Characterisation and mapping of chromosome regions associated with improved growth and grain yield of barley on sandy soils of low fertility

A thesis submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy at the University of Adelaide, Australia

By

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In loving memory

of my dad,

Alan

7 July 1933 to 13 July 2002

Declaration

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Publications

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Abstract

Sandy soils of low fertility constitute approximately 30% of the total area (968,600ha) sown to barley in South Australia (SA). With such a significant proportion of the barley sown on sandy soils, the development of specifically adapted cultivars for this soil type is a very important barley breeding objective in the SA Barley Improvement Program (SABIP).

It is generally recognised that barley displays better adaptation on these soil types than wheat, triticale and oats, but is inferior to cereal rye. Even so, the inherently poor properties of sandy soils make the production of barley unreliable. A large part of the lower grain yield potential of crops grown on these soils is associated with the poor establishment and growth typically observed in these environmnets. This compares with the superior yield potential of crops grown on heavier soil types and in more favourable environments. Furthermore, the efficiency and progress in breeding and selecting varieties with superior adaptation on sand is impeded by the low heritability of traits important to adaptation to this environment. The low heritability is related to low genetic variance, partly related to the germplasm available, and the high error variance of yield trials conducted on sandy sites. In addition, genetic gain for sand adaptation has been limited by traditional selection methods that tend to discriminate against low yield potential environments.

Despite these limitations, some genetic gain for adaptation has been achieved. Yagan, an introduction of unknown pedigree from the International Maize and Wheat Improvement Center (CIMMYT), was released by the Western Australian (WA) Department of Agriculutre barley breeding program in 1988 because of improved yield and superior agronomic features on sandy soils in low rainfall environments. In 1996 Mundah, a selection developed from a simple cross between Yagan and O'Connor (WA bred variety of feed quality), was commercially released by WA. Since being introduced into SA Research and Development Institute (SARDI) field evaluation trials Mundah has consistently shown superior grain yield potential over Yagan and SA bred varieties on sand, but has ranked lower than SA selections

in high yield potential environments. Such genotype x environment interaction for adaptation response provides evidence of genotypic variability for sand adaptation. While potential genetic variation for sand adaptation has been observed, there has been no concerted effort to identify the physiological, morphological or biochemical characteristics of Mundah and Yagan that contribute to their superior performance on sand. Neither has there been efforts directed at understanding the genetic control of these characteristics.

The objective of this study was to re-address this deficiency in our knowledge of sand adaptation and use molecular marker technology to characterise the genetic basis of adaptation.

A comprehensive review of the literature on the inherent properties of sandy soils and the mechanisms likely to be associated with improved growth and grain yield on these soils led to the definition of a putative barley 'ideotype' (*i.e.* ideal plant type) for sand adaptation.

Field and controlled environment experiments were designed to characterise the traits associated with genotypic differentiation for adaptation on sandy soils, and to test the validity of the putative ideotype previously defined. In these experiments, the superior performance and grain yield of Mundah on sandy soils was found to be associated with improved establishment, early vigour (both in terms of dry matter production and leaf area development), phosphorus utilisation efficiency, and a deep root system.

A study of the impact of seed size on growth and productivity on sandy soils led to the conclusion that establishment, early vigour and grain yield could be improved by selecting the large seed size fraction for sowing. Large seed size was also associated with longer coleoptile length and higher seed nutrient content. The control of seeding depth on sandy soils is difficult and consequently longer coleoptiles set the potential for improved establishment and early vigour, which can ultimately lead to improved productivity.

Varieties with superior adaptation on sandy soils exhibited an erect growth habit and an earlier flowering phenology, while poorly adapted varieties had a more prostrate growth habit. It is likely that an erect growth habit balances the necessity for moisture conservation and

improved water use efficiency, and crop photosynthesis. The leaf architecture provides sufficient ground cover to reduce evaporative loss from the soil surface, while minimizing transpiration loss (*i.e.* improved transpiation efficiency). In addition, such a leaf structure may also provide an effective leaf area for efficient light capture following full canopy closure to maintain adequate crop photosynthesis. The reallocation of carbohydrates from the stem to the developing grain post-anthesis was also found to be a mechanism associated with superior adaptation on these soils. Invariably, adaptation is a complex inter-relationship or combination of traits, and it seems unlikely that a variety will be developed that possesses the optimum level of expression for any one trait. Rather a balanced portfolio of traits associated with adaptation appears to be the key to improved growth and grain yield on sandy soils.

While the studies presented here identified a suite of important traits, the unreliable phenotypic expression of traits in low yield potential environments has necessitated the development of an effective and efficient system of selecting germplasm possessing a superior combination of these traits. In this regard, marker assisted selection (MAS) is an attractive option because selection of superior breeding lines with improved adaptation is based solely on the presence of alleles for molecular makers cosegregating with key quantitative trait loci (QTL) (*i.e.* genotypic expression), and not on the phenotypic expression of a trait, which is strongly influenced by environmental pressures. The development of a mapping population, from a cross between Mundah (very good adaptation) and Keel (moderately poor adaptation), for this study has allowed statistically significant marker-trait associations to be identified, and QTL conferring adaptation to be mapped (Chapter 5).

Research aimed at understanding the genetic basis for adaptation response to sand was confounded by the prevailing environmental conditions, particularly moisture stress. This illustrated the likely importance of traits for superior grain yield under moisture stress conditions, and the interaction between these traits and those important for sand adaptation. The high level of trait by environment interaction in these environments provides further support for the use of marker-assisted selection (MAS) as a valid selection tool. Problems

and limitations encountered with this mapping population, due to limited marker availability, low polymorphism and an incomplete map, were also discussed. Significant QTL for traits associated with adaptation on sandy soils were identified and our understanding of the genetic and physiological mechanisms for sand tolerance has improved. However, the implementation of MAS for sand adaptation is not, at this stage, feasible. Recommendations for further studies aimed at achieving this goal are made.

Both the wild progenitor of cultivated barley and landrace germplasm can provide a rich resource of novel genes for adaptation with the potential contribution to genetic gain for abiotic stress tolerance both speculated and clearly demonstrated. Accordingly, a preliminary evaluation of germplasm from the International Centre for Agricultural Research in Dry Areas (ICARDA) was conducted and described in Chapter 6. Although the ICARDA material was found to offer no immediate commercial value, two breeders lines have been found to provide significant potential as parents. A strategy to identify superior genetic variation for sand adaptation is presented and discussed.

Chapter 1. Literature Review

1.1 Introduction

This review of the literature will focus on the factors likely to have a role in determining, and potentially improving, the growth and yield of barley on sandy soils. Root morphology, early growth and vigour, which influence water uptake, use and efficiency, will be considered. Molecular marker technology can be applied to mapping chromosome regions associated with traits of importance for growth, yield, quality and tolerance/resistance to biotic and abiotic stresses. Their growing adoption as a tool in assisting plant breeding and in the dissection of complex traits will be reviewed.

1.2 Geology of South Australian Soils

In geological terms, Australia has a very old landscape. The period from the Archaean to the end of the Mesozoic, 75 Ma (Ma = million years before present; Parker *et al.*, 1985), has provided the geological and structural background that has determined the form of the continent, and the rock types from which the soils have been derived (Beckmann, 1983). However, many of the soil types distributed across Australia have been formed from transported pre-weathered materials during the Tertiary and Quaternary periods, 'rather than only from *in situ* weathering of parent materials' (Sheard, 1995). Further, the soils also reflect local vegetation and the climatic conditions impacting during their formation (Sheard, 1995). The Tertiary and Quaternary periods are said to be the 'key' to the development of Australian soils (Northcote, 1983; Taylor, 1983; Wright, 1985; Blackburn and Wright, 1989; McCord, 1995). Consequently the parental rock material and the soils formed from them

have, over a very long period of time, been subjected to many cycles of weathering, leaching, transport and deposition (Chittleborough, 1982). This contrasts with the northern hemisphere where soil development is generally less than 100,000 years old due to Pleistocene glacial events which exposed fresh parent material to weathering (Chittleborough, 1982).

Soils also reflect climatic conditions. Oscillations in climate during the Tertiary and Quaternary periods, with some periods of severe aridity, have had profound influences on sea level and also on the weathering, transport, deposition and leaching of sediments. Because of these fluctuations in climate and their significant effect on the Australian landscape, many soils are not in equilibrium with their modern environment (Sheard, 1995).

'The geological processes imposed on the South Australian landscape have produced a broad range of soil types that are dominated by sand and calcium carbonate, with lesser areas of loam and clay' (Sheard, 1995). South Australia (SA) comprises three basic geological structures (Reuter *et al.*, 1988).

The Gawler platform (Eyre Peninsula, EP) consists of the oldest parental rock materials, which were formed during the late Archaean to the earliest Proterozoic (2700-2300 Ma) (Northcote, 1983; Parker *et al.*, 1985). Despite the geological age of the EP, soil development is related mainly to processes occuring during the Quaternary period (Blackburn and Wright, 1989; Wright, 1985). The calcareous nature of the soils on the EP is not related to marine deposition, except for along the west coast, since there is little evidence of sea incursions during the Cainozoic (Northcote, 1983; Noble and Bradstock, 1989). Rather they are based on Pleistocene re-sorted carbonate-rich sandy sediments swept inland by aeolian (wind) activity from an exposed continental shelf, following lowering of the sea level (Johns, 1958; Northcote, 1983; Wright, 1985; Blackburn and Wright, 1989). In the process, sand ridges of northwest (NW)-southeast (SE) orientation were formed from siliceous sand blown inland

(Johns, 1958; French, 1958). These Pleistocene sediments 'cover widespread deposits of Tertiary fluviatile (formed by river action) sands' (Blackburn and Wright, 1989).

The second basic structure is the Adelaide Geosyncline, which extends from Kangaroo Island to the Flinders Ranges (Beckmann, 1983; Reuter *et. al.*, 1988). Much of this area has basement rock dating back to the Cambrian and Ordovician period (600-440 Ma) with small areas of Permian (270-Ma) origin (Beckmann, 1983). The Central highlands chain includes the Lower, Mid and Upper North agricultural zones. Between this chain and the Gawler Platform lies the Yorke Peninsula (YP). In this region calcareous aeolinites and sands of Quaternary origin overlie the basement rock (Reuter *et. al.*, 1988). On the southern tip 'calcareous sands have formed on recent coastal shell sands' (Northcote, 1983).

The third basic structure is the basins and depositional zones (Reuter *et. al.*, 1988). Included in this group is the Murray basin, which encompasses the Murray Mallee (MM) and South-East (SE) agricultural regions. Basement rock is Cambrian (600-500 Ma) metamorphosed sediment (Northcote, 1983; McCord, 1995). Frequent incursions of the sea led to the deposition of marine sediments that give the soils of the Murray Mallee their calcareous nature (Blackburn and Wright, 1989; McCord, 1995). With the recession of the sea, NW to SE aligned sand ridges were left at ancient coastlines (Butler *et. al.*, 1983; McCord, 1995), producing an undulating landscape that extends across the whole region. Formation of these ridges has been a gradual process that has also involved the accession of carbonate-rich aeolian sediments (Blackmann and Wright, 1989). In the SE there are stranded Pleistocene coastal beach ridges, within 100 km of the sea, lining almost parallel to the modern coastline (Butler *et. al.*, 1983). 'While Tertiary non-marine sands and clays and marine limestones have contributed significantly to the surface soils, the present surface geomorphology was largely formed during the Quaternary period' (Blackburn and Wright, 1989; McCord, 1995). During this time there was 'considerable re-working of the more recent calcareous and sandy sediments' by aeolian activity (Reuter *et. al.*, 1988). Deposition of sediments under fluvial (river action), lacustrine (produced by lakes) and estuarine (formed in estuaries) conditions

also had an influence on the development of soils (Blackburn and Wright, 1989; McCord, 1995).

