Brajcich *et al.*, 1983; Pfeiffer, 1996). However, in regions such as South Australia (with semi-arid and low input growing conditions) productivity is low. Many constraints limit production in marginal areas (eg. disease, insects, drought, salinity, soil infertility), such that genetic progress in highly variable stress environments is less efficient compared to that in high potential production conditions. Nevertheless, genetic improvements can be made if these constraints can be overcome. This necessitates identifying the constraint, establishing whether genetic variation exists for the trait, and incorporating resistance and/or tolerance to the trait into otherwise adapted material.

Currently, most of the durum in South Australia is produced on the red-brown earths and related soils in high rainfall areas. The areas which have the greatest potential to grow durum wheat with high grain protein concentrations are the more marginal regions of the state, with mostly duplex and sodic soils (Rathjen *et al.*, 1996). However, durum lines have been found to produce only half the grain yield of bread wheat in some field trials in these locations. Limitations to production are predominantly edaphic, and include high concentrations of B,

Na, and deficiencies of Mn, Zn and Fe in the soil. In these soils, Yallaroi has shown poor adaptation, in particular to B toxicity. When tolerance to B was incorporated into Australian germplasm, a yield advantage of up to 19% was obtained under B toxic conditions. The development of these B tolerant lines is likely to expand durum production into areas where B toxicity is a yield limiting factor.

Discrepancies in the quality of pasta manufactured from the durum variety Yallaroi produced from the different regions in Australia has been observed in the local pasta industry. The cooler grain ripening conditions in southern Australia are associated with lower gluten and lower dough strength. Gluten properties are influenced by the environment and by genetic composition of the kernel. In the absence of genetic variation, the environment is the major factor contributing to dough strength. Manipulation of grain protein composition offers the best approach to reducing the effect of the environment on gluten strength and, hence, on the quality of the end product. Incorporating genetic variation for the HMW glutenin subunits (including the subunits 5+10 by substitution of chromosome 1D for 1A) into Yallaroi increased and improved the range of values obtained in the tests for assessing gluten strength. The consistent production of strong gluten varieties (with high mixograph peak resistance) will give growers an advantage in the world market due to the positive correlation with cooked pasta firmness.

Appendix A

Table A1. Monthly rainfall (mm) at experimental sites used in 1994.

Month	Lowbanks 1994	Palmer 1994	Kapunda 1994	Mallala 1994	Roseworthy 1994	Winulta 1994
Jan	0	0	17.8	16	28.4	12.7
Feb	15.2	12.4	13.2	5	12.4	7.1
Mar	1.5	12.7	0	0	0.0	0.0
Apr	0	0	0	0	10.8	0.0
May	6.1	11.4	22.9	21	17.2	13.7
June	59.7	102.1	89.4	95	75.1	115.3
July	14.7	15.0	41.7	32	31.8	40.4
Aug	1.0	7.1	15.7	18	11.1	20.1
Sep	15.2	20.3	29.2	9	8.8	22.4
Oct	7.1	18.0	29.0	28	24.3	21.8
Nov	26.2	18.8	58.4	27	33.5	20.8
Dec	6.9	6.4	0	7	6.6	8.1
Apr-Oct total	103.9	174.0	227.8	201	179.6	233.7
Annual total	153.7	224.5	317	256	260.5	282.4
Average	250.7	418.9	494.8	399.1	443.1	398.5

Table A2. Monthly rainfall (mm) at experimental sites used in 1995.

Month	Rudall 1995	Palmer 1995	Walker Flat 1995	Two Wells 1995
Jan	37.0	33.9	32.8	2.75
Feb	2.5	27	20.6	21.64
Mar	7.4	6	5.6	17.94
Apr	9.5	29	35.0	38.79
May	38.7	17.5	23.2	28.64
June	55.4	36	59.4	46.03
July	54.6	62.3	40.6	98.61
Aug	14.3	4	9.0	15.13
Sep	29.5	18	9.6	31.74
Oct	25.9	55.2	69.0	37.68
Nov	23.5	4.5	3.2	13.57
Dec	4.7	15.5	7.0	77.51
Apr-Oct total	227.9	222	245.8	296.62
Annual total	303.0	308.9	315	430.03
Average	343.3	418.9	306.6	399.20

Month	Kapunda 1995	Mallala 1995	Roseworthy 1995	Winulta 1995
Jan	30.2	24	18.2	14.2
Feb	22.4	26	29.0	15.2
Mar	8.1	11	14.1	16.5
Apr	57.9	35	27.1	29.7
May	37.1	49	44.9	41.1
June	44.2	66	55.1	83.1
July	69.6	76	87.5	88.4
Aug	41.7	14	16.7	17.3
Sep	42.2	37	24.5	40.6
Oct	68.1	61	49.2	31.5
Nov	24.4	13	7.6	1.5
Dec	1.0	4	8.1	15.7
Apr-Oct total	421.6	341	306	331.7
Annual total	446.8	419	382	395.0
Average	494.8	399.1	443.1	398.5

Table A3. Monthly rainfall (mm) at experimental sites used in 1996.

Month	Minnipa 1996	Jamestown 1996	Walker Flat 1996	Two Wells 1996
Jan	1.2	0.0	11.2	2.36
Feb	6.0	0.0	11.2	12.19
Mar	4.8	20.3	46.0	6.68
Apr	9.9	2.3	10.4	30.48
May	5.4	6.4	6.6	8.06
June	47.3	40.9	53.4	8.16
July	63.5	80.3	61.2	75.62
Aug	62.4	52.8	48.4	73.91
Sep	55.8	67.8	53.6	78.46
Oct	17.0	11.4	19.6	5.90
Nov	16.8	7.9	3.6	4.57
Dec	10.4	0.0	7.0	15.54
Apr-Oct total	261.3	261.9	253.2	360.59
Annual total	300.5	290.1	332.2	401.93
Average	330	457.2	306.6	399.20

Month	Kapunda 1996	Mallala 1996	Roseworthy 1996	Winulta 1996
Jan	20.3	44.5	65.7	3.6
Feb	21.1	16.0	28.4	13.2
Mar	33.8	15.0	18.3	8.6
Apr	6.9	18.5	20.1	20.1
May	3.6	2.0	5.0	6.1
June	79.2	53.5	72.2	88.9
July	68.3	85.0	85.9	90.2
Aug	56.4	82.5	81.0	89.9
Sep	71.6	82.5	72.2	72.1
Oct	7.1	23.0	26.4	25.9
Nov	10.2	0.0	12.2	0.0
Dec	8.1	15.0	15.8	12.2
Apr-Oct total	293.1	347	362.8	393.2
Annual total	386.6	437.5	503.2	430.8
Average	494.8	399.1	443.1	398.5

Appendix B

Table B1. Cropping rotations for three years prior to trials conducted at site, and soil pH at trial sites in environment Sets 4 and 5.

Locality	Year	pH	Three year cropping history
Kapunda	1994	8.2	Faba beans (1991) - wheat (1992) - peas (1993)
"	1995	8.3	Peas (1992) - wheat (1993) - faba beans (1994)
"	1996	6.8	Canola (1993) - lupins (1994) - wheat (1995)
Mallala	1994	6.5	Peas (1991) - wheat (1992) - failed peas (1993)
"	1995	6.5	Wheat (1992) - failed peas (1993) - durum (1994)
"	1996	7.3	Wheat (1993) - medic (1994) - barley (1995)
Roseworthy	1994	7.7	Pasture (oats and medic) (1991) - hay (vetch and medic) (1992) - beans (1993)
"	1995	7.2	Peas (1992) - wheat (1993) - pasture (regenerated) (1994)
"	1996	7.7	Wheat (1993) - barley (1994) - peas (1995)
Winulta	1994	6.5-7	Peas (1991) - barley (1992) - 6 row barley/medic (1993)
"	1995	6.5-7	Medic (1992) - barley (1993) - medic (1994)
"	1996	8	Medic (1993) - barley (1994) - lathyrus/medic (1995)

Appendix C

Table C1. Locations of experimental trials in the years 1994 - 1996.

Trial No.	Code	Year	Locality
1	L94	1994	Lowbanks
2	PE94	1994	Palmer - Eichler
3	TW94	1994	Two Wells
4	RD94	1994	Rudall
5	K94	1994	Kapunda
6	M94	1994	Mallala
7	R94	1994	Roseworthy
8	W94	1994	Winulta
9	RD95	1995	Rudall
10	PK95	1995	Palmer - Krause
11	WF95	1995	Walker Flat
12	TW95	1995	Two Wells
13	K95	1995	Kapunda
14	M95	1995	Mallala
15	R95	1995	Roseworthy
16	W95	1995	Winulta
17	MR96	1996	Minnipa
18	J96	1996	Jamestown
19	WF96	1996	Walker Flat
20	TW96	1996	Two Wells
21	K96	1996	Kapunda
22	M96	1996	Mallala
23	R96	1996	Roseworthy
24	W96	1996	Winulta

Appendix D

Table D1. Site mean yields for environment Set 4.

Locality	1995		1996	
	Trial	Yield (t/ha)	Trial	Yield (t/ha)
Walker Flat	11	1.43	19	1.64
Two Wells	12	1.78	20	1.72
Kapunda	13	3.12	21	2.41
Mallala	14	1.84	22	2.59
Roseworthy	15	2.63	23	2.49
Winulta	16	2.60	24	2.59
LSD (0.05) 0.09				

Table D2. Site mean yields for environment Set 5.

Locality	1994		1995		1996	
	Trial	Yield (t/ha)	Trial	Yield (t/ha)	Trial	Yield (t/ha)
Kapunda	5	1.27	13	3.04	21	2.41
Mallala	6	0.91	14	1.81	22	2.52
Roseworthy	7	0.95	15	2.51	23	2.44
Winulta	8	1.80	16	2.55	24	2.57
LSD (0.05) 0.09						

Table D3. Mean performance of durum and bread wheat varieties in environment Sets 4 and 5.