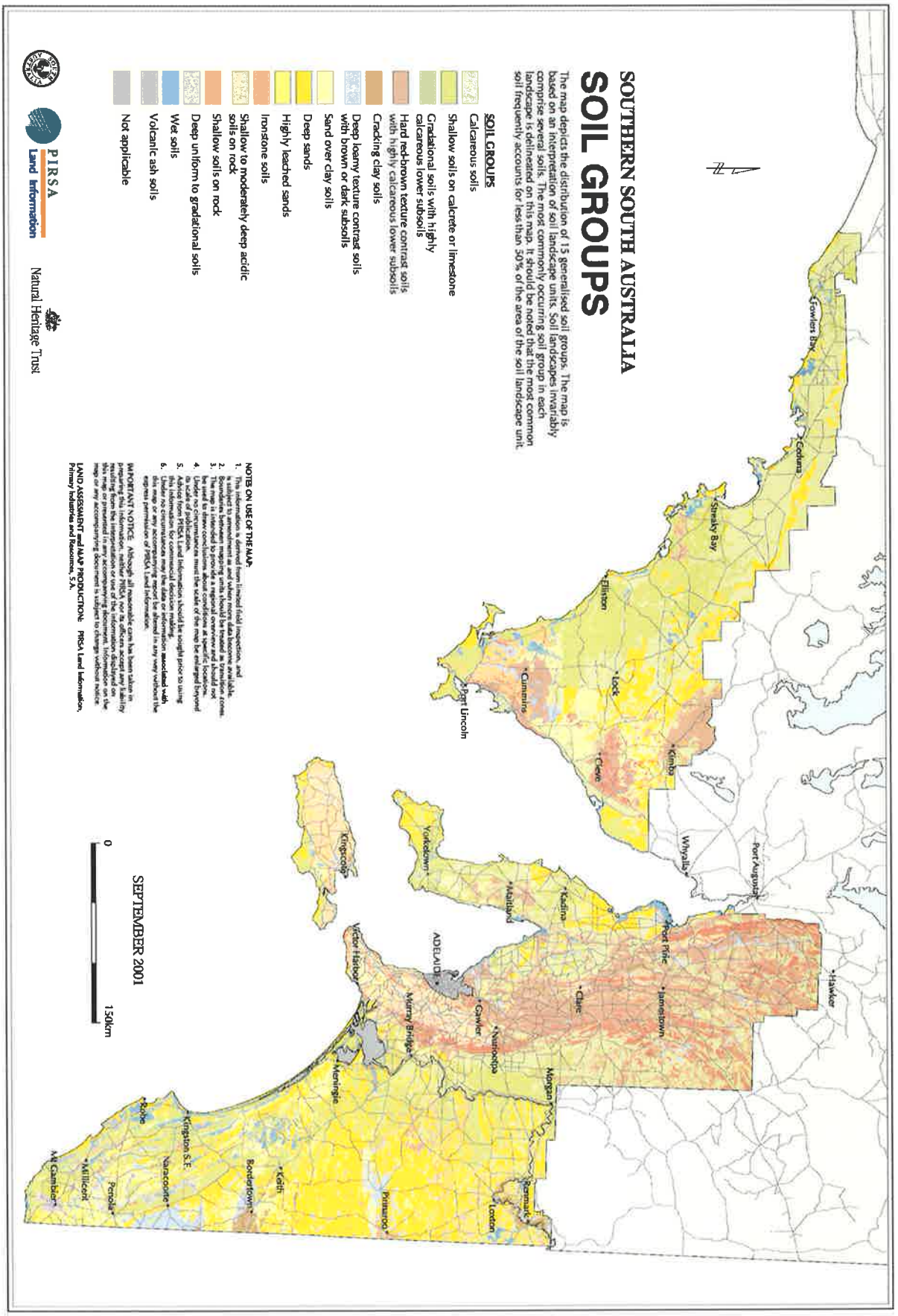
Aeolian sands and associated soils are a distinct feature of the landscape in Murray basin (Murray Mallee and South-East) and on the Eyre Peninsula in SA. Even the Yorke Peninsula, and Mid and lower north agricultural zones, which are predominately characterized by loam to clay-loam soils, are interspersed with sands (Map 1). The widespread occurrence of these soils in the agricultural regions of SA, and the fact that approximately 30% of the area sown to barley is on soils of a sandy texture, make them a defining issue in terms of crop production in those areas in which the exist.

1.3 Characteristics of Sandy Soils

Texturally, sandy soils are a group that have poorly developed profiles and include soils classified as sands, loamy sands and sandy loams (Kadry, 1975). Typically, sandy soils contain less than 5% clay (Hamblin *et. al.*, 1988) and are low in organic matter content, but can differ in terms of colour, pH and vertical heterogeneity (Northcote, 1979). The key features in common among 'sandy soils' are discussed in the following sections.

Map 1: Map depicting the main soil groups of the agricultural regions of SA.

Sandy soils are shown as yellow shaded areas (see key below).



Map provided by DWLBC, Soil and Land Information, SA

1.3.1 Fertility

Low soil fertility is a widespread problem throughout the cereal production areas of SA, with sandy soils being particularly deficient in all nutrients. Limited surface uplift, restriction of glaciation and minor post-Tertiary volcanic activity has limited the exposure of new parent material to weathering (Williams and Colwell, 1977). Soils have mostly developed from transported pre-weathered material that cover this parent material (Shead, 1995). The exposure of these deposited sediments, and their subsequent soils, to further cycles of severe and deep weathering and leaching (Taylor, 1983, Thompson *et. al.*, 1983) has produced soils chronically deficient in essential plant nutrients (Williams and Colwell, 1977; Williams and Raupach, 1983). Deficiencies in nitrogen and phosphorus have long been recognised, but deficiencies in micro-nutrients, which are also key problems in many areas (Williams, 1979; Taylor, 1983), were not recognised/identified until the 1920s and 1930s (Williams and Raupach, 1983). Soil fertility problems were further exacerbated by the widespread clearing of native vegetation for agriculture and the exploitative nature of cropping (Thompson *et. al.*, 1983).

1.3.2 Water retaining capacity and subsoil permeability

Low water retaining capacity and high subsoil permeability are perennial problems in sandy soils. The capillary forces acting to absorb water to the surface of soil particles, and retain it in soil pores, are weakened by the relatively large pore diameters that are a direct result of the coarse nature of sand particles. This prevents moisture from being uniformly distributed through the soil profile (Erickson, 1972) because moisture is lost through rapid drainage below the root zone, particularly during winter, when the crop is small and vegetative (Turner and Nicolas, 1987). The free draining nature of sandy soils also makes them highly prone to leaching losses of mobile nutrients and to water deficits in spring when precipitation is declining and soil evaporation is large (Turner and Nicolas, 1987). Consequently, sandy soils

require frequent rain to maintain productivity (Erickson, 1972). Plants with a deeper root morphology are likely to better access moisture in the lower horizons of the soil profile.

1.3.3 Water Repellency

Water repellency is a common feature of sandy soils throughout southern Australia (Bond, 1969) and once established becomes a permanent feature (Wetherby, 1984). Water repellency is a response to the coating of soil particles by hydrophobic organic substances originating either from the presence of organic matter and/or from microbial associations (Bond, 1969). The role of organic matter in promoting water repellency however, diminishes with increasing clay content (Harper and Gilkes, 1994). Hydrophobic substances weaken the attractive forces between the soil particle surface and water molecules, such that the water to water forces are considerably greater, thereby creating a contact angle of wetting of the particle surface greater than zero degrees and inducing repellency (Bond, 1964; DeBano, 1969).

The susceptibility of sandy soils to water repellancy is associated with their low clay content (Bond, 1964, 1969; Harper and Wilkes, 1994), although the degree of repellency *per se* is not closely correlated to clay content (Harper and Wilkes, 1994). In addition the relatively small surface area of sand particles readily facilitates coating by organic substances or fungal hyphae. Their reduced water holding capacity also acts to increase the potential for water repellency. Depth of sand may also exacerbate the effect of water repellency, particularly if roots are unable to tap into heavier textured subsoils for moisture (Bond, 1969).

Water repellency alters the moisture properties of the soil. Infiltration rate can be reduced initially (DeBano, 1969) resulting in increased water run off and exposing the soil to erosion (Dekker and Ritsema, 1994). The patterns of wetting through the soil profile can be irregular and incomplete (Bond, 1964; Wetherby, 1984; Dekker and Ritsema, 1994) causing considerable variation in moisture content (Dekker and Ritsema, 1994). Water movement tends to channel through preferential flow paths (Bond, 1971; Dekker and Ritsema, 1994; Ritsema and Dekker, 1994), formed in places that have the lowest degree of potential

repellency, with the intervening soil being quite dry (Ritsema and Dekker, 1994). The reduced capillary forces in these channels are not strong enough, however, to allow sufficient water movement through the whole profile. The soil's capacity to hold water is diminished, and solutes can be easily lost via leaching (Dekker and Ritsema, 1994).

The poor moisture properties of these soils, a product of water repellency, impact adversely on grain yield through reducing germination potential and emergence (Bond, 1971; Butler *et al.*, 1994). Poor plant establishment will also expose the soil to wind erosion. Management techniques such as clay spreading (Ma'Shum *et al.*, 1989; Ward and Oades, 1993), spraying wetting agents while furrow seeding and the use of press wheels (Crabtree and Gilkes, 1999) have been shown to improve the establishment, growth and yield of crops on water repellent sands. Clay spreading has been particularly successful in the South-East of SA, however the cost and time needed to rehabilitate the soils through this method are important considerations for farmers. The cost especially may prove inhibitory (Ward and Oades, 1993), particularly in lower rainfall, lower yield potential areas dominated by sandy soils.

1.3.4 Cation exchange capacity

Sandy soils have a low cation exchange capacity (CEC). CEC is the ability of a soil to adsorb positively charged cations (e.g., Cu^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, NH_4^+, Al^{3+}, Co^{3+}) to negatively charged soil particle surfaces partly through electrostatic attraction. Typically cations of higher charge are more strongly adsorbed to surfaces. Organic matter and clay, which have highly negatively charged surfaces, facilitate the improvement in CEC of soil particles. Therefore, soils with high organic matter and clay contents have a greater capacity to adsorb cations than soils that are low in both. Consequently, CEC plays a vital role in the storage of nutrient cations and in the prevention of their loss through leaching (McLaren and Cameron, 1994). A feature of sandy soils is the low availability of cationic nutrients such as nitrogen (NH_4^+), copper, zinc and manganese and this is a result, in part, of their low CEC.

1.3.5 Leaching loss of nutrients

Loss of nitrogen (nitrate, NO_3^-) and, to some extent, phosphorus (phosphate, $H_2PO_4^-$ or HPO_4^{2-}) with the movement of water down through the soil profile (leaching), reflects many of the properties of sandy soils already discussed, such as low water holding capacity, high soil permeability and water repellency. The impact of nutrient losses via leaching will, however, depend on the depth of sand to any underlying clay layer. If the movement of water and nutrients do not extend beyond the potential rooting zone then the impact of leaching is diminished.

1.3.6 Root Diseases

Sandy textured soils commonly have a high incidence of root diseases, such as cereal cyst nematode (*Heterodera avenae*), take-all (*Gaeumannomyces graminis*), rhizoctonia (*Rhizoctonia solani*) and root lesion nematode (*Pratylenchus neglectus*); and this may be linked to deficiencies in both macro- and micro-nutrients. Indeed, the association between adequate nutrition and disease tolerance/resistance is well recognized (Graham and Webb, 1991). Adequate nutrition increases the tolerance of a crop to root diseases, through ensuring maximum growth and yield, rather than affecting the expression of resistance directly (Graham, 1983; Wilhelm *et al.*, 1984; Brennan, 1992). Brennan (1992) noted that the form of nitrogen applied could influence the incidence of take-all. This implied that certain forms of nitrogen fertilizer can be toxic to the take-all fungus. In addition, micro-nutrients such as manganese, copper, boron and iron have been implicated in the metabolism of lignin and phenols in plants (Graham, 1983; Graham and Webb, 1991). Both lignin and phenols have a role in disease resistance (Graham and Webb, 1991). The role of zinc in disease resistance/tolerance is a little more complex then the other micro-nutrients (Graham, 1983). Zinc is known to be important for the integrity and stability of biological membranes (Welch *et al.*, 1982). Maintaining the integrity of root cell membranes will prevent leakage of

metabolites and or amino acids that attract diseases (Graham and Webb, 1991) and provide a physical barrier to soil-borne pathogen infection.

Root cell viability can also be undermined by a phenomenon called root cortical death (RCD). Evidence has suggested that RCD, which is a natural process in young cereal plants, is genetically controlled, and that it influences the potential for pathogen infection (for a full review of this topic see Deacon, 1987). Differences in the rate of RCD exist between cereal species (wheat>barley>oats=rye) (Lewis and Deacon, 1982; Deacon, 1987; Liljeroth, 1995), and these differences seem to coincide with susceptibility to root pathogens (Deacon and Mitchell, 1985). Variation in the rate of RCD has also been reported between barley (*Hordeum vulgare* L.) and its wild relatives, and amongst wheat (*Triticum aestivum*) and its wild relatives (Liljeroth, 1995). Genetic control of RCD is demonstrated in triticale, an intercross between wheat and rye, which has been observed to have an intermediate rate of RCD to wheat and rye (Liljeroth, 1995). Environmental factors, such as mineral nutrition, have a direct, localised effect on the rate of RCD. Deficiencies in nitrogen, calcium and to a lesser extent phosphorus and potassium will enhance the rate of RCD (Gillespie and Deacon, 1988; Lascaris and Deacon, 1991). Conversely, adequate nutrition can improve cortical viability, although the effectiveness of the mineral is dependent on the form in which the mineral is available (Gillespie and Deacon, 1988).

1.3.7 Wind erosion

The exposure of sandy soils to wind erosion is influenced not only by the properties of the soil type itself but also through poor crop management of these fragile soils (Hughes and Wetherby, 1992; Butler *et al.*, 1994). Poor crop management and the properties of sandy soils can impact on the development of an adequate crop cover that would otherwise stabilize the soil, preventing erosion.