Genotype	Yield (t/ha)	
	Set 4	Set 5
Durum wheat		
Kamilaroi	2.10	1.99
Yallaroi	2.12	1.92
Wollaroi	2.23	2.11
Kronos	2.34	
WLYY9/1/3/4	2.26	
WLYY9/2/L1/1	2.32	
WLYY9/3/1/5	2.29	
WLYY9/3/2/1	2.40	
WLYY9/3/2/3	2.38	
WLYY9/3/2/4	2.43	
WLYY9/3/2/5	2.45	
RH 900453	2.25	2.05
RH 911913	2.13	2.00
RH 911926	2.20	2.08
RH 911996	2.30	2.17
RH 912025	2.46	2.29
RH 920314	2.13	1.98
RH 920318	2.29	2.16
RH 920325	2.06	
RH 920334	2.08	2.02
RH 920351	2.20	2.04
RH 920356	2.24	2.08
RH 920528	2.16	2.04
RH 920532	2.08	1.95
RH 920615	2.22	2.14
RH 920618	2.26	2.14
RH 920621	2.25	
RH 920672	2.16	2.08
RH 920680	2.09	1.98
LSD (0.05)#	0.13	0.12
Bread wheat		
Spear	2.54	2.59
Molineux	2.42	2.43

calculated for durum only

Table D4. Site mean yields for environment Sets 6 and 7.

Trial	Year	Locality	Yield (t/ha)	
			Set 6	Set 7
1	1994	Lowbanks		0.39
2	1994	Palmer - Eichler		0.61
5	1994	Kapunda		1.27
6	1994	Mallala		0.91
7	1994	Roseworthy		0.95
8	1994	Winulta		1.80
9	1995	Rudall	0.72	0.71
10	1995	Palmer - Krause	0.80	0.78
11	1995	Walker Flat	1.43	1.36
12	1995	Two Wells	1.79	1.75
13	1995	Kapunda	3.12	3.06
14	1995	Mallala	1.84	1.79
15	1995	Roseworthy	2.62	2.53
16	1995	Winulta	2.59	2.56
17	1996	Minnipa	0.84	0.83
18	1996	Jamestown	1.67	1.63
19	1996	Walker Flat	1.64	1.62
20	1996	Two Wells	1.72	1.69
21	1996	Kapunda	2.41	2.36
22	1996	Mallala	2.59	2.50
23	1996	Roseworthy	2.49	2.42
24	1996	Winulta	2.59	2.55
LSD (0.05)			0.08	0.09

Table D5. Mean yield from ANOVA, regression coefficient (b), and standard error (SE_b) from adaptation analysis for durum and bread wheat genotypes in Set 6.

Genotypes	Yield (t/ha)	b	SE_b	Yield‡ (t/ha)	b‡	SE_b‡
Durum wheat						
Kamilaroi	1.82	.570**	.141	1.89	.645**	.181
Yallaroi	1.82	(1)		1.90	(1)	
Wollaroi	1.92	.705**	.166	1.99	.764**	.214
Kronos	2.02	.440*	.192	2.09	.460ns	.249
WLYY9/1/3/4	1.98	.447*	.154	2.06	.396ns	.199
WLYY9/2/L1/1	2.02	.804**	.168	2.10	.802**	.219
WLYY9/3/1/5	1.98	.745**	.122	2.06	.717**	.158
WLYY9/3/2/1	2.05	1.008**	.141	2.14	1.103**	.179
WLYY9/3/2/3	2.03	1.018**	.177	2.11	1.161**	.221
WLYY9/3/2/4	2.07	1.035**	.158	2.16	1.062**	.206
WLYY9/3/2/5	2.10	.878**	.159	2.18	1.018**	.197
RH 900453	1.97	.557**	.121	2.05	.486**	.155
RH 911913	1.83	.715**	.119	1.91	.713**	.155
RH 911926	1.94	.331**	.109	2.00	.433**	.134
RH 911996	1.99	.723**	.120	2.07	.828**	.148
RH 912025	2.13	.855**	.107	2.21	.988**	.126
RH 920314	1.82	.801**	.0985	1.90	.771**	.128
RH 920318	1.97	.578**	.113	2.05	.599**	.146
RH 920325	1.75	.857**	.191	1.83	.813**	.248
RH 920334	1.75	.913**	.194	1.83	.861**	.251
RH 920351	1.85	1.023**	.169	1.94	.934**	.216
RH 920356	1.90	.967**	.120	1.99	.930**	.155
RH 920528	1.90	.408*	.143	1.96	.519*	.179
RH 920532	1.81	.640**	.102	1.88	.676**	.131
RH 920615	1.94	.508**	.0926	2.01	.492**	.120
RH 920618	1.95	.701**	.139	2.02	.798**	.176
RH 920621	1.94	.678**	116	2.01	.803**	.140
RH 920672	1.87	.680**	.0960	1.94	.709**	.124
RH 920680	1.82	.477**	.120	1.88	.614**	.144
LSD# (0.05)	0.11			0.11		
Bread wheat						
Spear	2.45	(0)		2.52	(0)	
Molineux	2.13	.134ns	.148	2.19	.165ns	.192

‡ Mean excluding Trial 10 (Palmer - Krause). ** P<0.01 * P<0.05 ns P>0.05

calculated for durum only

Table D6. Mean yield from ANOVA, regression coefficient (b), and standard error (SE_b) from adaptation analysis for durum and bread wheat genotypes in Set 7.

Genotypes	Yield (t/ha)	b	SE_b	Yield‡ (t/ha)	b‡	SE_b‡
Durum wheat						
Kamilaroi	1.60	.534**	.148	1.69	.526*	.185
Yallaroi	1.55	(1)		1.65	(1)	
Wollaroi	1.66	.694**	.155	1.76	.720**	.195
RH 900453	1.69	.569**	.113	1.78	.533**	.140
RH 911913	1.60	.602**	.141	1.69	.548**	.167
RH 911926	1.69	.327**	.109	1.78	.361**	.121
RH 911996	1.73	.698**	.111	1.83	.731**	.131
RH 912025	1.81	.895**	.108	1.92	.981**	.123
RH 920314	1.57	.729**	.0978	1.67	.660**	.119
RH 920318	1.58	.464**	.131	1.67	.413*	.159
RH 920334	1.56	.701**	.224	1.66	.517ns	.270
RH 920351	1.59	1.014**	.161	1.70	.896**	.195
RH 920356	1.63	.884**	.118	1.73	.829**	.146
RH 920528	1.66	.303*	.135	1.74	.329ns	.170
RH 920532	1.57	.578**	.104	1.66	.581**	.123
RH 920615	1.71	.414**	.143	1.81	.297ns	.173
RH 920618	1.68	.659**	.142	1.77	.687**	.179
RH 920672	1.65	.537**	.134	1.74	.466*	.167
RH 920680	1.60	.383**	.130	1.69	.415*	.164
LSD# (0.05)	0.09			0.09		
Bread wheat						
Spear	2.13	(0)		2.23	(0)	
Molineux	1.88	.033ns	.147	1.97	.008ns	.184

‡ Mean excluding Trials 2 and 10. ** P<0.01 * P<0.05 ns P>0.05

calculated for durum only

References

Aarssen, L.W., and Burton, S.M. 1990. Maternal effects at four levels in *Senecio vulgaris* (Asteraceae) grown on a soil nutrient gradient. *Am. J. Bot.* 77: 1231-1240.

Abou-El-Fittouh, H.A., Rawlings, J.O., and Miller, P.A. 1969. Classification of environments to control genotype by environment interactions with an application to cotton. *Crop Sci.* 9: 135-140.

American Association of Cereal Chemists. 1984. "Approved Methods of the AACC." The Association, St. Paul, MN.

Ammar, K., Lukaszewski, A.J., and Banowetz, G.M. 1997. Effect of *Glu-D1*$_{(5+10)}$ on gluten strength and polymeric protein composition in durum wheat. *Cereal Foods World* 42:610.

Archer, M.J., and O'Brien, L. 1987. A comparative study of the quality status of Condor wheat grown in northern Victoria and southern New South Wales. *Aust. J. Agric. Res.* 38: 465-471.

Autran, J.-C., Abecassis, J., and Feillet, P. 1986. Statistical evaluation of different technological and biochemical tests for quality assessment in durum wheats. *Cereal Chem.* 63: 390-394.

Autran, J.-C., and Feillet, P. 1987. Genetic and technological basis of protein quality for durum wheat in pasta. *In* "Proc. EEC Symp. on Protein Evaluation in Cereals and Legumes" pp. 59-71. (*Ed.* V. Pattakou). 23-24 Oct., 1985, Cereal Institute, Thessaloniki, Greece.

Autran, J.-C., and Galterio, G. 1989. Associations between electrophoretic composition of proteins, quality characteristics and agronomic attributes of durum wheats. II. Protein-quality associations. *J. Cereal Sci.* 9: 195-215.

Axford, D.W.E., McDermott, E.E., and Redman, D.G. 1979. Note on the sodium dodecyl sulfate test of breadmaking quality: Comparison with Pelshenke and Zeleny tests. *Cereal Chem.* 68: 582-584.

Bagheri, A., Paull, J.G., Rathjen, A.J., Ali, S.M., and Moody, D.B. 1992. Genetic variation in the response of pea (*Pisum sativum* L.) to high soil concentrations of boron. *Plant and Soil* 146: 261-269.

Bianchi, A., and Boggini, G. 1996. Evoluzione varietale del frumento duro in Italia. *In* "Premio Barilla: dal grano alla pasta" pp. 23-28. Barilla Alimentare, Parma, Italy.

Bhatt, G.M., and Derera, N.F. 1975. Genotype × environment interactions for heritabilities of and correlations among quality traits in wheat. *Euphytica* 24: 597-604.

Blumenthal, C.S., Batey, I.L., Bekes, F., Wrigley, C.W., and Barlow, E.W.R. 1991a. Seasonal changes in wheat-grain quality associated with high temperatures during grain filling. *Aust. J. Agric. Res.* 42: 21-30.

Blumenthal, C.S., Bekes, F., Batey, I.L., Wrigley, C.W., Moss, H.J., Mares, D.J., and Barlow, E.W.R. 1991b. Interpretation of grain quality results from wheat variety trials with reference to high temperature stress. *Aust. J. Agric. Res.* 42: 325-334.

Blumenthal, C.S., Gras, P.W., Bekes, F., Barlow, E.W.R., and Wrigley, C.W. 1995. Possible role for the *Glu-D1* locus with respect to tolerance to dough-quality change after heat stress. *Cereal Chem.* 72:135-136.

Blumenthal, C.S., Stone, P.J., Gras, P.W., Bekes, F., Clarke, B., Barlow, E.W.R., Appels, R., and Wrigley, C.W. 1998. Heat-shock protein 70 and dough-quality changes resulting from heat stress during grain filling in wheat. *Cereal Chem.* 75: 43-50.

Boggini, G. 1985a. Breadmaking quality of some durum wheat culitvars (In Italian). *Tec. Molitoria* 36: 579-587.

Boggini, G. 1985b. Durum wheat bread making quality (In Italian). *Monogr. Genet. Agra.* 7: 407-416.

Boggini, G., Palumbo, M., and Biancardi, A.M. 1988. Bread making quality of Italian durum wheat cultivars. Results of three years trials (In Italian). *Tec. Molitoria* 39: 609-617.

Boggini, G., and Pogna, N.E. 1989. The breadmaking quality and storage protein composition of Italian durum wheat. *J. Cereal Sci.* 9: 131-138.

Boggini, G., Tusa, P., and Pogna, N.E. 1995. Bread making quality of durum wheat genotypes with some novel glutenin subunit compositions. *J. Cereal Sci.* 22: 105-113.

Boyacioglu, M.H., and D'Appolonia, B.L. 1994. Characterization and utilization of durum wheat for breadmaking. I. Comparison of chemical, rheological, and baking properties between bread wheat flours and durum wheat flours. *Cereal Chem.* 71: 21-28.

Bozzini, A. 1988. Origin, distribution, and production of durum wheat in the world. *In* "Durum Wheat: Chemistry and Technology" pp. 1-16. (*Eds.* G. Fabriani and C. Lintas). Am. Assoc. Cereal Chem., St. Paul, MN.

Bozzini, A. 1970. Scope and methods of breeding durum wheat. *In* "Proc. Third FAO/Rockefeller Foundation Wheat Seminar" pp. 154-157. Ankara, Turkey.

Brajcich, P., Vazquez, G., and Pfeiffer, W.H. 1983. Durum wheat. CIMMYT Report on Wheat Improvement 1983. CIMMYT, Mexico.

Branlard, G., Autran, J.-C., and Monneveux, P. 1989. High molecular weight subunits in durum wheat (*T. durum*). *Theor. Appl. Genet.* 78: 353-358.

Brennan, J.P. 1986. Impact of the Wheat Varieties from CIMMYT on Australian Wheat Production. Agricultural Economics Bulletin 5. Department of Agriculture New South Wales.

Breth, S.A. 1975. Durum wheat: New age for an old crop. CIMMYT Today. CIMMYT, Mexico.

Brooks, B.J. 1991. "The adaptation of *Triticum turgidum* L. var. *durum* (durum wheat) to South Australia. Honours Thesis." University of Adelaide, South Australia.

Burnouf, T., and Bouriquet, R. 1980. Glutenin subunits of genetically related European hexaploid wheat cultivars: Their relation to bread-making quality. *Theor. Appl. Genet.* 58: 107-111.

Byth, D.E., Eisemann, R.L., and DeLacy, I.H. 1976. Two-way pattern analysis of a large data set to evaluate genotypic adaptation. *Heredity* 37: 215-230.

Cakmak, I., Torun, B., Erenoglu, B., Öztürk, L., Marschner, H., Kalayci, M., Ekiz, H., and Yilmaz, A. 1998. Morphological and physiological differences in the response of cereals to zinc deficiency. *In* "Wheat: Prospects for Global Improvement. Proc. 5th Int. Wheat Conf." pp. 427-435. (*Eds.* H.-J. Braun, F. Altay, W.E. Kronstad, S.P.S. Beniwal, and A. McNab). 10-14 June, 1996, Ankara, Turkey. Kluwer Academic Publishers, The Netherlands.

Campbell, T.A., Rathjen, A.J., and Jefferies, S.P. 1994. Breeding wheat (*Triticum aestivum* L.) for tolerance to boron toxicity. *In* "Proc. Seventh Assembly Wheat Breeding Society of

Australia" (*Eds.* J.G. Paull, I.S. Dundas, K.W. Shepherd, and G.J. Hollamby). 25-30 Sept., 1994, Adelaide. University of Adelaide, South Australia.

Carrillo, J.M., Vazquez, J.F., and Orellana, J. 1990. Relationship between gluten strength and gluten proteins in durum wheat cultivars. *Plant Breeding* 104: 325-333.

Cartwright, B., and Hirsch, M. 1986. Boron toxicity in barley and wheat - a disorder resembling foliar disease. Dept. Agric. South Aust. FS 8/86, Adelaide.

Cartwright, B., Rathjen, A.J., Sparrow, D.H.B., Paull, J.G., and Zarcinas, B.A. 1987. Boron tolerance in Australian varieties of wheat and barley. *In* "Genetic aspects of plant mineral nutrition" pp. 139-151. (*Eds.* H.W. Gabelman and B.C. Loughman). Martinus Nijhoff, Dordrecht, The Netherlands.

Cartwright, B., Zarcinas, B.A., and Spouncer, L.R. 1986. Boron toxicity in South Australian barley crops. *Aust. J. Agric. Res.* 37: 351-359.

Cartwright, B., Zarcinas, B.A., and Mayfield, A.H. 1984. Toxic concentrations of boron in a red-brown earth at Gladstone, South Australia. *Aust. J. Soil Res.* 22: 261-272.

Ceccarelli, S. 1997. Adaptation to low/high input cultivation. *In* "Adaptation in Plant Breeding" pp. 225-236. (*Ed.* P.M.A. Tigerstedt). Kluwer Academic Publishers, The Netherlands.

Chantachume, Y. 1995. "Genetic studies on the tolerance of wheat to high concentrations of boron. PhD Thesis." University of Adelaide, South Australia.

Chantachume, Y., Rathjen, A.J., Paull, J.G., and Shepherd, K.W. 1993. Genetic studies on boron tolerance of wheat. *In* "Focused Plant Improvement: Towards Responsible and Sustainable Agriculture. Proceedings of Tenth Australian Plant Breeding Conference. Vol.2" pp. 74-75. (*Eds.* B.C. Imrie and J.B. Hacker). 18-23 April, 1993, Gold Coast, Queensland. Organising Committee, Australian Convention and Travel Service, Canberra.

Chantachume, Y., Smith, D., Hollamby, G.J., Paull, J.G., and and Rathjen, A.J. 1995. Screening for boron tolerance in wheat (*T. aestivum*) by solution culture in filter paper. *Plant and Soil* 177: 249-254.

Chapman, S.C., Crossa, J., and Edmeades, G.O. 1997a. Genotype by environment effects and selection for drought tolerance in tropical maize. I. Two mode pattern analysis of yield. *Euphytica* 95: 1-9.

Chapman, S.C., Crossa, J., Basford, K.E., and Kroonenberg, P.M. 1997b. Genotype by environment effects and selection for drought tolerance in tropical maize. II. Three-mode pattern analysis. *Euphytica* 95: 11-20.

Chapman, V., Miller, T.E., and Riley, R. 1976. Equivalence of the A genome of bread wheat and that of *Triticum urartu*. *Genet. Res., Cambridge* 27: 69-76.

Ciaffi, M., Benedettelli, S., Giorgi, B., Porceddu, E., and Lafiandra, D. 1991. Seed storage proteins of *Triticum turgidum* ssp. *dicoccoides* and their effect on the technological quality in durum wheat. *Plant Breeding* 107: 309-319.

Ciaffi, M., Lafiandra, D., Turchetta, T., Ravaglia, S., Bariana, H., Gupta, R., and MacRitchie, F. 1995. Breadbaking potential of durum wheat lines expressing both X- and Y-type subunits at the *Glu-A1* locus. *Cereal Chem.* 72: 465-469.

Ciaffi, M., Lafiandra, D., Porceddu, E., and Benedettelli, S. 1993. Storage-protein variation in wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) from Jordan and Turkey. I. Electrophoretic characterization of genotypes. *Theor. Appl. Genet.* 86: 474-480.

Clarke, J., Marchylo, B., Kovacs, M., Noll, J., and McCaig, T. 1996. Screening of durum wheats for pasta quality: The Canadian system. *In* "Abstracts Proc. 5th Int. Wheat Conf." pp. 233-234. 10-14 June, 1996, Ankara, Turkey.

Comstock, R.E., and Moll, R.H. 1963. Genotype-environment interactions. *In* "Statistical Genetics and Plant Breeding" pp. 164-196. (*Eds.* W.D. Hanson and H.F. Robinson). National Academy of Sciences - National Research Council, Washington D.C.

Cooper, M., Byth, D.E., and Woodruff, D.R. 1994a. An investigation of the grain yield adaptation of advanced CIMMYT wheat lines to water stress environments in Queensland. I. Crop physiological analysis. *Aust. J. Agric. Res.* 45: 965-984.

Cooper, M., Byth, D.E., and Woodruff, D.R. 1994b. An investigation of the grain yield adaptation of advanced CIMMYT wheat lines to water stress environments in Queensland. 2. Classification analysis. *Aust. J. Agric. Res.* 45: 985-1002.

Cooper, M., DeLacy, I.H., and Eisemann, R.L. 1993. Recent advances in the study of genotype × environment interactions and their application to plant breeding. *In* "Focused Plant Improvement: Towards Responsible and Sustainable Agriculture. Proceedings of Tenth Australian Plant Breeding Conference. Vol.1" pp. 116-131. (*Eds.* B.C. Imrie and

J.B. Hacker). 18-23 April, 1993, Gold Coast, Queensland. Organising Committee, Australian Convention and Travel Service, Canberra.

Cornish, E.A. 1950. The influence of rainfall on the yield of wheat in South Australia. *Aust. J. Scient. Res. B.* 3: 178-218.

Cox, M.C., Qualset, C.O., and Rains, D.W. 1985. Genetic variation for nitrogen assimilation and translocation in wheat. II. Nitrogen assimilation in relation to grain yield and protein. *Crop Sci.* 25: 430-435.

Crossa, J., Fox, P.N., Pfeiffer, W.H., Rajaram, S., and Gauch, H.G. 1991. AMMI adjustment for statistical analysis of an international wheat yield trial. *Theor. Appl. Genet.* 81: 27-37.

Crossa, J., and Franco, J. 2004. Statistical methods for classifying genotypes. *Euphytica.* 137: 19-37.