Wind erosion particularly affects the top 10 cm of the soil profile where soil nutrients are concentrated. The removal of nutrients from sandy soils, which are already highly infertile, is

probably the most devastating consequence of erosion. Fine soil materials of less than 90 μm (clays and silts) are also removed from the top soil by a process called winnowing, further reducing the low CEC of sandy soils (Leys and McTainsh, 1994). Leys and Heinjus (1991) and Leys *et al.* (1993) showed that eroded material can contain up to four times the concentration of nitrogen, phosphorus and organic carbon than the top soil from which it was derived. This is because the concentration of nutrients is higher in material smaller than 90 μm than in larger particles (Leys and McTainsh, 1994). Further, the water holding capacity of the soil is diminished to levels that can be half that of undisturbed soils under native vegetation (Leys and McTainsh, 1994). All these factors will reduce the productivity of the land subjected to erosion.

1.3.8 Sand Blasting

'Sand blasting' is a phenomenon that causes physical damage to plant tissue. The plant is 'blasted' by sand particles carried along by wind erosion. Ultimately, the damage caused to plant tissue will adversely impact on growth and yield. Crops grown on sandy soils are often vulnerable to 'sand blasting'. Early sowing into dry sandy soil reduces the emergence potential of the crop, which can expose bare soil to wind erosion. Plants that do emerge are generally quite weak and can subsequently be 'sand blasted' if high winds occur and dry conditions continue (Hughes and Wetherby, 1992). Cereal rye, which is better adapted to sandy soils than wheat and barley, exhibits a capacity to tolerate 'sand blasting'.

1.3.9 Favourable Features

Much of the focus on the characteristics of sandy soils has been on their limitations/deficiencies for crop production. However, favourable aspects for crop production on these soils do exist. French and Ewing (1989) showed the benefit of light textured soils on yield potential in low rainfall regions, particularly during moisture stress. The relatively larger mean pore size of sandy soils provides good aeration, favouring root development, high

infiltration rates, reduced evaporative loss and reduced water erosion potential. The free-draining nature of these soil types also reduces the potential for waterlogging, particularly in high rainfall areas, and during periods of increased rainfall incidence. The durability of the surface of sandy soils means they can, to a certain extent, withstand the abuses of tillage and farming (Erikson, 1972).

1.4 Root morphology and the availability of moisture and nutrients, and genetic variability for root traits

The inherent infertility, and poor moisture and nutrient relations, of sandy soils outlined in section 1.3, and water-limiting conditions, suggest that root morphology will be a crucial component in improving the adaptation of cereals on sandy soils. An associated aspect of a well-developed root system is as a stabilizing structure against erosion and sand 'blasting'. To understand how the root system can potentially improve adaptation on sandy soils, it is essential to discuss root morphology in general terms, and how specific root parameters and genetic variation for root traits influence moisture and nutrient availability and uptake.

1.4.1 Root Morphology

Barley, as with other cereals, has a fibrous network of roots that extend vertically and horizontally in the soil matrix. Two types of root system exist (Troughton, 1962; Klepper *et. al.*, 1983); seminal roots and nodal (adventitious) roots. The seminal roots are the first initiated in the germinating seed and originate from the embryo (Troughton, 1962; Barley, 1970; Glinski and Lipiec, 1990), with the final seminal root appearing shortly after coleoptile emergence (Richards and Passioura, 1981a; Klepper *et. al.*, 1983). Cultivated cereals may have three to several seminal roots (Robertson et. al., 1979; Richards and Passioura, 1981a,b;

Grando and Ceccarelli, 1995) and they tend to grow straight down through the soil profile (Klepper *et. al.*, 1983). Early work by Sallans (1942), reviewed in Troughton (1962), suggested that wheat plants that produced the greatest number of seminal roots, also produced the greatest grain yield. This highlighted potential genetic variability for seminal root number. But as with many other aspects of plant development, environment has a major effect. Seminal roots seem to be quite sensitive to adverse environmental conditions, particularly during the early stages of development (Troughton, 1962). Given that the number of seminal root axes is determined by the time seedlings have emerged, environmental conditions prior to emergence are critical to seminal root morphology (Richards and Passioura, 1981a). Drought during seedling establishment inhibits seminal root number, however this is unlikely to occur in practice, since germination and establishment occurs during cool, wet conditions. Other factors of importance to seminal root number and diameter are seed size and drought during grain filling (Richards and Passioura, 1981a). Richards and Passioura (1981a) also noted that sowing depth and soil type (two sandy textured soils, and swelling and non-swelling clay were compared) had no significant effect on seminal root number.

The fact that seminal roots are responsible for the initial absorption of moisture and nutrients (Troughton, 1962; Grando and Ceccarelli, 1995), suggests that the number and length of seminal roots may play a key role in establishment and early growth on sandy soils. A vigourous and deep root system is essential for adequate growth, where water holding capacity is low and nutrients have reduced availability due to adsorption to soil particles or are rapidly lost from the soil profile via leaching. Mattsson *et al.* (1993) concur that the contribution of seminal roots to nutrient uptake in early growth is critical. They determined that greater than 50% of the total nitrate taken up was attributed to the seminal roots during the vegetative phase of growth, declining to 20% just after anthesis and to less than 5% during grain filling.

Nodal roots originate from the basal nodes of the stem (main and tillers), becoming prominent at the onset of tillering (Barley, 1970). Nodal roots grow horizontally for a few centimeters before turning downwards, allowing the plant to explore progressively larger areas of the soil profile (Klepper *et. al.*, 1983). As with the seminal roots, nodal roots are sensitive to moisture stress which may inhibit or prevent their development and growth (Gregory, 1987; Gorny, 1992), depending on the timing of the stress.

Roots arising from primordia that develop in the pericycle of the root are called laterals and they account for much of the length of the system and much of the uptake of moisture and nutrients (Barley, 1970). Those that develop from the main axes (seminal or nodal) are called first order laterals. In turn, these roots can produce laterals (secondary) and so on.

As roots extend into the soil, a zone of elongation forms behind the root apex. Towards the proximal end of the zone of elongation, small protuberances (root hairs) form out of the epidermal cells. Root hairs are thin walled and usually short lived because they can be readily decomposed by micro-organisms. However, root hairs have been observed to persist if they become thicker and lignified, although they may not necessarily continue to absorb ions (Barley, 1970). The development of root hairs increases the effective surface area of the plant's root system in contact with the soil. This is critical for improving accessibility to moisture, particularly upon soil drying, because they are able to enter narrow voids where water retreats (Barley, 1970). Root hairs also facilitate enhanced nutrient uptake, especially those of low mobility, such as phosphorus (Barley, 1970; Glinski and Lipiec, 1990).

1.4.2 Root morphology and moisture and nutrient uptake

1.4.2.1 Root parameters and moisture uptake

Water absorption by roots is a function of factors that affect the resistance to water flow in both the soil and in the plant (Glinski and Lipiec, 1990). On the basis of this, 'plants with finitely dense roots' will be able to take up water if the resistance to flow is overcome; the rate of which is governed by transpirational demand (Van Noordwijk and De Willigen, 1991).

Evidence provided by Ehlers *et al.* (1981) supports the theory that the major resistance to flow is in the plant and more specifically in the roots, and that the role of the soil in controlling the resistance to water absorption has been overestimated. However as the soil dried out to water potentials approaching –1.5 MPa, soil resistance indirectly increase plant resistance by restricting root growth and reducing root density (Ehlers *et al.*, 1980). Poor hydraulic continuity between roots and the soil, resulting from roots growing in pores much larger than themselves (Passioura, 1985), or when the soil dries out (Ehlers *et al.*, 1981), will increase the resistance to moisture flow and further reduce the ability to extract water (Herkelrath *et al.*, 1977). This resistance to moisture flow may be a problem in sandy soils where the contact between roots and the soil is reduced due to the large mean pore size in the profile and the small surface area of soil aggregates.

Water movement in roots flows via the radial (cortex to xylem) and axial (xylem to stem) pathways (Hamblin, 1985; Glinski and Lipiec, 1990). Therefore resistance to water flow can occur in either one or both pathways. Axial resistance is greatest in narrow xylem vessels (eg roots of graminaceous plants) and will increase with root age and depth (Hamblin, 1985) whereas radial resistance will result from osmotic barriers across the cortex cell walls (Glinski and Lipiec, 1990). Resistance to flow in the axial direction has been suggested as an advantageous trait for cereals especially when grown on stored water in environments subjected to frequent periods of moisture stress (Passioura, 1972). Passioura's (1972) theory was that plants with seminal roots of high hydraulic resistance would conserve water during early growth thereby allowing adequate moisture to be available for the critical period of grain filling. Passioura (1972) observed that single-rooted plants exhibited higher axial resistance and used substantially less water then normal plants, but they produced significantly higher grain yields. However he speculated that resistance was primarily a function of xylem diameter. Richards and Passioura (1981a), using a range of wheat genotypes, found that xylem vessel diameter varied considerably between and within genotypes. In investigating 1000 accessions of modern and primitive wheats, Richards and

Passioura (1981b) noted that while no genotype had fewer seminal roots than the average, there was evidence of genetic variation for vessel diameter among landraces. The lack of significant genotype by environment interaction for xylem diameter suggested selection for this trait could be undertaken in controlled environment conditions (Richards and Passioura, 1981a).

Water absorption by roots is related to the distribution of the root system in the soil and the depth of water uptake related to rooting depth (McGowan, 1974). In turn, root morphology is likely to be influenced by the moisture status of the soil. Richner *et al.* (1997) showed that a highly branched root system was critical to ensure adequate water supply to maize genotypes. In general, the greatest density of roots is confined to the top 10 cm of the soil profile, with density declining with soil depth (Barley, 1970; Gregory *et al.*, 1978a; Proffitt *et al.*, 1985). This may be attributed to root growth being weighted in favour of roots in the upper parts of the profile rather than to the current root mass (Adiku *et al.*, 1996). Under conditions of adequate moisture the root system will usually be shallower, but highly branched (Proffitt *et al.*, 1985). In contrast, moderate moisture stress, brought about by drought or rapid drainage, as with sandy soils, will enhance root growth by inducing plants to develop longer roots and therefore reach a greater rooting depth (Gregory *et al.*, 1978a; Eghball and Maranville, 1983; Proffitt *et al.*, 1985). Consequently there is a reduced emphasis on branching throughout the soil profile, such that the total length of roots in specific soil intervals (root length density) decreases (Gregory *et al.*, 1978a). Steingrobe *et al.* (2001) similarly found in sandy soils that net root length was lower, but that total root production was greater, than in loamier soil types. This increase in root production was also accompanied by an increase in the rate of root mortality, indicating a higher turnover of roots in the sandy soil. Indeed, Steingrobe *et al.* (2001) were able to show that the turnover rate of roots was correlated with sand content. The redistribution in the pattern of root growth suggests that plants 'may provide their own adaptation mechanism to moderate stress' that allows them to explore the soil for more favourable moisture conditions (Eghball and Maranville, 1983). This mechanism may allow

plants to maximise water uptake at depth in the soil profile, especially when the soil is subjected to drying out of the upper soil layers, to increase early growth and maintain yield potential (Turner and Nicolas, 1987). A reduced emphasis on root mass in favour of a greater rooting depth may also improve the water use efficiency (WUE) of the plant (Hamblin and Tennant, 1987; Richards, 1991). Proffitt *et al.* (1985) working with wheat however, highlighted that extracting moisture from depth was less efficient than extraction from the full soil profile. Water movement from depth will encounter greater root flow resistance (Hamblin, 1985). Severe stress, on the other hand, will cause considerable damage to roots, reducing their growth, and inhibiting water uptake (Ehlers *et al.*, 1980; Eghball and Maranville, 1983).

1.4.2.2 Root length density and moisture uptake

Root length density (RLD) is widely used to describe the density of roots in the soil profile; either as L_v (length of root per unit volume of soil, cm cm^{-3}) or L_a (length of root per unit area of soil, cm cm^{-2}). Both measures can be used to assess overall density in the soil, whereas L_v can also be used to assess density in discrete zones of the soil profile. Since RLD is used to define the distribution of the root system of a plant, it has been used as the 'sink term' in water uptake models. Many of these models are critically reviewed in Molz (1981), who determined that several were 'conceptually wrong' because they assumed that the dominant resistances to water flow resided in the soil. Some authors have been questioned the relationship between water uptake and RLD (Gregory *et al.*, 1978b; Hamblin and Tennant, 1987; Ehlers *et al.*, 1991), while others (Willatt and Olsson, 1982) have shown water uptake to be directly related to RLD. Gregory *et al.* (1978b) established no clear correlation between the proportion of roots in a particular layer and water extraction from that layer. This was borne out by the fact that as the topsoil dried out, the predominant proportion of water was extracted from parts of the soil profile (below 100cm) where root density was low. Ehlers *et al.* (1980) obtained similar results. Gregory *et al.* (1978b) illustrated the importance of deeper

roots, particularly during dry periods, with data that showed while deeper roots only constituted 3% of the total root weight, they accounted for 20% of moisture uptake. Follow up work by Hamblin and Tennant (1987) and Ehlers *et al.* (1991) determined that water uptake was dependent more on maximum rooting depth than on rooting density.

A number of reasons for caution when using RLD to determine water uptake exist. (1) RLD models assume an even distribution of roots with each soil volume; an assumption Hamblin (1985) questions since many field data show the predominance of non-uniform rooting patterns related to the moisture profile of the soil. (2) Field data show that 'specific' water uptake (uptake rate per unit length of root) is higher with low RLD to compensate for lower root density, even though models suggest a slight increase with higher RLD (Ehlers *et al.*, 1991). (3) There is an assumption that all roots are equally effective in taking up water (Gregory *et al.*, 1978b). Gregory *et al.* (1978b) lists a series of authors whose opinions differ in terms of the fraction of total root length most effective in water extraction. Gregory *et al.* (1978b) attributed these differences to the fact that water uptake can vary considerably between and along roots due to resistance to flow that reduce the effective root length. Resistance can either be within the root (Ehlers et al, 1991) and/or through root-soil contact resistance (Herkelrath *et al.*, 1977) resulting from incomplete contact between the root and the soil, or caused by suberization (thickening of the cortex cell layer). The exposure of different parts of the root system to different soil water potentials, even at the same depth of soil (Hamblin, 1985) will affect the uptake of moisture. (4) As discussed above, under moisture stress, the uptake of water is shifted to deeper roots that are not so well branched (low RLD) as topsoil roots. (5) RLD does not take into account the role of root hairs in absorbing moisture, since root hair measurements are difficult.

1.4.2.3 Root parameters and nutrient uptake

In many respects, the availability of nutrients is defined in similar terms to that of moisture availability (Van Noordwijk and De Willigen, 1991). Both the physical and chemical

properties of the soil, and the configuration and physiological activity (exudates) of the root system have a role in determining the degree of resistance to nutrient transfer (Barley, 1970; Glinski and Lipiec, 1990). The chemical nature, concentration, and location of nutrients in the soil will also contribute to availability (Glinski and Lipiec, 1990). Indeed nutrient supply or availability *per se* can have a significant influence on the configuration of the root system (Barley, 1970).

Drew *et al.* (1973), Drew (1975), and Gleiser and Krutzfeldt (1983) have showed that considerable modifications in the root system occur in response to increases in nitrogen, phosphorus and potassium. While it has been suggested that seminal root extension is little affected by the concentration of a single nutrient ion, lateral root growth can be affected (Drew *et al.*, 1973; Drew, 1975). The proliferation of lateral root growth proved to be localised to those regions of the root system that received higher amounts of nitrogen and phosphorus (Drew *et al.*, 1973; Gleiser and Krutzfeldt, 1983). In contrast, regions of the soil profile deficient in potassium showed lateral root growth equivalent to conditions where the whole root system received an ample supply of potassium (Drew, 1975). A critical observation of Drew (1975) was that in addition to an increase in the growth of laterals, there was a compensatory increase in uptake rate such that plants receiving a localised supply of nutrient only showed a marginal reduction in shoot growth. In the same study he noted that root proliferation was apparent only when all nutrients were present; omission of one would inhibit the growth of laterals.

Under limiting soil nitrogen, so long as it is not severe, it has been shown that a proliferation in root growth occurs down the soil profile (Comfort *et al.*, 1988; Eghball and Maranville, 1993). Rooting depth is especially important for sandy soils where nitrogen deficiency can also be related to the rapid leaching of nitrate that results in a deeper distribution of nitrate or a complete loss from the system (Andren *et al.*, 1993; Van Noordwijk and DeWilligen, 1991). According to Comfort *et al.* (1988), nitrogen management has particular implications for low rainfall areas. In low rainfall areas, depth of soil water use and root growth were shown to be

influenced by nitrogen rate for some genotypes of wheat. Drew *et al.* (1973) and Drew (1975) however, suggested that the rapid extension of the seminal roots under nitrogen deficiency was only temporary. Their explanation for this was that seminal root growth was due to shoot/root growth interactions. During the early periods of establishment and growth the plant is reliant on the seed reserves for growth and, under nitrogen stress, there is a preferential shift in assimilate allocation to the roots over the shoots. Upon depletion of the seed reserves, the rate of extension declined because the reduced photosynthetic ability of the nitrogen deficient leaves was unable to adequately supply assimilate to the roots.

1.4.2.4 Root parameters and uptake of low mobility nutrients

Root growth that increases the root-soil contact is essential to maximise the potential availability of nutrients that have a characteristically low mobility in the soil, such as phosphorus (Schjorring and Nelson, 1987). Drew *et al.* (1973) showed that a localised supply of phosphorus stimulated a proliferation in lateral root growth. This response allowed a compensatory increase in phosphorus accumulation to overcome deficiencies in other regions of the soil profile. Experiments by Bhat and Nye (1974a) with Brassica ssp showed laterals did not deplete the soil of phosphorus despite considerable phosphorus accumulation. Under phosphorus deficiency they noted that there was a continuous decline in phosphorus concentration toward the root surface, within the root hair zone. This suggested that root hairs were important contributors to the uptake of phosphorus in rape. Bhat and Nye (1974b) compared onion (no root hair) with rape and found that onion had a narrower depletion zone and consequently reduced uptake of phosphorus. Other authors have also shown the importance of root hairs for the uptake of phosphorus (Itoh and Barber, 1982; Schubert and Mengel, 1988; Gahoonia and Nielson, 1997; Gahoonia *et al.*, 1997). In a similar study, Fohse *et al.* (1991) was unable to find a correlation between phosphorus influx and root hairs. The inclusion of root hairs in the prediction models, however, allowed differences in phosphorus

influx to be accounted for. In addition, their calculations showed that in low phosphorus soils, the contribution to phosphorus uptake by root hairs was up to 90% of total uptake.

1.4.3 Genetic variation for root morphology traits

An understanding of the nature and extent of genetic variation for root system parameters is essential for genetic improvement in crops adapted to diverse environments. The ability to cope with the temporal and spatial variability that exists throughout a soil profile associated with uncertain soil water and nutrient status, is of particular importance in water limiting environments (O'Toole and Bland, 1987).

Listed below (Table 1.1) are of examples of genetic variation for root parameters in three cereal species. Wild relatives, landraces and accessions of modern cereals have proved to be a rich source of genetic variation in root morphology, that have, in many instances, given them better adaptation to abiotic stresses (*e.g.* water limiting conditions) than modern cultivated genotypes (Brown *et al.*, 1987; Cooper *et al.*, 1987). Further examples, including other plant species, are reviewed in O'Toole and Bland (1987)

Table 1.1: Examples of genetic variation for root parameters.

Trait	Root Parameters	Range in trait	Species	References
Drought tolerance/ WUE	Xylem vessel diameter	38-78 μm	Wheat	Richards and Passioura (1981b)
Genetic variability	Angle between seminals Number of first order laterals Length of first order laterals Maximum depth	36-124° 3.0-4.7 53.7-159 cm 34-69 cm	Wheat	O'Brien (1979)
Drought tolerance	Root number	2.5-6.5	Wheat	Robertson *et al.* (1979)
Drought tolerance	Seminal root number Maximum seminal length	3.3-5.9 69.8-125.3 mm	*H. spontaneum*/ Landrace barley	Grando and Ceccarelli (1995)
Yield stability	Root volume	25-40 sums of intersects	Barley & Oats	Schwarz *et al.* (1991)
Genetic variability	Root volume Root length Root weight	14.3-35 μl 5.3-15.1 cm 11.1-24.9 mg	Oats	Murphy *et al.* (1982)
Phosphorus uptake	Root hairs-length -total length	0.48-1.27 mm 12-48 mm/mm root	Wheat	Gahoonia (1997) Gahoonia *et al.* (1997)
Water use efficiency	Root weight Root length or depth	Landrace v cultivated	Barley	Brown *et al.* (1987) Cooper *et al.* (1987)

1.5 Traits potentially associated with improved performance on sand

1.5.1 Early Vigour

Improvements in grain yield potential through conventional plant breeding have been primarily a result of increased harvest index (HI). However, improvement via selection for HI is believed to be reaching its upper limit (Richards, 2000). Consequently other avenues for improving grain yield, and the stability of yield, need to be addressed. This includes selecting traits that may improve water uptake, use and water use efficiency (WUE) in water limiting conditions (Passioura, 1977) and increased crop photosynthesis (net carbon gain/unit ground/per unit time)(Richards, 2000). Richards (1991 & 2000) and Bort *et al.* (1998) reported that both features could be improved by a vigourous early growth and canopy development phase (early vigour) that increased the above-ground biomass. Traits that have been reported to contribute to increased early vigour and growth are listed in Table 1.2.

While it is clear that genetic factors contribute significantly to improved early vigour, appropriate management practices also play a key role in terms of early vigour. Higher seeding rates, nitrogen management, controlled seeding depth (problematic on sandy soils) and larger average grain size, through screening out small seed prior to sowing, may all contribute to improved early vigour (Richards *et al.*, 2002). Early vigour has been measured as dry matter production (Brown *et al.*, 1987; Turner and Nicolas, 1987; Whan *et al.*, 1991) and visual scores related to crop density (Ceccarelli, 1987; Acevedo *et al.*, 1991; Annicchiarico and Pecetti, 1995).

Richards (1987 & 1991) proposed that increasing the rate of leaf area development, and thereby increasing the rate of canopy closure and ground cover, would increase the amount of light interception by the crop and reduce the amount of radiant energy reaching the soil surface. WUE would be improved because transpiration efficiency would be increased and soil evaporation reduced. This is particularly important for Mediterranean environments where crop growth between sowing and stem elongation is characterized by high soil evaporation, as a proportion of transpiration (Richards *et al.*, 2002). Crop photosynthesis is also advantaged because full light interception (leaf area index (LAI)=3.5) is achieved more rapidly (Richards, 2000). This is particularly so for crops of high specific leaf area (SLA) during early growth because they exhibit a higher net assimilation rate for a given leaf weight (*e.g.* barley v wheat) (Richards, 2000). In addition, crops with a higher SLA also display a higher leaf area ratio (LAR, ratio of leaf area and total plant weight), because LAR is a product of SLA and leaf weight ratio (ratio of leaf weight and total plant weight). Furthermore, Poorter and Remkes (1990) were able to demonstrate that high SLA was the major contributor to improved relative growth rate (RGR); a function of NAR and LAR. It is likely that early vigour is correlated with RGR, via LAR and SLA (Table 1.2).

Table 1.2: Traits associated with increased early vigour (adapted from Richards, 1991 & 2000).

Trait	Additional References
Germination rate (fast)	Peterson *et al.*, 1989 Lopez-Castaneda *et al.*, 1996
Seed size (large)	Peterson *et al.*, 1989 Lopez-Castaneda *et al.*, 1996
Embryo size (large)	Ogilvie and Kaltsikes, 1977 Djisbar and Gradner, 1989 Lopez-Castaneda *et al.*, 1996 Pandey *et al.*, 1994
Coleoptile length (long)	Gul and Allan, 1976 Gorny and Patyna, 1981 Redona and Mackill, 1996 Rebetzke and Richards, 1996 Rebetzke *et al.*, 1999
Emergence rate (time)	
Leaf habit (erect v prostrate)	Acevedo *et al.*, 1991 Richards *et al.*, 2001
Leaf breadth (wider) Specific leaf area (higher) Leaf appearance rate (fast) Leaf area ratio (higher) Leaf expansion rate (faster) Relative leaf expansion rate (faster) Leaf area of 1st leaf (high)	Lopez-Castaneda *et al.*, 1995, 1996 Richards, 1991 & 2000 Rebetzke and Richards, 1999 Liang and Richards, 1994
Coleoptile tiller appearance (earlier)	Liang and Richards, 1994
Floral initiation (early)	
Crown depth (shallow)	Richards (unpublished; in Richards, 2000)
Leaf area index (high)	El Hafid *et al.*, 1998
Seed nutrient reserves (large)	Bolland and Baker, 1989 (phosphorus) Longnecker *et al.*, 1991(Manganese) Rengel and Graham, 1995a,b (Zinc) Genc *et al.*, 2000 (Zinc)

Upon canopy closure, however, high SLA becomes a hindrance because continued leaf area expansion will not increase photosynthesis capacity, and net assimilation rate (NAR) will be reduced. Richards (2000) proposed that improved photosynthesis and light interception following canopy closure would be reliant on canopy architecture (leaf posture), the maintenance of green leaf area for a longer time and rate of photosynthesis. In addition, an increased partitioning of carbon and nitrogen to reproductive meristems, to establish a higher number of fertile florets with a potential for a large grain size would be necessary as a sink for the products of photosynthesis. Accordingly, good early vigour could be associated with improved performance on sandy soils through improving water use efficiency and crop photosynthesis. The associated increase in ground cover would also increase competitiveness

with weeds, and therefore reduce herbicide usage, and reduce wind and water erosion and sandblasting.