Cubadda, R. 1989. Current research and future needs in durum wheat chemistry and technology. *Cereal Fd. World* 34: 206-209.

Cunin, C., Handschin, S., Walther, P., and Escher, F. 1995. Structural changes in starch during cooking of durum wheat pasta. *Lebensm. Wiss. Technol.* 28: 323

Damidaux, R., Autran, J.-C., Grignac, P., and Feillet, P. 1980a. Determinisme genetique des constituants gliadines de *Triticum durum* DESF associes a la qualite culimaire intrinseque des varietes. *C. R. Acad. Sci. Paris, serie D* 291: 585-588.

Damidaux, R., Autran, J.-C., Grignac, P., and Feillet, P. 1978. Evidence of relationships useful for breeding between the electrophoretic patterns of gliadins and the viscoelastic properties of the gluten in *Triticum-durum* Desf. (in French). *C. R. Acad. Sci. Paris, serie D* 287: 701-704.

Damidaux, R., Autran, J.-C., and Feillet, P. 1980b. Gliadin electrophoregrams and measurements of gluten viscoelasticity in durum wheats. *Cereal Fd. World* 25: 754-756.

Damidaux, R., and Feillet, P. 1978. Relation entre les proprietes viscoelastiques du gluten cuit, la teneur en proteines et la qualite culinaire des bles durs. *An. Techn. Agric.* 28: 799-808.

Day, G.E., Paulsen, G.N., and Sears, R.G. 1985. Nitrogen relations in winter wheat cultivars differing in grain protein percentage and stature. *J. Plant Nutr.* 8: 555-566.

Debbouz, A., and Doetkott, C. 1996. Effect of process variables on spaghetti quality. *Cereal Chem.* 73: 672-676.

D'Egidio, M.G., Fortini, S., Galterio G., Mariani, B.M., Sgrulletta, D., and Volpi, M. 1979. Proteines totales et composition proteique de semoules de bles durs italiens; correlation avec la qualite des pates alimentaires. *Qual. Plant. Pl. Fds. Hums. Nutr.* 14: 333-348.

D'Egidio, M.G., Mariani, B.M., Nardi, S., and Novaro, P. 1993. Viscoelastograph measures and total organic matter test: Suitability in evaluating textural characteristics of cooked pasta. *Cereal Chem.* 70: 67-72.

DeLacy, I.H., Basford, K.E., Cooper, M., Bull, J.K., and McLaren, C.G. 1996 Analysis of Multi-environment Trials - An Historical Perspective. *In* "Plant Adaptation and Crop Improvement" pp. 39-124. CAB International.

Dexter, J.E., Matsuo, R.R., Preston, K.R., and Kilborn, R.H. 1981. Comparison of gluten strength, mixing properties, baking quality and spaghetti quality of some Canadian durum and common wheats. *Can. Inst. Fd. Sci. Technol. J.* 14: 108-111.

Dexter, J.E., and Matsuo, R.R. 1980. Relationship between durum wheat protein properties and pasta dough rheology and spaghetti cooking quality. *J. Agric. Food Chem.* 28: 899-902.

Dexter, J.E., Matsuo, R.R., and Morgan, B.C. 1983. Spaghetti stickiness and realtionship to other cooking quality characteristics. *J. Fd. Sci.* 48: 1545-1551.

Dick, J.W. 1988. Evaluation of durum wheat for bread. *In* "Proc. Int. Symp. on Durum wheat for bread making" pp. 177-183. Canatania, Sicily, Italy.

Dick, J.W., and Quick, J.S. 1983. A modified screening test for rapid estimation of gluten strength in early generation wheat breeding lines. *Cereal Chem.* 60: 315-318.

du Cros., D.L. 1987. Glutenin proteins and gluten strength in durum wheat. *J. Cereal Sci.* 5: 3-12.

du Cros, D.L., Wrigley, C.W., and Hare, R.A. 1982. Prediction of durum wheat quality from gliadin protein composition. *Aust. J. Agric. Res.* 33: 429-442.

Dvorak, J. 1976. The relationship between the genome of *Triticum urartu* and the A and B genomes of *Triticum aestivum*. *Can. J. Genet. Cytol.* 18: 371-377.

Dvorak, J., di Terlizzi, P., Zhang, H.-B., and Resta, P. 1993. The evolution of polyploid wheats: Identification of the A genome donor species. *Genome* 36: 21-31.

Dvorak, J., Zhang, H.-B., Kota, R.S., and Lassner, M. 1989. Organization and evolution of the 5S ribosomal RNA gene family in wheat and related species. *Genome* 32: 1003-1016.

Dvorak, J., and Zhang, H.-B. 1990. Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. *Proc. Natl. Acad. Sci. USA* 87: 9640-9644.

Eagles, H.A., Hollamby, G.J., Gororo, N.N., and Eastwood, R.F. 2002. Estimation and utilisation of glutenin gene effects from the analysis of unbalanced data from wheat breeding programs. *Aust. J. Agric. Res.* 53: 367-377.

Eaton, F.M. 1944. Deficiency, toxicity and accumulation of boron in plants. *J. Agric. Res.* 69: 237-277.

Eisemann, R.L. 1981. Two methods of ordination and their application in analysing genotype-environment interactions. *In* "Interpretation of Plant Response and Adaptation to Agricultural Environments" pp. 293-307. (*Eds.* D.E. Byth and V.E. Mungomery). Queensland Branch, Australian Institute of Agricultural Science, Brisbane.

Eisemann, R.L., Cooper, M., and Woodruff, D.R. 1990. Beyond the analytical methodology - better interpretation and exploitation of genotype-by-environment interaction in breeding. *In* "Genotype-by-Environment Interaction and Plant Breeding." pp. 108-117. (*Ed.* K.S. Lang). Louisiana State University, Baton Rouge.

Feillet, P. 1984. The biochemical basis of pasta quality: Its consequences for durum wheat breeders. *Sciences Des Aliments* 4: 551-566.

Feillet, P., Abecassis, J., and Alary, R. 1977. Description d'un nouvel appareil pour mesurer les proprietes viscoelastiques des produits cerealiers. Application a l'appreciation de la qualite du gluten, des pates alimentaires et du riz. *Bull. ENSMIC* 278: 97-101.

Feillet, P., and Abecassis, J. 1976. Valeur d'utilisation des bles durs. *Sem. Etudes Cereal., Faculte Sciences Agronomiques Gembloux, Belgium* 551-560.

Feldman, M., Lupton, F.G.H., and Miller, T.E. 1995. Wheat. *In* "Evolution of Crop Plants" pp. 184-192. (*Eds.* J. Smartt and N.W. Simmonds). Longman Group UK Ltd., London.

Feldman, M. and Millet, E. 1993. Methodologies of identification, allocation and transfer of quantitative genes from wild emmer into cultivated wheat. *In* "Proc. 8th Int. Wheat Genet. Symp." pp. 19-27. 20-25 Aug., 1993, Beijing, China. China Agricultural Scientech Press, China.

Finlay, K.W., and Wilkinson, G.N. 1963. The analysis of adaptation in a plant-breeding programme. *Aust. J. Agric. Res.* 14: 742-54.

Finney, K.F., and Barmore, M.A. 1948. Loaf volume and protein content of hard winter and spring wheats. *Cereal Chem.* 25: 291-312.

Fleming, G.A. 1980. Essential micronutrients I: Boron and molybdenum. *In* "Applied Soil Trace Element" pp. 155-197. (*Ed.* B.E. Davies). John Wiley & Sons, Chichester.

Fox, P.N., Rosielle, A.A., and Boyd, W.J.R. 1985. The nature of genotype × environment interactions for wheat yield in Western Australia. *Field Crops Res.* 11: 387-398.

Fox, P.N., Skovmand, B., Thompson, B.K., Braun, H.-B., and Cormier, R. 1990. Yield and adaptation of hexaploid spring triticale. *Euphytica* 47: 57-64.

Franco, J., and Crossa, J. 2002. The Modified Location Model for classifying genetic resources: I. Association between categorical and continuous variables. *Crop Sci.* 42: 1719-1726.

French, R.J., and Schultz, J.E. 1984. Water use efficiency of wheat in a Mediterranean-type environment. I. The relation between yield, water use and climate. *Aust. J. Agric. Res.* 35: 743-764.

Galterio, G., Codianni, P., Novembre, G., Saponaro, C., Di Fonzo, N., and Pogna, N.E. 1998. Storage protein composition of F_6 lines from the cross *Triticum turgidum* spp *durum* x *Triticum turgidum* spp *dicoccum*. *In* "Proc. 9th Int. Wheat Genet. Symp." Vol. 4. pp. 148-150. (*Ed.* A.E. Slinkard). 2-7 August, 1998, Saskatoon, Saskatchewan, Canada. University Extension Press, University of Saskatchewan.

Genstat 5 Reference Manual. 1987. Genstat 5 Committee, Rothamsted Experimental Station, Clarendon Press, Oxford.

Gilmour, A.R., Cullis, B.R., and Verbyla, A.P. 1997. Accounting for natural and extraneous variation in the analysis of filed experiments. *Journal of Agricultural, Biological and Environmental Statistics*. 2: 269-273.

Gomez, K.A., and Gomez, A.A. 1984. "Statistical procedures for agricultural research" John Wiley & Sons, Canada.

Goodchild, N.A., and Boyd, W.J.R. 1975. Regional and temporal variations in wheat yield in Western Australia and their implications in breeding. *Aust. J. Agric. Res.* 26: 209-217.

Gorham, J., Bridges, J., Dubcovsky, J., Dvorak, J., Hollington, P.A., Luo, M.-C., and Khan, J.A. 1997. Genetic analysis and physiology of a trait for enhanced K/Na discrimination in wheat. *New Phytol.* 137: 109-116.

Gorham, J., Hardy, C., Wyn Jones, R.G., Joppa, L.R., and Law, C.N. 1987. Chromosomal location of a K/Na discrimination charater in the D genome of wheat. *Theor. Appl. Genet.* 74: 584-588.

Graham, R.D. 1988. Development of wheats with enhanced nutrient efficiency: progress and potential. *In* "Proc. CIMMYT/UNDP Int. Symp. on Tropical Wheat Production" pp. 305-320. (*Ed.* A.R. Klatt). 19-23 Jan., 1987, Chiang Mai, Thailand.

Graybosch, R.A., Peterson, C.J., and Shelton, D.R. 1994. Environmental modification of wheat flour quality and protein composition in the northern great plains of North America. *In* "Proc. 44th Cereal Chem. Conf." pp. 14-17. (*Eds.* J.F. Panozzo and P.G. Downie). Royal Australian Chemical Institute, Ballarat, Australia.

Grewal, H.S., Graham, R.D., and Rengel, Z. 1996. Genotypic variation in zinc efficiency and resistance to crown rot disease (*Fusarium graminearum* Schw. Group 1) in wheat. *Plant and Soil* 186: 219-226.

Grzybowski, R.A., and Donnelly, B.J. 1979. Cooking properties of spaghetti: Factors affecting cooking quality. *J Agric. Fd. Chem.* 27: 380-384.

Gupta, R.B., Paul, L.G., Cornish, G.B., Palmer, G.A., Bekes, F., and Rathjen, A.J. 1994. Alleic variation at glutenin subunits of gliadin loci, *Glu-1*, *Glu-3* and *Gli-1*, of common wheats. I. Its additive and interaction effects on dough properties. *J. Cereal Sci.* 19: 9-17.

Gupta, U.C. 1979. Boron nutrition of crops. *Adv. Agron.* 31: 273-307.

Hanson, W.D. 1963. Heritability. *In* "Statistical Genetics and Plant Breeding" pp. 125-140. H.F. NAS-NRC Publ. 982, Wash. DC.

Hare, R.A. 1994. National Durum Wheat Improvement Program. *In* "Proc. Seventh Assembly Wheat Breeding Society of Australia" pp. 81-84. (*Eds.* J.G. Paull, I.S. Dundas, K.W. Shepherd, and G.J. Hollamby). 25-30 Sept., 1994, Adelaide. University of Adelaide, South Australia.

Hare, R.A. 1996. Australian durum wheat improvement. *In* "Proc. 46th Australian Cereal Chem. Conf." pp. 353-355. (*Ed.* C.W. Wrigley). 4-6 Sept., 1996, Sydney. RACI, N. Melbourne.

Hare, R.A. 1998. Tamaroi. *Plant Varieties Journal* 11: 81.

He, G.Y., Rooke, L., Steele, S., Békés, F., Gras, P., Tatham, A.S., Fido, R., Barcelo, P., Shewry, P.R., and Lazzeri, P.A. 1999. Transformation of pasta wheat (*Triticum turgidum* L. var. *durum*) with high-molecular-weight glutenin subunit genes and modification of dough functionality. *Molecular Breeding* 5: 377-386.

Hollamby, G.J. 1973. "The measurement of genotype-environment interaction in plant breeding. Master's Thesis." University of Adelaide, South Australia.

Hollamby, G.J. 1999. The use of probe genotypes to improve selection efficiency - Examples. *In* "Proc. 11th Australian Plant Breeding Conference Volume 2 - Contributed Papers" pp. 87-88. 19-23 April, 1999, Glenelg, South Australia. CRC for Molecular Plant Breeding, South Australia.

Hollamby, G.J. 1996. Wheat Breeding in South Australia. *In* "Proc. Eighth Assembly Wheat Breeding Society of Australia" pp. O40-O42. (*Eds.* R.A. Richards, C.W. Wrigley, H.M. Rawson, G.J. Rebetzke, J.L. Davidson, and R.I.S. Brettell). 29 Sept.- 4 Oct., 1996, Canberra. The Australian National University, Canberra, ACT.

Hollamby, G.J., Bayraktar, A., and Wilson, R.E. 1983. An effective breeding procedure for improving yield, adaptation, disease resistance and quality in wheat for Australia. *In* "Proc. 6th Int. Wheat Genet. Symp." pp. 1163-1169. (*Ed.* S. Sakamoto). Kyoto, Japan. Plant Germplasm Institute, Kyoto University, Japan.

Hollamby, G.J., Rathjen, A.J., and Bayraktar, A. 1994. Wheat Breeding in South Australia in the 1990's. *In* "Proc. Seventh Assembly Wheat Breeding Society of Australia" pp. 101-106. (*Eds.* J.G. Paull, I.S. Dundas, K.W. Shepherd, and G.J. Hollamby). 25-30 Sept., 1994, Adelaide. University of Adelaide, South Australia.

Holloway, R.E., and Alston, A.M. 1992. The effect of salt and boron on growth of wheat. *Aust. J. Agric. Res.* 43: 987-1001.

Huang, C., and Graham, R.D. 1990. Resistance of wheat genotypes to boron toxicity is expressed at the cellular level. *Plant Soil* 126: 295-300.

Imrie, B.C., Drake, D.W., DeLacy, I.H., and Byth, D.E. 1981. Analysis of genotypic and environmental variation in international mungbean trials. *Euphytica* 30: 301-311.

Imrie, B.C., and Shanmugasundaram, S. 1987. Source of variation in yield in international mungbean trials. *Field Crop Res.* 16: 197-208.

International Wheat Council. 1994. World Wheat Statistics. The Council, London.

Isbell, R.F. 1996. "The Australian Soil Classification." CSIRO, Australia.

Jackson, P.A., Byth, D.E., Fischer, K.S., and Johnston, R.P. 1993. Genotype × environment interactions in progeny from a barley cross I. Patterns of response among progeny lines for grain yield and time to anthesis. *Aust. J. Expt. Agric.* 33: 619-627.

Jackson, P.A., Byth, D.E., Fischer, K.S., and Johnston, R.P. 1994. Genotype × environment interactions in progeny from a barley cross II. Variation in grain yield, yield components and dry matter production among lines with similar times to anthesis. *Field Crops Res.* 37: 11-23.

Jamjod, S. 1996. "Genetics of boron tolerance in durum wheat. PhD Thesis." University of Adelaide, South Australia.

Jamjod, S., Paull, J.G., Brooks, B.J., and Rathjen, A.J. 1997. Genetic variation in the tolerance of durum wheat (*Triticum turgidum* L. var *durum*) to high concentrations of boron. *In* "Boron in Soils and Plants" pp. 111-115. (*Eds.* R.W. Bell and B. Rerkasem). 7-11 Sept., 1997, Chiang Mai, Thailand. Kluwer Academic Publishers, The Netherlands.

Jefferies, S.P., Barr, A.R., Karakousis, A., Kretschmer, J.M., Manning, S., Chalmers, K.J., Nelson, J.C., Islam, A.K.M., and Langridge, P. 1999a. Mapping of chromosome regions conferring boron toxicity tolerance in barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* 98: 1293-1303.

Jefferies, S.P., Barr, A.R., Langridge, P., Chalmers, K.J., and Gianquitto, P. 1999b. Backcrossing - A traditional breeding method with a new lease of life. *In* "Proc. 11th

Australian Plant Breeding Conference Volume 2 - Contributed Papers" pp. 89-91. 19-23 April, 1999, Glenelg, South Australia. CRC for Molecular Plant Breeding, South Australia.

Jefferies, S.P., Pallotta, M.A., Paull, J.G., Karakousis, A., Kretschmer, J.M., Manning, S., Islam, A.K.M.R., Langridge, P. and Chalmers, K.J., 2000. Mapping and validation of chromosome regions conferring boron toxicity tolerance in wheat (*Triticum aestivum*). *Theor. Appl. Genet.* 101: 767-777.

Jenkin, M.J. 1993. "The genetics of boron tolerance in barley. PhD Thesis." University of Adelaide, South Australia.

Joppa, L.R., Hareland, G.A., and Cantrell, R.G. 1991. Quality characterisitics of the Langdon durum-*dicoccoides* chromosome subsitution lines. *Crop Sci.* 31: 1513-1527.

Joppa, L.R., Josephides, C., and Youngs, V.L. 1983. Chromosomal location of genes affecting quality in durum wheat. *In* "Proc. 6th Int. Wheat Genet. Symp." pp. 297-301. (*Ed.* S. Sakamoto). Kyoto, Japan. Plant Germplasm Institute, Kyoto University, Japan.

Joppa, L.R., and Williams, N.D. 1988. Langdon durum disomic substitution lines and aneuploid analysis in tetraploid wheat. *Genome* 30: 222-228.

Josephides, C.M., Joppa, L.R., and Youngs, V.L. 1987. Effect on chromosome 1B on gluten strength and other characterisitics of durum wheat. *Crop Sci.* 27: 212-216.

Joshi, Y.C., Snehi Dwivedi, R., Qadar, A., and Bal, A.R. 1982. Salt tolerance in diploid, tetraploid and hexaploid wheat. *Indian J. Plant Physiol.* 25: 421-422.

Kaan, F., Branlard, G., Chihab, B., Borries, C., and Neveux, P. 1993. Relations between genes coding for grain storage protein and two pasta cooking quality criteria among world durum wheat (*Triticum durum* Desf.) genetic resources. *J. Genetics & Breeding* 47: 151-156.

Kaltsikes, P.J., Evans, L.E., and Bushuk, W. 1968. Durum-type wheat with high bread-making quality. *Science* 159: 211-213.

Kempton, R.A. 1984. The use of bi-plots in interpreting variety by environment interactions. *J. Agric. Sci.* 103: 123-135.

Kerber, E.R., and Tipples, K.H. 1969. Effects of the D genome on milling and baking properties of wheat. *Can. J. Plant Sci.* 49: 255-263.

Kihara, H. 1944. Discovery of the DD analyser, one of the ancestors of *T. vulgare*. *Agric. Hort.* 19: 889-890.

King, P.M., and Alston, A.M. 1975. Diagnosis of trace element deficiencies in wheat on Eyre Peninsula, South Australia. *In* "Trace Elements in Soil-Plant-Animal Systems" pp. 339-352. (*Eds.* D.J.D. Nicholas and A.R. Egan). Academic Press, New York.

Knight, R. 1970. The measurement and interpretation of genotype-environment interactions. *Euphytica* 19: 225-235.

Kosmolak, F.G., Dexter, J.E., Matsuo, R.R., Leisle, D., and Marchylo, B.A. 1980. A relationship between durum wheat quality and gliadin electrophoregrams. *Can. J. Plant Sci.* 61: 149-151.

Kovacs, M.I.P., Howes, N.K., Leisle, D., and Zawistowski, J. 1995a. Effect of two different low molecular weight glutenin subunits on durum wheat pasta quality parameters. *Cereal Chem.* 72: 85-87.