Barley and wheat, which differ in their 'adaptation' to sandy soils, have also been demonstrated to differ in terms of leaf area growth and above-ground biomass production (Lopez-Castaneda *et al.*, 1995). Barley was shown to have 40% more dry matter and two times greater leaf area than wheat by the two leaf stage due to earlier emergence, a generally larger embryo and greater SLA (Lopez-Castaneda *et al.*, 1995). Accordingly, barley's better 'adaptation' on sandy soils could potentially be due, in part, to increased rates of dry matter production and leaf area development.

Several reports showed that improvements in dry matter production at anthesis and grain yield in low rainfall environments were associated with increases in early vigour (Turner and Nicolas, 1987; Brown *et al.*, 1987; Ceccarelli, 1987; Acevedo *et al.*, 1991; Whan *et al.*, 1991; Annicchiarico and Pecetti, 1995; El Hafid *et al.*, 1998; Simane *et al.*, 1998). There is however a potential penalty for increased transpiration associated with greater early vigour; that is, the exhaustion of soil water reserves by anthesis which can result in 'haying off' and pinched grains of poor quality (Richards, 1991; El Hafid *et al.*, 1998). Even so, some evidence has been published that suggests an increase in dry matter production does not always result in higher pre-anthesis water use (Doyle and Fischer, 1979; Cooper *et al.*, 1987; Shephard *et al.*, 1987). In addition, Turner and Nicolas (1987) concluded that improved early vigour on deep sandy soils enables greater root development, so yields are not restricted by water limitations post-anthesis.

1.5.1.1 Genetic variation for early vigour

Genetic variation for early vigour has been identified in modern introductions and cultivars, landraces and wild types of spring wheats (Whan et al, 1991; Richards, 1992 a,b; Rebetzke and Richards, 1996, 1999), durum wheats (Annicchiarico and Pecetti, 1995; El Hafid et al, 1998) and barley (Ceccarelli, 1987; Cooper et al, 1987; Acevedo *et al.*, 1991;

Hadjichristodoulou, 1993). Leaf breadth in particular has been found to have a high heritability and a low genotype by environment interaction (Rebetzke and Richards, 1999), making it a prime candidate trait for selection to improve leaf area *per se*, and early vigour.

1.5.2 Contribution of non-structural carbohydrate to grain filling

Increased dry matter production prior to anthesis associated with early vigour, may overcome, to an extent, the impact of moisture stress post anthesis, because assimilate (non-structural carbohydrate) stored in the stem pre-anthesis may contribute to grain filling (Richards, 1991). Non-structural carbohydrates consist of ethanol soluble, low molecular weight, mono-, di- and some oligo-saccharides (sucrose, glucose and fructose) and water soluble, high molecular weight fractions (fructans). Fructans are storage carbohydrates that can vary in structure and size based on the degree of polymerisation (DP) of fructan units, and are the main component of assimilates in the stem pre-anthesis (Virgona and Barlow, 1991). Fructans are initially synthesised from sucrose by fructosyltransferases and there tends to be a significant correlation between fructan accumulation and high sucrose concentration in plant cells (Vijn and Smeekens, 1999). Pre-anthesis assimilate in the stem can be a substantial source of carbohydrate for grain filling (Bonnett and Incoll, 1992). Its contribution can potentially account for between 11% and 44% of the increase in grain weight in cereals under favourable conditions, and the proportion can be even greater with increased drought severity (Austin *et al.*, 1980; Richards and Townley-Smith, 1987; Blum *et al.*, 1994; Blum, 1998; Gebbing *et al.*, 1999). Virgona and Barlow (1991) identified that drought conditions resulted in a shift in the composition of assimilate in the stem from fructans to ethanol soluble carbohydrates. This adjustment, according to Virgona and Barlow (1991), occurs because fructan depolymerisation seems to be sensitive to moisture stress. The products of depolymerisation (*e.g.* sucrose, glucose and fructose) can either be remobilised to the developing grains and/or partly contribute to osmotic adjustment to maintain cell turgor during moisture stress (Virgona and Barlow, 1991). The utilization of pre-anthesis reserves is also dependent on the

mobilization efficiency of assimilate from vegetative plant parts to the filling grain (Gebbing *et al.*, 1999). While this may be the case, early heading (flowering), in combination with early vigour, is an important phenological trait in moisture limiting environments. Early heading can assist in the avoidance of drought and heat stress and can increase the length of the grain filling period (Ceccarelli, 1987; Acevedo *et al.*, 1991; Hadjichristodoulou, 1993).

1.5.3 Inter-relationships of traits associated with establishment and early vigour

Limitations on the growth and yield of cereals on sandy soils are also related to poor emergence and establishment associated with uneven seeding depth, poor inherent fertility, water repellency and poor water-retaining properties of the soil. Deep sowing, a common occurrence on sandy soils in SA, can increase the time to seedling emergence, and reduce the length of leaves due to low relative growth rates (Kirby, 1993) resulting in reduced canopy development (leaf area). In overcoming these problems, traits such as coleoptile length, seed (embryo) weight, endosperm weight and seed nutrient reserves may provide avenues to improve emergence and establishment on sandy soils. A long coleoptile will improve emergence (Whan, 1976; Bacaltchuk and Ulrich, 1990) and establishment (Hughes and Mitchel, 1987; Radford, 1987; Radford and Wildermuth, 1987; Sharma, 1990) especially when seed is sown at depth. A good example of the effect of coleoptile length on emergence was demonstrated with semi-dwarf wheats, because plant height is related to coleoptile length (Whan, 1976; Agrawal *et al*, 1977; Ceccarelli *et al*, 1980; Bacaltchuk and Ulrich, 1990). The reduced coleoptile length was associated with the major dwarfing genes (Rht 1 & 2). Semi-dwarf wheats with these genes have been shown to exhibit slower leaf growth, and delayed and poor emergence (Richards, 1992b; Rebetzke and Richards, 1996; Schillinger *et al.*, 1998). Consequently, semi-dwarf wheats display quite poor early vigour. These features have particular importance when semi-dwarf wheats are sown too deep, into stubble and when pre-emergent herbicides are used (Rebetzke *et al.*, 1999). Seed and embryo weight, which are correlated (Richards and Lukacs, 2002), and endosperm weight can also influence coleoptile

length (Djisbar and Gardner, 1989; Ceccarelli and Pegiati, 1980; Cornish and Hindmarsh, 1988) and improve the rate of emergence and establishment (Wood *et al.*, 1977).

During the early establishment phase, the supply of mineral nutrients can come partly from seed reserves and partly from the soil (Genc *et al.*, 2000), therefore on sandy soils, which are characteristically low in fertility, large seed reserves will be especially important to improve emergence and early growth. The importance of seed reserves on early growth, under nutrient deficient conditions, has been clearly demonstrated (Bolland and Baker, 1989; Longnecker *et al.*, 1991; Rengel and Graham, 1995a,b; Genc *et al.*, 2000). Seed nutrient reserves are only temporary, such that the rapid development of roots into the soil profile is essential to find and take up nutrients to maintain adequate plant growth.

1.6 Application of molecular marker technology in plant breeding

Conventional breeding is based on early generation selection for phenotype, with selection for yield and quality occurring in later generation where seed quantities are adequate to cover quality assessment and ensure enough seed for yield evaluation trials. Genetic progress for morphological, physiological and agronomic traits considered important to abiotic stress tolerance and improving grain yield potential in low yielding environments is, however, hindered. This is because the genetic variance for key traits is low, and the environment can have a profound impact on their expression. As a result the heritability (h^2) of important traits is diminished, and potential new lines with superior adaptation may go unnoticed. Genetic progress for important traits may also be stalled due to the measurement of traits being time consuming, difficult, laden with error, and expensive.

One solution to this conundrum has been the advent of molecular marker technology. Molecular marker technology is developing at a rapid rate and is having a significant impact

on all areas of modern biology (Jones *et al.*, 1997), including cereal breeding. The potential and realised application of molecular markers in cereal breeding is extensive (Langridge and Paul, 1993; Langridge *et al.*, 1996; Jefferies *et al.*, 1997; Jones *et al.*, 1997). The advantage is that early generation material can be screened with markers of known linkage to a trait of interest for target environments without the need for full-scale sampling, which is often destructive. Molecular marker technology also improves breeding efficiency (marker assisted selection), because initial selection is based on genotype rather than on the phenotypic expression of traits, which is confounded by environmental factors and low genetic variance, common in low yielding environments. However, extensive field evaluation is essential to establish genotype by environment effects and ensure trait expression is of benefit to adaptation in the target evironment. Sandy soils are a prime example of a low yielding environment, where yield and agronomic trials are subverted by low genetic variance and high error variance (environmental variability), and therefore molecular marker technology is likely to have a significant role in developing superior varieties.

1.6.1 Molecular Markers and their identification

Molecular markers are neutral sites of variation at the DNA sequence level (Jones *et al.*, 1997). They are numerous, are not expressed in the phenotype, do not alter the physiology of the organism and may be nothing more than a single nucleotide difference in a gene or a piece of repetitive DNA (Jones *et al.*, 1997). The ability to access and use markers has resulted from the development of tools such as restriction enzymes, electrophoretic separation of DNA fragments, southern hybidisation, the polymerase chain reaction (PCR) and labelled probes (Jones *et al.*, 1997). Molecular markers are further classed by the techniques used to identify the neutral site of variation. Restriction fragment length polymorphisms (RFLPs) have, until recently, been the most extensively used system (Beckmann and Stoller, 1983). However, PCR based methods such as amplified fragment length polymorphisms (AFLPs, Vos *et al.*, 1995), and more particularly simple sequence repeats (SSRs, microsatellites) (Roder *et al.*,

1995) have become the methods of choice for developing molecular markers. Another PCR system is randomly amplified polymorphic DNA (RAPDs, Williams *et al.*, 1990). Table 1.3 lists the advantages and disadvantages of these four techniques. SSRs offer the highest level of polymorphism, but it is well established that AFLPs have a greater level of efficiency due to the ability to simultaneously analyse a large number of loci (multiplex ratio) (Powell *et al.*, 1996; Russell *et al.*, 1997). In a study directly comparing the marker systems listed in table 1.3 in determining the levels of genetic relationship between barley accessions, Russell *et al.* (1997) found that 70% of the pairwise comparisons between RFLPs and AFLPs ranked accessions for genetic relatedness in the same order. In contrast, SSRs only ranked 50% of the accessions in the same order as RFLPs and AFLPs. This result led Russell *et al.* (1997) to determine that SSRs, despite their high level of polymorphism, were ineffectual for assessing genetic relationships among cultivars. RAPDs were the least comparable with the other marker systems.

Other marker systems include those derived from RFLPs and AFLPs such as sequence tagged sites (Olson *et al.*, 1989) and cleaved amplified polymorphic sequence markers, which are more rapid and efficient PCR detection methods. In addition there are variants of the RAPDs technique that have been developed (arbitrary primed PCR, DNA amplification fingerprinting and sequence characterised amplified regions).

1.6.1.1 Restriction fragment length polymorphisms

The early development of genetic maps of cultivated crop species relied almost entirely on screening populations with restriction fragment length polymorphisms (RFLPs). As molecular marker technology has advanced, the use of RFLPs in creating high-density genetic maps has, to a large extent, become obsolete. Even so, selective use of RFLPs, of known location on the genome, can provide a useful mechanism to fill regions not sufficiently saturated with other markers systems for appropriate quantitative trait loci (QTL) analysis.

RFLPs are based on the generation of DNA fragments via the action of restriction enzymes. Individual restriction enzymes recognize specific and unique nucleotide sequences, and cleave the DNA at these sites by splicing between amino acids. Polymorphisms for RFLP markers are distinguished by differences in the size of the fragments, between genotypes, generated by the action of these restriction enzymes. Variation in fragment sizes arise from mutations that result in the addition/deletion of amino acids altering the length of the DNA sequence, or through modifying sequences to create a new cleavage site or remove a cleavage site (Jones *et al.*, 1997). Individuals heterozygous (*e.g.* F_1's) for a specific RFLP marker display two fragments corresponding to each parent in the cross, so long as the parents are polymorphic for that marker.

Table 1.3: Advantages and disadvantages of the most common molecular marker systems.