Kovacs, M.I.P., Noll, J.S., Dahlke, G., and Leisle, D. 1995b. Pasta viscoelasticity: Its usefulness in the Canadian durum wheat breeding program. *J. Cereal Sci.* 22: 115-121.

Kovacs, M.I.P., Poste, L.M., Butler, G., Woods, S.M., Leisle, D., Noll, J.S., and Dahlke, G. 1997. Durum wheat quality: Comparison of chemical and rheological screening tests with sensory analysis. *J. Cereal Sci.* 25: 65-75.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage. *Nature* 227: 680-685.

Lafiandra, D., Margiotta, B., Colaprico, G., Masci, S., Roth, M.R. and MacRithchie, F. 2000. Introduction of the D-genome related high- and low-Mr glutenin subunits into durum wheat and their effect on technological properties. *In* "Proc. 7th International Workshop Gluten 2000" (*Eds.* P.R. Shewry & A.S. Tatham) pp. 51-54. Royal Society Chemistry, Cambridge, UK.

Lawrence, G.J., MacRitchie, F., and Wrigley, C.W. 1988. Dough and baking quality of wheat lines deficient in glutenin subunits controlled by the *Glu-A1*, *Glu-B1* and *Glu-D1* loci. *J. Cereal Sci.* 7: 109-112.

Lawrence, G.J., and Shepherd, K.W. 1980. Variation in glutenin protein subunits of wheat. *Aust. J. Biol. Sci.* 33: 221-233.

Lemerle, D., Verbeek, B., Cousens, R.D., and Coombes, N.E. 1996. The potential for selecting wheat varieties strongly competitive against weeds. *Weed Res.* 36: 505-513.

Levy, A.A., and Feldman, M. 1989. Genetics of morphological traits in wild wheat, *Triticum turgidum* var. *dicoccoides*. *Euphytica* 40: 275-281.

Lill, W.J., Gleeson, A.C., and Cullis, B.R. 1988. Relative accuracy of a neighbour method for field experiments. *Journal of Agricultural Science, Cambridge*. 111: 339-346.

Lin, C.S., Binns, M.R., and Lefkovitch, L.P. 1986. Stability analysis: Where do we stand? *Crop Sci.* 26: 894-900.

Liu, C.-Y. 1995a. Preliminary results of the *Glu-D1* alleles on quality characteristics of durum wheat cultivar Langdon. (1). 1D (1B) substitution lines. *J. Genet. & Breed.* 49: 269-276.

Liu, C.-Y. 1995b. Preliminary results of the *Glu-D1* alleles on quality characteristics of durum wheat cultivar Langdon. (2). 1D (1A) substitution lines. *J. Genet. & Breed.* 49: 277-283.

Liu, C.-Y. 1994. "Variation and genetic control of prolamins in tetraploid wheats and their association with quality in durum wheat. Ph.D Thesis." University of Adelaide, South Australia.

Liu, C.-Y., Paull, J.G., and Rathjen, A.J. 2000. Shoot mineral composition and yield of wheat genotypes grown on a sodic and a non-sodic soil. *Aust. J. Expt. Agric.* 40: 69-78.

Liu, C.-Y., Rathjen, A.J., Shepherd, K.W., Gras, P.W., and Giles, L.C. 1995. Grain quality and yield characteristics of D-genome disomic substitution lines in 'Langdon' (*Triticum turgidum* var. *durum*). *Plant Breeding* 114: 34-39.

Liu, C.-Y., and Rathjen, A.J. 1994. Grain yield and quality characteristics of durum wheats. *In* "Proc. Seventh Assembly Wheat Breeding Society of Australia" pp. 279-282. (*Eds.* J.G. Paull, I.S. Dundas, K.W. Shepherd, and G.J. Hollamby). 26-30 Sept., 1994, Adelaide, South Australia.

Liu, C.-Y., and Shepherd, K.W. 1996. Variation of B subunits of glutenin in durum, wide and less-widely cultivated tetraploid wheats. *Plant Breeding* 115: 172-178.

Liu, C.-Y., Shepherd, K.W., and Gras, P.W. 1994a. Grain yield and quality characteristics of chromosome 1D and 1B substitution lines in durum wheat and their F2-derived progeny lines. I. Comparisons among the tetraploid phenotypes. *J. Cereal Sci.* 20: 20-32.

Liu, C.-Y., Shepherd, K.W., and Gras, P.W. 1994b. Grain yield and quality characteristics of chromosome 1D and 1B substitution lines in durum wheat and their F2-derived progeny lines. II. Comparisons with the durum and bread wheat controls. *J. Cereal Sci.* 20: 227-234.

Liu, C.-Y., Shepherd, K.W., and Rathjen, A.J. 1996. Improvement of durum wheat pastamaking and breadmaking qualities. *Cereal Chem.* 73: 155-166.

López-Castañeda, C., and Richards, R.A. 1994. Variation in temperate cereals in rainfed environments. III. Water use and water-use efficiency. *Field Crops Res.* 39: 85-98.

Loss, S.P., and Siddique, K.H.M. 1994. Mediterranean wheat yield increases. *In* "Advances in Agronomy" (*Ed.* D.L. Sparks). Academic Press,

MacRitchie, F. 1984. Baking quality of wheat flours. *Adv. Fd. Res.* 29: 207-277.

MacRitchie, F., du Cros, D.L., and Wrigley, C.W. 1990. Flour polypeptides related to wheat quality. *In* "Advances in Cereal Science and Technology" pp. 79-146. (*Ed.* Y. Pomeranz). Am. Assoc. Cereal Chem., St. Paul, MN.

Mangels, C.E. 1925. Effect of climate and other factors on the protein content of North Dakota wheat. *Cereal chem.*. 2: 288-297.

Marchylo, B., and Dexter, J.E. 1996. Durum wheat and pasta quality now and into the 21st century. *In* "Proc. 46th Australian Cereal Chem. Conf." pp. 345-352. (*Ed.* C.W. Wrigley). 4-6 Sept., 1996, Sydney. RACI, N. Melbourne.

Marschner, H. 1986. "Mineral Nutrition of Higher Plants" 674 pp. Academic Press, London.

Matsuo, R.R., and Irvine, G.N. 1970. Effect of gluten on the cooking quality of spaghetti. *Cereal Chem.* 47: 173-180.

Matveef, M. 1966. Influence du gluten des bles durs sur la valeur des pates alimentaires. *Bull. ENSMIC* 213: 133-138.

McFadden, E.S., and Sears, E.R. 1946. The origin of *Triticum spelta* and its free threshing hexaploid relatives. *J. Hered.* 37: 81-89.

McIntosh, M.S. 1983. Analysis of combined experiments. *Agron. J.* 75: 153-55.

McKenzie, E. 1994. Processing requirements in respect of end products - semolina/pasta. *In* "Durum Wheat Improvement" 30-31 Aug., 1994, Canberra. GRDC, ACT.

Menger, A. 1974. Über das Kochen und die Bewertung des Garezustandes von Teigwaren. *Getr. Mehl U. Brot* 28: 236-241.

Moody, D.B., Rathjen, A.J., Cartwright, B., Paull, J.G., and Lewis, J. 1988. Genetic diversity and geographical distribution of tolerance to high levels of soil boron. *In* "Proc. 7th Int. Wheat Genet. Symp." pp. 859-866. (*Eds.* T.E. Miller and R.M.D. Koebner). 13-19 July, 1988, Cambridge. Oxford University Press, Oxford, England.

Moody, D.B., Rathjen, A.J., and Cartwright, B. 1993. Yield evaluation of a gene for boron tolerance using backcross-derived lines. *In* "Genetic Aspects of Plant Mineral Nutrition" pp. 363-366. (*Eds.* P.J. Randall, E. Delhaize, R.A. Richards and R. Munns). Kluwer Academic Publishers, The Netherlands.

Moonen, J.H.E., Scheepstra, A., and Graveland, A. 1983. The possible effects of the high molecular weight subunits 3+10 and 2* of glutenin on the bread-making quality of wheat cultivars. *Euphytica* 32: 735-742.

Moss, H.J., Randall, P.J., and Pendleton, V. 1986. Growth temperature and grain quality in wheat. *In* "26th Annu. General Meeting, Aust. Soc. Plant Physiol." Abstract No. 101. Melbourne, Australia.

Mungomery, V.E., Shorter, R., and Byth, D.E. 1974. Genotype × environment interactions and environmental adaptation. I. Pattern analysis-application to soya bean populations. *Aust. J. Agric. Res.* 25: 59-72.

Nable, R.O. 1988. Resistance to boron toxicity amongst several barley and wheat cultivars: A preliminary examination of the resistance mechanisms. *Plant Sci.* 112: 45-52.

Nable, R.O., Lance, R.C.M., and Cartwright, B. 1990a. Uptake of boron and silicon by barley genotypes with differing susceptibilities to boron toxicity. *Ann. Bot.* 66: 83-90.

Nable, R.O., and Moody, D.B.. 1992. Effects of rainfall on the use of foliar analysis for diagnosing boron toxicity in field grown wheat. *Plant and Soil* 140: 311-314.

Nable, R.O., Paull, J.G., and Carwright, B. 1990b. Problems associated with the use of foliar analysis for diagnosing boron toxicity in barley. *Plant and Soil* 128: 225-232.

Norrish, K. 1975. Geochemistry and mineralogy of trace elements. *In* "Trace Elements in Soil-Plant-Animal Systems" pp. 55-81. (*Eds.* D.J.D. Nicholas and A.R. Egan). Academic Press Inc., New York.

Northcote, K.H., and Skene, J.K.M. 1972. "Australian Soils with Saline and Sodic Properties." Aust. CSIRO Soil Publ. No. 27.

Nyquist, W.E. 1991. Estimation of heritability and prediction of selection response in plant populations. *Critical Reviews in Plant Sciences* 10: 235-322.

Oertli, J.J., and Kohl, H.C. 1961. Some considerations about the tolerance of various plant species to excessive supplies of boron. *Soil Sci.* 92: 243-247.

Panozzo, J.F., and Eagles, H.A. 2000. Cultivar and environmental effects on quality characters in wheat. II. Protein. *Aust. J. Agric. Res.* 51: 629-636.