	RFLPs	RAPDs	AFLPs	SSRs
Advantages	-Co-dominant -Reliable	-Simpler and less expensive then RFLPs (Jones *et al.*, 1997) -No need for radioactive probes (Jones *et al.*, 1997)	-High multiplex ratio (Powell *et al.*, 1996, Russell *et al.*, 1997) -Reveals high degree of polymorphism -Well dispersed (fill gaps in established maps without interrupting RFLP clusters) -Chromosome specific (Waugh *et al.*, 1996) -Require less DNA than RFLPs	-Co-dominant -High level of polymorphism (Powell *et al.*, 1996) -Highly informative (allele detection) (Powell *et al.*, 1996) -Ability to distinguish between closely related individuals
Disadvantages	-Expensive -Time consuming -Require large amounts of genetic material -Single locus specific (slow to detect infrequent (polymorphisms) (Vogel *et al.*, 1996)	-Dominant markers (Jones *et al.*, 1997) -Poor reliability and reproducibility (Jones *et al.*, 1997) -Sensitive to experimental conditions -Low levels of polymorphism (Powell *et al.*, 1996)	-Dominant markers -Technically difficult -Expensive	-Expensive to establish -Long development time -Need specific primers (Jones *et al.*, 1997) -Primers require cloning and sequencing (Gupta and Varshney, 2000)

The detection of polymorphisms is facilitated through separating the DNA fragments by electrophoresis on an agarose gel, and transferring and fixing the fragments onto a nylon membrane via a process termed southern transfer (Southern, 1975). Once transferred, the DNA contained on the nylon membrane can be analysed with at least 10 DNA probes to detect and score polymorphisms with the stripping of probes between each analysis. The DNA probes (markers) are cloned genomic DNA complimentary to the DNA sequence, either in whole or part, of the fragments created by the restriction enzymes. The probes are radioactively labelled (^{32}P CTP), denatured and then hybridised to the DNA on the nylon membrane. The labelling of the probes with ^{32}P CTP illuminates the hybridised bands on the membrane so they can be analysed by autoradiography.

1.6.1.2 Microsatellites or simple sequence repeats (SSRs)

SSRs are repeated sequences of DNA, composed of dinucleotides, trinucleotides, tetranucleotides and so on, occurring at many different loci scattered throughout the genome (Jones *et al.*, 1997). Detection of SSRs centres on the application of primers (forward and reverse primers) matching specific sequences flanking the repeated sequence. PCR is utilised to amplify the sequence (SSR) between the primers, and a DNA banding pattern is generated by electrophoresis on an 8% polyacrylamide gel. The banding patterns are visualised by staining the DNA with ethidium bromide and illuminating under UV light. Polymorphism for a particular SSR is based on the size (length) of the repeats structure.

As with RFLPs and AFLPs, SSRs have been used in map construction, determining genetic diversity and relationships, and as a diagnostic tool. In fact SSRs have, in recent years, become the markers of choice (Gupta and Varshney, 2000). Table 1.4 highlights some examples of the use of SSRs and the wide range of species in which they have been applied.

Table 1.4: Examples of the application of SSRs in various crop species.

Species	Application	References
Olive	Genetic diversity	Rallo, P., Dorado, G. and Martin, A. (2000)
Soybean	Genetic diversity QTLs controlling seed weight	Narvel *et al.* (2000) Maughan *et al.* (1996)
Arabidopsis	SSR type and frequency	Cardle *et al.* (2000)
Barley	barley mild mosaic virus (BaMMV) resistance Abiotic stress tolerance Genetic diversity	Werner *et al.* (2000) Forster et al. (1997) Sanchez de la Hoz *et al.* (1996)
Cucumis (melon & cucumber)	Evaluation and mapping	Danin-Poleg *et al.* (2000)
Alfalfa	Genetic relationships	Mengoni *et al.* (2000)
Larch	Characterisation and inheritence of SSR loci	Khasa *et al.* (2000)
Wheat	Characterisation of SSRs	Varshney *et al.* (2000)
Cotton	Chromosomal assignment of SSRs	Liu *et al.* (2000)
Maize	Detection of genetic variation	Li-XinHai *et al.* (2000)
Sunflower	Genetic relationships	Dehmer & Friedt (1998)
Watermelon	Genetic relatedness	Jarret *et al.* (1997)
Sorghum	Characterisation and mapping of SSRs	Taramino *et al.* (1997)
Avocado	Genetic linkage map	Sharon *et al.* (1997)
Potato	Genetic relationships	Milbourne *et al.* (1997)
Rice	Rice nuclear restorer gene Genetic analysis	Akagi *et al.* (1996) Panaud *et al.* (1996)
Tomato	Characterising and mapping of SSRs	Broun & Tanksley (1996)
Black cherry	Genetic diversity	Downey & Iezzoni (2000)
Coconut	Genetic diversity	Perera *et al.* (2000) Teulat *et al.* (2000)
Coffee	Identification of polymorphic SSRs	Mettulio *et al.* (1999)

1.6.1.3 Amplified fragment length polymorphisms (AFLPs)

AFLPs employ a combination of restriction enymes to produce DNA fragments and PCR techology to produce banding patterns on a denaturing polyacrylamide gel (Vos et al., 1995; Jones et al., 1997). Two restriction enzymes; a rare cutter (e.g. Pst1, 6 base cutter) and a frequent cutter (e.g. Mse1, 4 base cutter), used in AFLP analysis result in three types of DNA fragments with ends characterised by the restriction site. The majority (>90%) are Mse1-

Mse1 fragments, a small number are Pst1-Pst1 fragments, and the Pst1-Mse1 fragments constitute approximately twice the number of the Pst1-Pst1 fragments. Alternatively Pst1 can be substituted with EcoR1, also a rare cutter. The second step in the process is to ligate specific double-stranded adaptors to the ends of the fragments. Mse1 adaptors ligate to the Mse1 restriction sites and the Pst1 adaptors to the Pst1 ends. Pre-amplification of fragments follows the ligation of adaptors.

Pst1 and Mse1 primers, each consisting of a core sequence, an enzyme specific sequence, and a selective extension of 1-3 nucleotides at the 3' end, anneal to restriction/adaptor sites on the fragments. The primer combination facilitates the selective amplification of the DNA fragments consisting of both Pst1 and Mse1 ends, and complementary 1-3 nucleotide extension sequences flanking the restriction sites. The presence of the Pst1 primer in the reaction ensures that there is preferential amplification of the Pst1-Mse1 fragments and no amplification of the Mse1-Mse1 fragments (Vos *et al.*, 1995). Vos *et al.* (1995) implied from this observation that amplification of the Mse1-Mse1 fragments was inefficient in the presence of the Pst1 primer; either due to the Mse1 primer having a lower annealing temperature, or due to the stem-loop structure formed from the base pairing of the inverted repeat common at the ends of the Mse1-Mse1 fragments, which compete with primer annealing.

Each AFLP reaction has the potential to generate multiple banding patterns. The number of polymorphic bands equals the number of loci able to be detected by a specific AFLP primer combination. The number of nucleotides that make up the selective extension can be increased to reduce the number of bands amplified, however the selectivity (number of loci for polymorphism detection) of the AFLP reaction diminishes. The use of extensions with three nucleotides reduces the number of bands amplified while still retaining a high degree of selectivity (Vos *et al.*, 1995).

Typically the rare cutter (*e.g.* Pst1) is radioactively or fluoroescently labelled to score loci for polymorphisms. For radioactive labelling the bands are visualised using autoradiography, while the fluoroescently labelled bands are scored using a specifc software package.

A major draw back of AFLPs is that they do not allow for high throughput genotype determination. Therefore, further advances in QTL analysis and marker assisted selection (MAS) will require that AFLPs be converted to sequence specific markers (Meksem *et al.*, 2001)

1.6.2 Genetic analysis of mapping populations, and QTL mapping, characterization and validation

With the assistance of molecular tools, the genetic basis to the expression of economically important traits can be determined and molecular markers developed which can be used to select for them in the breeding program. To achieve this end chromosome regions or quantitative trait loci (QTL) involved in the expression of traits of interest must be identified and characterized. QTL are regions of the chromosome that have been shown to be statistically associated with the expression of quantitative traits. The term "Quantitative trait" describes those traits that display continuous variation, because they are typically controlled by multiple genes, and are affected by the environment (*e.g.* grain yield). These characteristics of quantitative traits can make successful identification, and often manipulation, difficult (Jones *et al.*, 1997). In addition, epistatic effects between QTL may vary the overriding phenotypic expression of a particular trait, and skew the evaluation of QTL effects (Ribaut *et al.*, 2002). This compares to qualitative traits (e.g. some disease resistances), which are generally controlled by major genes, and the character is expressed at discrete levels.

The characterization and mapping of QTL relies on statistical procedures, either interval mapping (Simple Interval Mapping or Composite Interval Mapping) that employs maximum likelihood, or regression analysis at each individual loci, to associate the expression of a

quantitative trait with molecular marker alleles. Through this process, the chromosomal location of the QTL can be established, the percentage phenotypic variance attributed to each QTL can be determined, and the genetic effect (*i.e.* additive or dominance) can be quantified (Ribaut *et al.*, 2002). A QTL that explains greater than 30% of the phenotypic variance is considered to be a major QTL (Ribaut *et al.*, 2002). The precision of QTL mapping relies heavily on the construction of a high-density genetic map to ensure marker coverage of the entire genome. For a more detailed review of QTL analysis refer to Kearsey and Farquhar (1998). The process for charcterising and mapping chromosome regions involved in the expression of QTL is also outlined in Ribaut *et al.* (2002). Briefly the steps include:

1. Construction of a segregating population from a cross where the parents contrast for the trait(s) of interest.
2. Genotyping the population based on the allelic segregation of a suite of molecular markers.
3. Development of a genetic map of the population based on linkage groups formed by statistical associations and recombination frequencies between markers.
4. In parallel, phenotypic evaluation for traits of interest is carried out on the mapping population.
5. Statistical procedures are used to identify markers closely linked to the expression of target trait(s).
6. Validation-confirmation that marker(s) are closely associated with the trait(s) in question through evaluation in alternate genetic backgrounds. The validation population needs to display segregation for the trait(s) and the potential marker. Lines with better performance for a given trait(s) will have alleles relating to the superior parent. Field evaluation of material is also required in the validation process to characterise the genotype by environment effects in the target environment.
7. Upon successful validation, the marker(s) can be applied in breeding programs (see section 1.6.3)

QTL analysis has been used extensively to map quantitative traits associated with abiotic factors. They include salt tolerance in tomato (Foolad and Chen, 1997; Foolad *et al.*, 1999), rice (Flowers *et al.*, 2000) and barley (Ellis *et al.*, 1997; Mano and Takedo, 1997; Forster *et al.*, 1997; Forster *et al.*, 2000); physiological, morphological and biochemical factors relating to drought tolerance (Teulat *et al.*, 1997), osmotic adjustment in barley (Teulat *et al.*, 1998; Zhang *et al.*, 1999; Teulat *et al.*, 2001), water use efficiency in soyabean (Specht *et al.*, 2001), anthesis-silking interval in maize (Agrama and Moussa, 1996; Ribaut *et al.*, 1996; Ribaut *et al.*, 1997), root morphology in rice (Champoux *et al.*, 1995; Price and Tomos, 1997; Price *et al.*, 1997a), stomatal conductance in rice (Price *et al.*, 1997b), abscisic acid concentration response in maize (Sanguineti *et al.*, 1999); heat shock proteins for thermo-tolerance in maize (Frova and Gorla, 1993; Frova *et al.*, 1995); winter hardiness/cold tolerance in barley (Hayes and Pan 1996) and tomato (Foolad *et al.*, 1998); boron toxicity in barley and wheat (Jefferies *et al.*, 1999b; Jefferies, 2000); and nitrogen use efficiency in maize (Bertin *et al.*, 1996).

1.6.3 Marker assisted selection

Marker assisted selection (MAS) is the use of molecular markers for indirect selection for a trait of interest. Alternatively a marker linked to an undesirable trait can be used to remove that gene from a breeding population (negative selection)(Jones *et al.*, 1997).

The relative efficiency of MAS in plant breeding programs, though, is a function of the cost of implementation compared to the cost of conventional methods of selection (e.g. CCN/Boron tolerance)(Jefferies *et al.*, 1997 & 1999a,b). In addition, the degree of linkage between the marker and the trait will be a major factor contributing to the effectiveness of markers as tools in plant breeding. Ultimately the time taken to select a homozygous line with the desired trait from the initial cross can be substantially reduced through MAS. MAS has particular benefits for application in accelerated backcrossing (see Jefferies, 2000). In accelerated backcrossing the molecular marker can play a pivotal role in reducing the number of backcrosses to produce a line that carries the desired gene(s) from the donor parent, while

still maintaining the genetic background of the recurrent parent. MAS for the recurrent parent background, however, requires a large number of polymorphic markers covering the entire genome (Jefferies *et al.*, 1997). AFLP analysis is particular well suited to selecting lines with the least amount of donor parent genome because a large number of loci can be detected with in a single reaction (high multiplex ratio) (Powell *et al.*, 1996). Powell *et al.* (1996) estimated that selection of an individual(s) with only 8% donor DNA in the BC_1 generation using this method would be equivalent to advancing a population to the BC_3 generation, effectively saving two generations of backcrossing. Examples of MAS in accelerated backcrossing in barley include the introgression of stripe rust resistance (Toojinda *et al.*, 1998) and CCN resistance (Jefferies *et al.*, 1997).

The advantage of MAS is that it is non-destructive; therefore early generation material can be screened without loss of seed. Furthermore, selection by MAS is based on genotype, not phenotype, and therefore is not subject to environmental interaction or high assay error (Jefferies *et al.*, 1999a). This last point is particularly relevant in terms of the potential for the application of MAS for sand adaptation. The genetic expression of traits for sand adaptation in breeding trials tends to be overwhelmed by the large environmental effects of sandy soils due to the incidence and variation of root disease, depth of sand, soil water properties (*e.g.* water repellency, non-uniform wetting patterns down the soil profile, drainage), soil nutrient properties, uneven seeding depth, and the error associated with small plots, and limited replication. Consequently the heritability (h^2) of important traits is very low, eliminating traditional selection strategies, based on phenology, as a viable process of improving sand adaptation. MAS overcomes this obstacle because it is based on genotype, and may significantly improve the development of barley varieties with superior combinations of traits for adaptation.

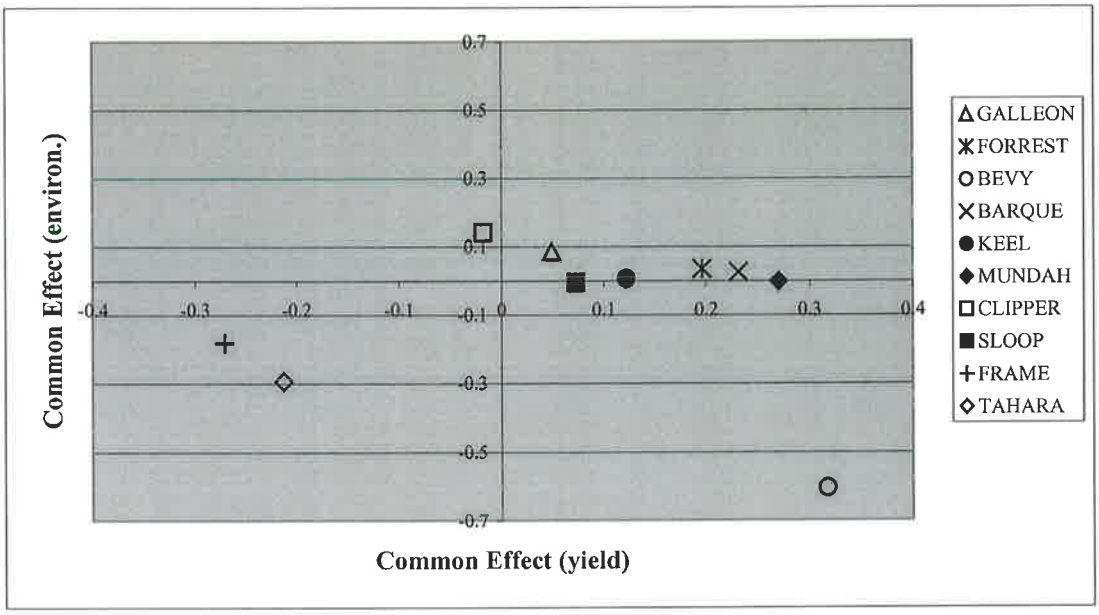
1.7 General discussion and a putative 'ideotype' for sand adaptation in barley

Barley is the second most important crop in SA, after wheat. The average area sown to barley is 968,600ha and, with an average annual production of 1.715 million tonnes (10 yr. Ave., 1988/89-1997/98, ABARE, 1998), SA is the major producer of barley in Australia. Of the area sown to barley (968,600ha), approximately 30% is grown on soils of a sandy texture. In addition, these soil types tend to be associated with dune/swale landscapes, where the dunes are sandy and the swales are of a loamier texture. Hence the ability to grow and yield well on sandy soils is an important characteristic to be selected for in the SA Barley Breeding Program.

Growing cereals on sandy textured soils often provide low and uncertain economic returns for growers because crop responses on sandy soils can be highly variable (Hamblin *et. al.*, 1988). The unreliability of grain yield on sandy soils reflect the potential limitations on adequate growth imposed by the inherently poor properties of sandy soils outlined in section 1.2 and their variable profile, and due to poor establishment which is associated with uneven seeding depth. Depth of sand may also influence crop performance (Hamblin *et. al.*, 1988) if plant roots are unable to tap into heavier textured sub-soils for moisture. Generally, dry matter production and grain yield is highest on the heaviest soils, and this is related to vigourous early growth resulting from better water use efficiency and greater fertility (Hamblin *et. al.*, 1988; French and Ewing, 1989). However, under drought conditions, French and Ewing (1989) were able to show an advantage, in terms of yield, of sandy soils over clay-textured soils. Plants performed relatively poorly on heavier soil compared to sandy soils and this was related to the lower soil water potentials and hydraulic conductivities, at the same volumetric water content, of the heavier soil. These soil conditions caused the plants to suffer water stress and therefore cease growth earlier, despite having a higher water holding capacity.

Trials conducted by the South Australian Field Crop Evaluation Program (SAFCEP) of the SA Research and Development Institute (SARDI) have also highlighted the differences in yield potential between sandy soils and heavier textured soils. Long term grain yield for the sand screening trials and sandy sites within S4 trials have averaged 1.58 and 2.07 t ha^{-1} respectively, while non-sandy S4 trial sites have averaged 2.87 t ha^{-1} (Rob Wheeler, *pers. Comm.*).

In general barley has better adaption to sandy soils than wheat, triticale and oats but is inferior to cereal rye (Figure 1.1). In dune/swale environments a typical management strategy is to sow the high value crop (wheat) in the swales and barley on the dunes, because of its superior adaptation (Plate 1.1). However, on deeper dunes, where even barley shows poor growth, it has been common practice to sow cereal rye. This is because rye has the ability to satisfactorily perform on deep sands of low fertility and to stabilize sand drift (erosion control). Historically, SA has grown the greatest proportion of cereal rye in Australia. Rye has good early vigour and competitive ability; tolerates unfavourable climatic conditions during the season; has limited soil nutrient requirements and an ability to use the available soil moisture supply; and has a vigourous and extensive root system (Lovett, 1987). Hamblin *et al.* (1988) showed that rye has better emergence and anthesis dry matter production than barley or wheat, but yielded less. However, wheat and barley were more sensitive to the depth of sand. Rye also had a deeper root system.



*Source: Rob Wheeler, SA Field Crop Evaluation Program (SARDI)

Figure 1.1: MET analysis of long-term grain yield data for barley (7 varieties), wheat, cereal rye and triticale in SARDI sand evaluation trials (1988-2000).

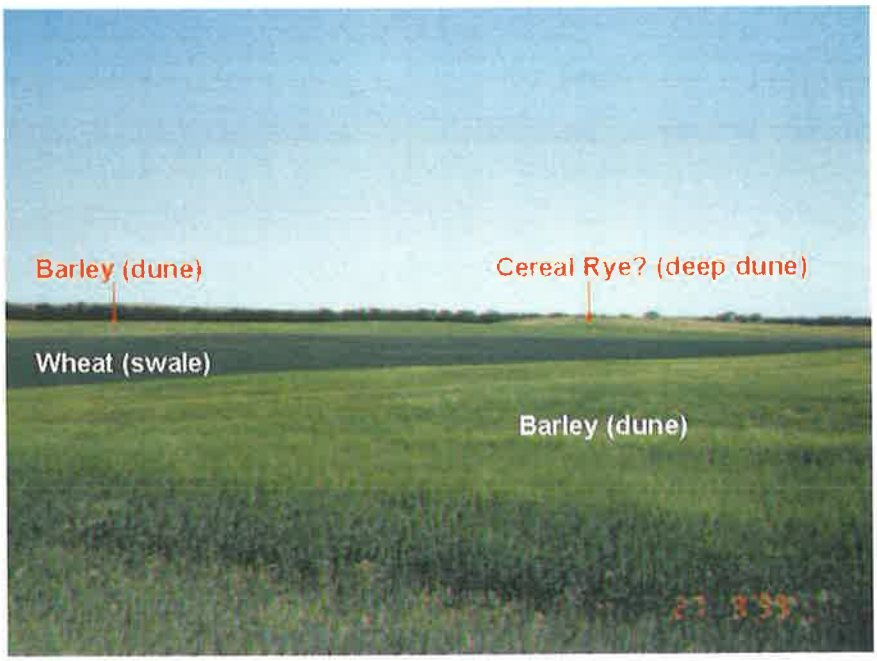


Plate 1.1: Typical management practice for cereal production in dune/swale environments.

An indication of genotype by environment effects for grain yield, have been characterised between barley cultivars grown on sandy soils versus heavier textured soils by SARDI (Table 1.5). Western Australian (WA) varieties such as Mundah and Forrest, which have very good early vigour, show superiority over most SA bred types on sandy soils (Figure 1.1). This is a reflection of their evaluation and selection, which is predominantly on sandy textured soils. In contrast these varieties do not show this superiority on heavy textured soils. Forrest and Keel show distinct variation in grain yield between sandy and non-sandy soil types. The grain yield of Mundah and Barque on the other hand, are relatively stable across soil types. Mundah's ability to perform well on sand has led to its recommendation as an excellent alternative for sandy soils in SA, although it is limited by a lack of significant disease resistance compared to SA counterparts.

Table 1.5: Long term grain yield (t ha^{-1}) and rankings of selected WA and SA cultivars on sandy and non-sandy soils in South Australia. (SAFCEP, 1988-2000). Data analysed by MET analysis.

Cultivar	Sandy Soils	Rank	Non-sandy Soils	Rank
Mundah (WA feed)	1.70	1	2.97	3
Forrest (WA feed)	1.64	**3**	2.68	**8**
Galleon (SA feed)	1.47	8	2.85	4
Keel (SA feed)	1.55	**5**	3.02	**1**
Sloop (SA malting)	1.49	7	2.83	5
Schooner (SA malting)	1.52	6	2.77	7
Barque (SA feed)	1.67	2	2.99	2
Chebec (SA feed/malting)	1.56	4	2.80	6
mean	1.58		2.87	

The lack of adaptation of SA bred varieties for performance on sandy soils, is probably related to a number of factors. Historically, the breeding objectives of the SA Barley Breeding program have focused on selection for improved malting quality. Because of this focus, a large proportion of the germplasm introduced into the program has been of European, Canadian and Japanese origin. This germplasm has been sourced from higher yielding

environments and as such are not agronomically suited to the soil types and growing conditions of SA. In addition, site selection, for varietal evaluation, has focused on producing malting quality and high yield. Consequently, until recently, field evaluation trials were predominately conducted on soils of loamy to clay texture and not on sandy soils. This has been re-addressed by the addition of sites on sandier textured soils such as at Callington, Geranium (Murray Mallee) and Tuckey (Eyre Peninsula). Traditional methods of selection have also inhibited varietal improvement for sandy soils. These methods were based on across site performance (across site mean) and therefore selection pressure was weighted against low yielding conditions, such as sandy sites, in favour of high yielding lines under high yielding conditions. In addition, low genetic variance, related to the germplasm available, and the high error variance of yield trials on sandy sites mean the heritability (h^2) of traits that may be desirable for sand adaptation is low. This can complicate the selection of lines with suitable characteristics for growth on sandy soils. The high error variance of barley yield trials on sandy soils is related to variability across the site due to factors such as uneven seeding depth, depth of sand, disease, the small size of breeding plots, and the limited replication number.

More modern statistical procedures for selection attempt to overcome the selection pressure being weighted towards high yielding environments by adjusting up low yielding sites and down high yielding sites. Nevertheless, these methods still tend to favour high genetic variance and low error variance. Selection, to a large extent, is still weighted against low yielding environments.

The very nature of sandy soils predisposes them to both moisture and nutrient limitations. Therefore the key to improving the growth and yield of barley on sandy soils may well be linked to crop characteristics that are essential to improving grain yield *per se* in water limiting environments. Turner and Nicolas (1987) provided a composite of desirable traits for drought tolerance on light textured soils; namely a deep root system, good early vigour, and a

high degree of assimilate transfer to the grain during grain fill. The conceptual model for drought tolerance in wheat illustrated in Reynolds *et al.* (2002) also prioritises traits such as large seed size, long coleoptile and high spike photosynthesis. In addition, Richards (1991) emphasised the contribution of an appropriate phenology to improvements in crop production and adaptation. An early flowering phenology will reduce the potential effect of moisture stress on grain development, by lowering the probability of premature cessation of grain filling. This review of the literature has expanded on morphological and physiological traits, centred on those suggested by Turner and Nicolas (1987), with a potential for improving the adaptation of barley on sandy soils of low fertility. Accordingly a putative barley ideotype for sand adaptation has been devised that reflects these traits (Figure 1.2). The purpose of the conceptual model is to encapsulate the hypothesis of the thesis, and to ensure that all experimental work focuses on testing those traits considered beneficial to improving growth and grain yield on sandy soils, and determining whether real genetic variance exists.