Paull, J.G. 1990. "Genetic studies on the tolerance of wheat to high concentrations of boron. PhD thesis." University of Adelaide, South Australia.

Paull, J.G., Cartwright, B., and Rathjen, A.J. 1988a. Responses of wheat and barley genotypes to toxic concentrations of soil boron. *Euphytica* 39: 137-144.

Paull, J.G., Holloway, R.E., McCarthy, D., and Nable, R.O. 1992c. Subsoil toxicities in alkaline soils of southern Australia. *In* "Proc. National Workshop on Subsoil Constraints to Root Growth and High Soil Water and Nutrient Use by Plants" 30 Aug.-2 Sept., 1992, Tanunda, South Australia.

Paull, J.G., Moody, D.B., and Rathjen, A.J. 1992d. Genetics and breeding of wheat for boron toxicity. *In* "Proc. CIMMYT Workshop on Boron in Plants" Chiang Mai, Thailand.

Paull, J.G., Nable, R.O., and Rathjen, A.J. 1992a. Physiological and genetic control of the tolerance of wheat to high concentrations of boron and implications for plant breeding. *Plant and Soil* 146: 251-260.

Paull, J.G., Nable, R.O., Lake, A.W.H., Materne, M.A., and Rathjen, A.J. 1992b. Response of annual medics (*Medicago* spp.) and field peas (*Pisum sativum*) to high concentrations of boron: Genetic variation and the mechanism of tolerance. *Aust. J. Agric. Res.* 43: 203-213.

Paull, J.G., Rathjen, A.J., and Cartwright, B. 1986. Boron tolerance in wheat varieties and advanced breeding lines. *In* "Proc. 5th Assembly of the Wheat Breeding Society of Australia" Perth/Merredin, Western Australia.

Paull, J.G., Rathjen, A.J., and Cartwright, B. 1988b. Genetic control of tolerance to high concentrations of soil boron for wheat. *In* "Proc. 7th Int. Wheat Genet. Symp." pp. 871-878. (*Eds.* T.E. Miller and R.M.D. Kroebner). 13-19 July, 1988, Cambridge. Oxford Press, Oxford University, Oxford, England.

Paull, J.G., Rathjen, A.J., and Cartwright, B. 1991. Major gene control of tolerance of bread wheat to high concentrations of soil boron. *Euphytica* 55: 217-228.

Paull, J.G., Rathjen, A.J., Cartwright, B., and Nable, R.O. 1990. Selection parameters for assessing the tolerance of wheat to high concentrations of boron. *In* "Genetic aspects of plant mineral nutrition" pp. 361-369. (*Eds.* N.E. Bassam, and B.C. Loughman). Kluwer Academic Press, The Netherlands.

Paull, J.G., Rathjen, A.J., Langridge, P., and McIntosh, R.A. 1993. Location of genes controlling boron tolerance of wheat. *In* "Proc. 8th Int. Wheat Genet. Symp." pp. 1065-1069. (*Eds.* Z.S. Li and Z.Y. Xin). Beijing.

Payne, P.I. 1987. The genetical basis of bread-making quality of wheat. *Aspects Appl. Biol.* 15: 79-90.

Payne, P.I., Holt, L.M., Krattiger, A.F., and Carrillo, J.M. 1988. Relationships between seed quality characteristics and HMW glutenin subunit composition determined using wheats grown in Spain. *J. Cereal Sci.* 7: 229-235.

Payne, P.I., Holt, L.M., Jackson, E.A., and Law, C.N. 1984a. Wheat storage proteins: their genetics and potential for manipulation by plant breeding. *Phil. Trans. Roy. Soc. Lond. B.* 304: 359-371.

Payne, P.I., Jackson, E.A., and Holt, L.M. 1984b. The association between gamma-gliadin 45 and gluten strength in durum wheat varieties: A direct casual effect or the result of genetic linkage? *J. Cereal Sci.* 2: 73-81.

Payne, P.I., Nightingale, M.A., Krattiger, A.F., and Holt, L.M. 1987. The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *J. Sci. Fd. Agric.* 40: 51-65.

Peña, R.J., Zarco-Hernandez, J., Amaya-Celis, A., and Mujeeb-Kazi, A. 1994. Relationship between chromosome 1B-encoded glutenin subunit compositions and bread-making quality characteristics of some durum wheat (*Triticum turgidum*) cultivars. *J. Cereal Sci.* 19: 243-249.

Pfeiffer, W.H. 1996. CIMMYT's future challenges in durum wheat breeding. *In* "Premio Barilla: dal grano alla pasta" pp. 53-69. Barilla Alimentare, Parma, Italy.

Pogna, N.E., Autran, J.-C., Mellini, F., Lafiandra, D., and Feillet, P. 1990. Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat: genetics and relationship to gluten strength. *J. Cereal Sci.* 11: 15-34.

Pogna, N.E., Lafiandra, D., Feillet, P., and Autran J.-C. 1988. Evidence for a direct causal effect of low molecular weight subunits of glutenins on gluten viscoelasticity in durum wheats. *J. Cereal Sci.* 7: 211-214.

Porceddu, E., Turchetta, T., Masci, S., D'Ovidio, R., Lafiandra, D., Kasarda, D.D., Impiglia, A., and Nachit, M.M. 1998. Variation in endosperm protein composition and technological quality properties in durum wheat. *Euphytica* 100: 197-205.

Preston, K.R., March, P.R., and Tipples, K.H. 1982. An assessment of the SDS-sedimentaion test for the prediction of Canadian bread wheat quality. *Can. J. Plant Sci.* 62: 545-553.

Quaglia, G.B. 1988. Other durum wheat products. *In* "Durum Wheat: Chemistry and Technology" pp. 263. (*Eds.* G. Farbriani and C. Lintas). Am. Assoc. Cereal Chem., St. Paul, MN.

Randall, P.G., Manley, M., McGill, A.E.J., and Taylor, J.R.N. 1993. Relationship between the high *Mr* subunits of glutenin of South African wheats and end-use quality. *J. Cereal Sci.* 18:251-258.

Randall, P.J., and Moss, H.J. 1990. Some effects of temperature regime during grainfilling on wheat quality. *Aust. J. Agric. Res.* 41: 603-617.

Rao, V.K., Mulvaney, S.J., Dexter, J.E., Edwards, N.M., and Peressini, D. 2001. Stress-relaxation properties of mixograph semolina-water doughs from durum wheat cultivars of

variable strength in relation to mixing characteristics, bread- and pasta-making performance. *J Cereal Sci.* 34: 215-232.

Rathjen, A.J. 1994. The biological basis of Genotype × Environment interaction - its definition and management. *In* "Proc. Seventh Assembly Wheat Breeding Society of Australia" pp. 13-17. (*Eds.* J.G. Paull, I.S. Dundas, K.W. Shepherd, and G.J. Hollamby). 25-30 Sept., 1994, Adelaide. University of Adelaide, South Australia.

Rathjen, A.J., Brand, J.D., Liu, C.-Y., Paull, J.G., and Cooper, D. 1999. Breeding for tolerance to soil toxicities. *In* "Proc. 11th Australian Plant Breeding Conference Volume 1 - Invited Papers" pp. 34-40. 19-23 April, 1999, Glenelg, South Australia. CRC for Molecular Plant Breeding, South Australia.

Rathjen, A.J., and Brooks, B.J. 1994. Durum. *In* "Agronomy Technical Conference" 22-23 March, 1994, Adelaide.

Rathjen, A.J., Cartwright, B., Paull, J.G., Moody, D.B., and Lewis, J. 1987. Breeding for the tolerance of mineral toxicities in Australian cereals with special reference to boron. *In* "Priorities in soil/plant relations research for plant production" pp. 111-130. (*Eds.* P.G.E. Searle and B.G. Davey). School of Crop Sciences, The University of Sydney, Sydney.

Rathjen, A.J., Eastwood, R.F., Lewis, J.G., and Dube, A.J. 1998. Breeding wheat for resistance to *Heterodera avenae* in Southeastern Australia. *Euphytica* 100: 55-62.

Rathjen, A.J., Hare, R.A., and Brooks, B.J. 1996. Durum breeding in Australia. *In* "Proc. Eighth Assembly Wheat Breeding Society of Australia" pp. O57-O58. (*Eds.* R.A. Richards, C.W. Wrigley, H.M. Rawson, G.J. Rebetzke, J.L. Davidson, and R.I.S. Brettell). 29 Sept.- 4 Oct., 1996, Canberra.

Rathjen, A.J., Paull, J.G., and Graham, R.D. 1993. Breeding for nutrient toxicities and deficiencies. *In* "Proc. Tenth Australian Plant Breeding Conf." (*Eds.* B.C. Imrie and J.B. Hacker). 18-23 April, 1993, Gold Coast.

Rathjen, A.J., and Pederson, D.C. 1986. Selecting for improved grain yield in variable environments. *In* "Proc. of Plant Breeding Symp." pp. 104-115. (*Eds.* T.A. Williams and G.S. Wratt). Agronomy Society of New Zealand Special Publication, Lincoln, New Zealand.

Regan, K.L., Siddique, K.H.M., Turner, N.C., and Whan, B.R. 1992. Potential for increasing early vigour and total biomass in spring wheat. II. Characteristics associated with early vigour. *Aust. J. Agric. Res.* 43: 541-553.

Reisenauer, H.M., Walsh, L.M., and Hoeft, R.G. 1973. Testing soils for sulpher, boron, molybdenum, and chloride. *In* "Soil Testing and Plant Analysis" pp. 173-200. (*Eds.* L.M. Walsh and J.D. Beaton). Soil Sci. Soc. Amer., Madison, Wisconsin.

Richards, R.A., Rebetzke, G.J., Condon, A.G., van Herwaarden, A.F., Duggan, B.L., and Munns, R. 1999. Targeting physiological traits to overcome environmental limitations and raise the yield potential of dryland crops. *In* "Proc. 11th Australian Plant Breeding Conference Volume 1 - Invited Papers" pp. 27-33. 19-23 April, 1999, Glenelg, South Australia. CRC for Molecular Plant Breeding, South Australia.

Riley, R., Unrau, J., and Chapman, V. 1958. Evidence on the origin of the B genome of wheat. *J. Hered.* 49: 91-99.

Romagosa, I., and Fox, P.N. 1993. Genotype × environment interaction and adaptation. *In* "Plant Breeding: Principles and prospects." , M.D. Hayward, N.O. Bosemark and I. Romagosa edpp. 373-390. Chapman & Hall, London.

Saberi, H.K., Graham, R.D., and Rathjen, A.J. 1996. Screening for Mn efficiency in durum wheat (*Triticum turgidum* L. *var. durum*). *In* "Abstracts Proc. 5th Int. Wheat Conf." pp. 206-207. 10-14 June, 1996, Ankara, Turkey.

Saberi, H.K., Graham, R.D., and Rathjen, A.J. 1997. Genotypic variation for Mn efficiency in durum wheat (*Triticum turgidum* L. *var. durum*). *In* "Plant Nutrition - for Sustainable Food Production and Environment" pp. 33-35. (*Eds.* T. Ando, K. Fujita, T. Mae, H. Matsumoto, S. Mori, and J. Sekiya). Kluwer Academic Publishers, The Netherlands.

Salinger, M.J., Jamieson, P.D., and Johnstone, J.V. 1995. Climate variability and wheat baking quality. *N.Z. J. Crop Hort. Sci.* 23: 289-298.

Sarkar, P., and Stebbins, G.L. 1956. Morphological evidence concerning the origin of the B genome in wheat. *Am. J. Botany* 43: 297-304.

Schipper, A., Jahn-Deesbaach, W., and Weipert, D. 1986. Untersuchungen zum Klimateinfluss auf die Weizenqualitat. *Getreide, Mehl und Brot* 40: 99-103.

Schultz, J.E., and French, R.J. 1976. Mineral content of herbage and grain of Halberd wheat in South Australia. *Aust. J. Exp. Agric. Anim. Husb.* 16: 887-892.

Seif, E., and Pederson, D.G. 1978. Effect of rainfall on the grain yield of spring wheat, with an application to the analysis of adaptation. *Aust. J. Agric. Res.* 29: 1107-15.

Shorter, R., Lawn, R.J., and Hammer, G.L. 1991. Improving genotypic adaptation in crops - a role for breeders, physiologists and modellers. *Expt. Agric.* 27: 155-157.

Siddique, K.H.M., Tennant, D., Perry, M.W., and Belford, R.K. 1990. Water use and water use efficiency of old and modern wheat cultivars in a Mediterranean type climate. *Aust. J. Agric. Res.* 41: 431-447.

Singh, N.K., Donovan, R., and MacRitchie, F. 1990. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. II. Relative quantity of glutenin as a measure of breadmaking quality. *Cereal Chem.* 67: 161-170.

Sissons, M.J., and Gianibelli, M.C. 2000. Use of reconstitution techniques to study the functionality of gluten proteins on durum wheat pasta quality. *In* "Proc. 7th International Workshop Gluten 2000" (*Eds.* P.R. Shewry & A.S. Tatham) pp. 347-351. Royal Society Chemistry, Cambridge, UK.

Sissons, M.J., Gianibelli, M.C., and Batey, I.L. 2002. Small-scale reconstitution of durum semolina components. *Cereal Chem.* 79: 675-680.

Smith, A.B., Cullis, B.R., and Thompson, R. 2001. Analysing variety by environment data using multiplicative mixed models and adjustments for spatial field trends. *Biometrics.* 57: 1138-1147.

Sparrow, D.H.B. 1972. "A study of genotypic differences in the malting quality of barley. PhD Thesis." University of Adelaide, South Australia.

Sparrow, D.H.B, and Graham, R.D. 1988. Susceptibility of zinc-deficient wheat plants to colonization by *Fusarium graminearum* Schw. Group 1. *Plant and Soil.* 112: 261-266.

Spouncer, L.R., Nable, R.O., and Cartwright, B. 1992. A procedure for the determination of soluble boron in soils ranging widely in boron concentrations, sodicity and pH. *Comm. Soil Sci. Plant Anal.* 23: 441-453.

Srivastava, J.P., Damania, A.B., and Pecetti, L. 1988. Landraces, primitive forms and wild progenitors of macaroni wheat, *Triticum durum*: their use in dryland agriculture. *In* "Proc. 7th Int. Wheat Genet. Symp." pp. 153-158. (*Eds.* T.E. Miller and R.M.D. Kroebner). 13-19 July, 1988, Cambridge, England. Oxford University Press, Oxford, England.

Steel, R.G.D., and Torrie, J.H. 1980. "Principles and Procedures of Statistics." McGraw-Hill, New York.

Thongbai, P.G., Graham, R.D., Neate, S.M., and Webb, M.J.. 1993. Interaction between nutrional status of cereals and Rhizoctonia root rot. II. Effect of Zn on disease severity of wheat under controlled conditions. *Plant and Soil* 153: 215-222.

Turchetta, T., Ciaffi, M., Porceddu, E., and Lafiandra, D. 1995. Relationship between electrophoretic pattern of storage proteins and gluten strength in durum wheat landraces from Turkey. *Plant Breed.* 114: 406-412.

Vallega, J., and Zitelli, G. 1973. New high yielding Italian *durum* wheat varieties. *In* "Proc. Symp. Genetics and Breeding of Durum Wheat" pp. 373-399. 14-18 May, 1973, Bari, Italy.

Vallega, V. 1986. High molecular weight glutenin subunit composition of Italian *Triticum durum* cultivars & spaghetti cooking quality. *Cereal Res. Commun.* 14: 251-257.

Vallega, V. 1988. Comparative analysis of high-molecular-weight glutenin subunit composition in various *Triticum* species. *Plant Breed.* 100: 241-246.

Vallega, V., and Waines, J.G. 1987. High-molecular-weight glutenin subunit variation in *Triticum turgidum* var. *dicoccum*. *Theor. Appl. Genet.* 74: 706-710.

van Oosterom, E.J., and Acevedo, E. 1992. Adaptation of barley (*Hordeum vulgare* L.) to harsh Mediterranean environments. I. Morphological traits. *Euphytica* 62: 1-14.

Vanstone, V.A., Rathjen, A.J., Ware, A.H., and Wheeler, R.D. 1998. Relationship between root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) and performance of wheat varieties. *Aust. J. Expt. Agric.* 38: 181-188.

Waddington, S.R., Osmanzai, M., Yoshida, M., and Ransom, J.K. 1987. The yield of durum wheats released in Mexico between 1960 and 1984. *J. Agric. Sci., Camb.* 108: 469-477.

Waines, J.G., and Payne, P.I. 1987. Electrophoretic analysis of the high-molecular-weight glutenin subunits of *Triticum monococcum, T. urartu*, and the A genome of bread wheat (*T. aestivum*). *Theor. Appl. Genet.* 74: 71-76.

Walsh, D.E., and Gilles, K.R. 1971. The influence of protein composition on spaghetti quality. *Cereal Chem.* 48: 544-554.

Wildermuth, G.B., McNamara, R.B., Sparks, T., and Davis, M. 1999. Sources and types of resistance to crown rot in wheat. *In* "Proc. Ninth Assembly Wheat Breeding Society of Australia" pp. 64-65. (*Eds.* P. Williamson, P. Banks, I. Haak, J. Thompson, and A. Campbell). 27 Sept.-1 Oct., 1999, Toowoomba. The Univerisity of Southern Queensland, Toowoomba, Qld.

Williams, P.C., Jaby El-Haramein, F., Sayegh, A., Nachit, M., and Srivastava, J.P. 1989. Durum wheat utilization in West Asia/North Africa. *In* "ICC'89 Wheat End-use Properties" pp. 555-565. Lahti, Finland.

Wood, J.A., Batey, I.L., Hare, R.A., and Sissons, M.J. 2001. A comparison of Australian and imported spaghettis. *Food Australia* 53: 349-354.

Wrigley, C.W. 1994. Developing better strategies to improve grain quality for wheat. *Aust. J. Agric. Sci.* 45: 1-17.

Wyn Jones, R.G., Gorham, J., and McDonnell, E. 1984. Organic and inorganic solute contents as selection criteria for salt tolerance in the Triticeae. *In* "Salinity tolerance in plants; strategies of crop improvement" pp. 189-203. (*Eds.* R.C. Staples and G.H. Toenniessen). Wiley, New York.

Yates, F., and Cochran, W.G. 1938. The analysis of groups of experiments. *J. Agric. Sci.* 28: 556-580.

Yau, S.K. 2002. Interactions of boron-toxicity, drought, and genotypes on barley root growth, yield, and other agronomic characters. *Aust. J. Agric. Res.* 53:347-354.

Yau, S.K., Hamblin, J., and Ryan, J. 1994. Phenotypic variation in boron toxicity tolerance in barley, durum and bread wheat. *Rachis* 13: 20-25.

Yau, S.K., Nachit, M.M., Ryan, J., and Hamblin, J. 1995. Phenotypic variation in boron-toxicity tolerance at seedling stage in durum wheat (*Triticum durum*). *Euphytica* 83: 185-191.

Yau, S.K., Nachit, M.M., and Ryan, J. 1997a. Variation in boron-toxicity tolerance in a durum wheat core collection. *In* "Boron in Plants and Soil" pp. 117-120. (*Eds.* R.W. Bell and B. Rerkasem). 7-11 Sept., 1997, Chiang Mai, Thailand. Kluwer Academic Publishers, The Netherlands.

Yau, S.K., Nachit, M., and Ryan, J. 1997b. Variation in growth, development, and yield of durum wheat in response to high soil boron II. Differences between genotypes. *Aust. J. Agric. Res.* 48: 951-957.

Yau, S.K., and Saxena, M.C. 1997. Variation in growth, development, and yield of durum wheat in response to high soil boron I. Average effects. *Aust. J. Agric. Res.* 48: 945-949.

Zarcinas, B.A., and Cartwright, B. 1983. Analysis of soil and plant material by inductively coupled plasma - optical emission spectrometry. Optimization of operating parameters, calibration of the spectrometer and quantification of inter-element interferences. *Aust. CSIRO. Div. Soils Tech. Pap. No. 45*

Zarcinas, B.A., Cartwright, B., and Spouncer, L.R. 1987. Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Commun. Soil. Sci. Plant Anal.* 18: 131-146.

Zubaidi, A. 1996. "Growth and yield of durum and bread wheat. Masters Thesis." University of Adelaide, South Australia.