The free-draining and leaching nature of sandy soils, suggests a deep root system would be critical to improve moisture and nutrient availability and acquisition, and therefore contribute to more efficient WUE. Improving early vigour (rapid leaf area development and dry matter production) will assist WUE, through efficient transpiration and reducing soil evaporation, and provide a greater capacity for photosynthesis. An erect growth habit will also facilitate improved photosynthesis through improving the leaf area able to capture light. Associated benefits of greater ground cover included an improved competitiveness with weeds, and therefore herbicide usage is reduced, and a reduction in the potential for erosion and sandblasting. Various authors have identified traits to improve establishment and early vigour such as large seed size, large embryo, a long coleoptile, leaf habit and development, and seed nutrient content (Table 1.2). In addition, evidence has been produced to illustrate how early vigour can improve grain yield potential (Brown *et al.*, 1987; Ceccarelli, 1987; Acevedo *et al.*, 1991). Increasing temperatures and moisture deficit following anthesis, common in Mediterranean environments, can have a significant impact on grain filling, and therefore

grain yield. Accordingly the potential contribution of carbohydrate, stored in the stem pre-anthesis, to grain filling needs to be considered. Finally, early flowering is an important attribute for sand adaptation, to limit the impact of drought and heat stress, and maximise the length of grain filling given prevailing conditions (Ceccarelli, 1987; Acevedo *et al.*, 1991; Hadjichristodoulou, 1993).

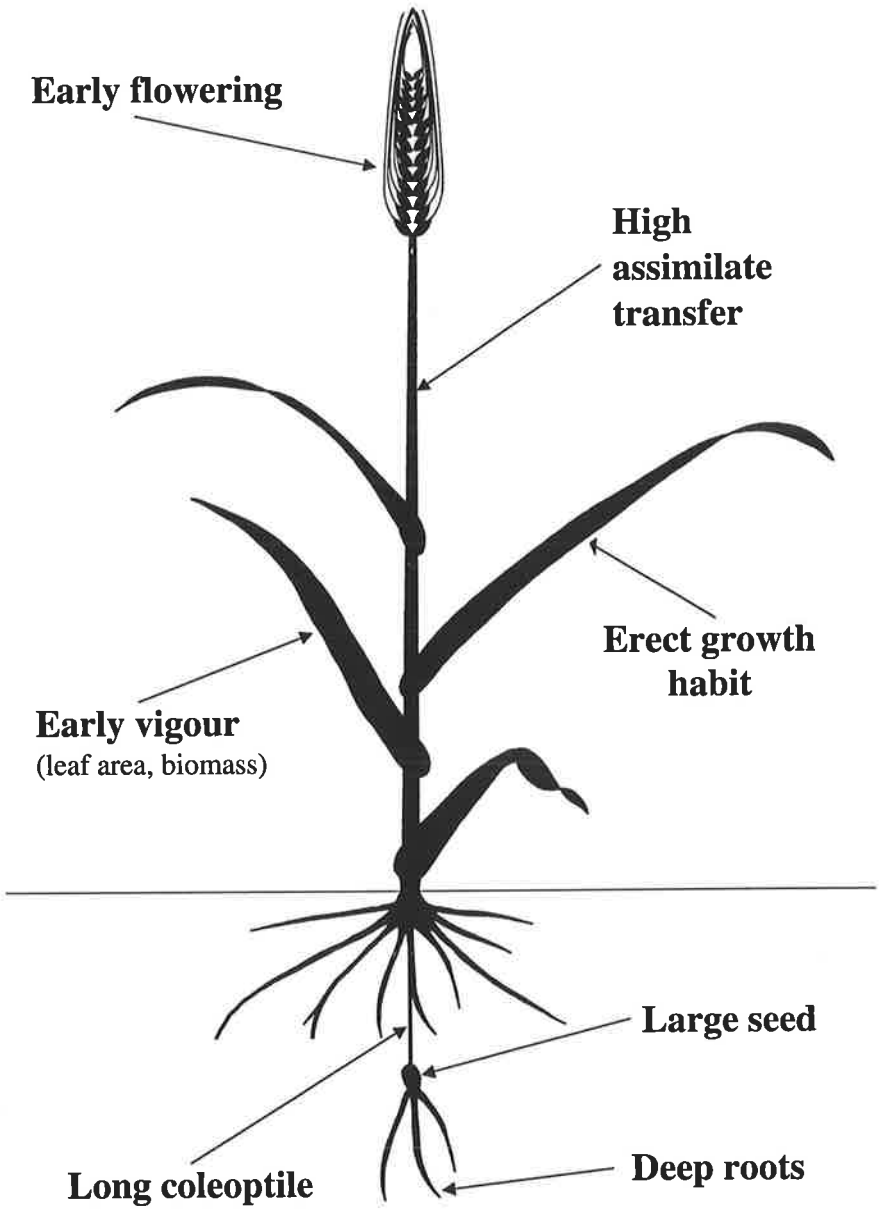


Figure 1.2: A putative barley 'ideotype' for sand adaptation

(cereal plant graphic adapted from Araus *et al.*, 2001)

Chapter 2. Traits associated with improved growth and grain yield of barley on sandy soils of low fertility: Field component

2.1 Introduction

Crop production limitations on sandy soils are believed to be associated with poor establishment and growth, which restrict grain yield potential. This reflects the characteristics of sandy soils such as low inherent fertility, both in terms of macro- and micro-nutrient status; low organic matter; low water retaining capacity and cation exchange capacity; water repellency; a high incidence of root disease; loss of nutrients through leaching; and the propensity to wind erosion. In addition, crops are often subject to sand "blasting". In contrast, heavier soil types have a greater yield potential, and this has been attributed largely to improved establishment and early vigour (Hamblin *et. al.*, 1988; French and Ewing, 1989). Barley is commonly regarded to have better adaptation to sandy soils than wheat, triticale and oats. This may be related to barley exhibiting greater early vigour than these other cereals, particularly wheat (Lopez-Castaneda *et al.*, 1995). However barley is inferior to cereal rye on sand. Historically, South Australia (SA) has grown the greatest proportion of cereal rye in Australia due mainly to rye's ability to satisfactorily perform on sandy soils of low fertility and to stabilize sand drift (erosion control). Rye has good early vigour and competitive ability; tolerates unfavourable climatic conditions during the season; is tolerant to sand "blasting"; has reduced soil nutrient requirements and an ability to use the available soil moisture supply; has a vigourous and extensive root system (Lovett, 1987); and displays good root disease resistance. Hamblin *et. al.* (1988) demonstrated that rye had better emergence and anthesis dry matter production than barley or wheat, but yielded less. However, wheat and barley were more sensitive to the depth of sand. Rye also had a deeper root system (Hamblin *et. al.*, 1988).

Varietal improvement and selection for sand 'adaptation' is a challenge. This is due to the low heritability (h^2) of traits on sandy soils. Heritability is a function of the genetic variance and the total variance in the form;

$$h^2 = \text{genetic variance} / \text{total variance}$$

where the total variance is the sum of the genetic, environmental and error variances.

The low h^2 is likely to result from the low genetic variance in Australian germplasm for sand adaptation, because the breeding objectives of the SA Barley Breeding program have, historically, focused on selection for improved malting quality in more favourable areas. The majority of germplasm has typically been of superior malting quality and sourced from higher yielding environments such as Europe, Canada and Japan, and as such are not agronomically suited to the soil types and growing conditions of SA. The high environmental and error variance associated with breeders' yield trials on sandy soils has also been a limitation for germplasm development. In addition, conventional statistical approaches to analysing yield differences do not adjust for variabilility within trials and tend to select towards high grain yield potential varieties and environments.

Despite this, lines developed in the Western Australian (WA) breeding program (*e.g.* Yagan, Forrest and Mundah) have shown an ability to perform well on sandy soils, and are generally superior to their SA bred counterparts, despite a lack of resistance to economically significant diseases such as spot form of net blotch (*Pyrenophora teres* f. sp. *maculata*), cereal cyst nematode (CCN) (*Heterodera avenae*), root lesion nematode (*Pratylenchus neglectus*) and leaf scald (*Rhynchosporium secalis*). The superior adaptation of the WA varieties is a reflection of their evaluation and selection, which is predominantly on sandy textured soils, and thereby indicates that potential genotypic variability for sand adaptation exists. This has been borne out in the long-term yield analysis of varieties in sand evaluation trials (Table 2.1)

conducted by the South Australian Research and Development Institute (Rob Wheeler, *pers. Comm.*). However, little information on characteristics potentially associated with their improved yield potential on sandy soils is available.

Table 2.1: Long term yield data of varieties on sandy soils and heavier soils in SA (1988-2000)*

Variety	Sandy Soils ($t\ ha^{-1}$)	Rank	Heavier Soils ($t\ ha^{-1}$)	Rank
Mundah (WA)	1.70	1	2.97	3
Barque (SA)	1.67	2	2.99	2
Forrest (WA)	1.64	3	2.68	6
Yagan (WA)	1.64	3	2.64	7
Bevy Rye (SA)	1.63	4	-	-
Keel (SA)	1.55	5	3.02	1
O'Connor (WA)	1.53	6	-	-
Sloop (SA)	1.49	7	2.83	5
Galleon (SA)	1.47	8	2.85	4
Clipper (SA)	1.43	9	2.63	8
Tahara triticale (SA)	1.25	10	-	-
Frame wheat (SA)	1.13	11	-	-

*Source: Rob Wheeler, SA Field Crop Evaluation Program (SARDI)

It is possible that traits related to drought tolerance also provide a mechanism to improve adaptation on sandy soils. A deep root system, good early vigour, and a high degree of assimilate transfer to the grain during grain fill has been suggested as desirable for drought tolerance on light textured soils (Turner and Nicolas, 1987). In addition, various authors have identified traits to improve early vigour (Lopez-Castaneda *et. al.*, 1996; Rebetzke and Richards, 1996 & 1999; El Hafid *et al.*, 1998; Rebetzke *et. al.*, 1999; Richards, 1991 & 2000) and illustrated how vigour can improve grain yield potential (Brown *et al.*, 1987; Ceccarelli, 1987; Acevedo *et al.*, 1991). The contribution of non-structural carbohydrates (Fructan and ethanol soluble carbohydrates), stored in the stem prior to anthesis, to grain filling, a key trait observed in drought tolerance (Austin *et al.*, 1980; Richards and Townley-Smith, 1987; Blum

et al., 1994; Blum, 1998; Gebbing *et al.*, 1999), may also be an important characteristic for sand adaptation because of the poor moisture relations of this soil type. This chapter reports on field experiments, using selected varieties that vary in adaptation on sandy soils, conducted to identify traits conferring improved growth and yield on sandy soils of low fertility.

2.2 Methods and Materials

2.2.1 Varieties

Seven barley varieties ranging in adaptation on sandy soils were selected as the core group for identifying the factors that confer improved growth and yield. The varieties considered 'good' performers on sand were Mundah and Forrest, the variety considered 'intermediate' was Barque and those considered 'poor' were Keel, Galleon, Clipper and Sloop (Table 2.1). Agronomic and disease reaction information for these core varieties is listed in table 2.2 (Rob Wheeler, *pers. Comm.*). In 1999, 20 entries were sown in field trials; 10 parents from the National Barley Molecular Marker Program (NBMMP) (Sahara, Chebec, Harrington, Haruna Nijo, Alexis, Tallon, Kaputar, Franklin, Skiff and Arapiles), Bevy (Cereal Rye), Tahara (Triticale) and Frame (Wheat). The inclusion of cereal rye, triticale and wheat varieties was for use as controls to compare adaptation between cereal species. In 2000, in addition to the seven core varieties, Yagan and O'Connor (parents of Mundah) were included.

2.2.2 Sites

Trials in 1999 and 2000 were established at three sites in SA (appendix 1); one north of Minnipa Agricultural Centre (Eyre Peninsula), and two in the Murray Mallee; at Lowbank (upper Murray Mallee) and Cooke Plains (lower Murray Mallee). In 1999 two sites were located on sand hills, either on a northern aspect, with plots running up and down the slope, as

at Minnipa, or plots running along the top of the ridge (Lowbank). Cooke Plains was sown on a sandy flat.

The 2000 trials were all located on sand hills, either on a northern aspect, with plots running up and down the slope, as at Minnipa, or plots running along the top of the ridge (Lowbank and Cooke Plains). A description of the sites is listed in Tables 2.3 and 2.4.

Table 2.2: Agronomic and disease reaction information for the seven core barley varieties, and Yagan and O'Connor*. (0-9 scale: a high figure indicates that the variety expresses the character to a high degree)

Variety	Early vigour	Tillering ability	Standing ability	Plant Height	Maturity	Head retention	CCN resistance	CCN tolerance	Net blotch (spot form)	Net blotch (net form)
Barque	6	9	6	6	mid	5	R	T	MR	
Clipper					mid					
Forrest	9	6	3	8	early	6	S	T	MS-S	-
Galleon	5	9	5	4	mid	5	R	T	MR	MR
Keel	6	9	5	4	early	5	R	T	MR	R
Mundah	9	6	6	5	early	3	S	T	S	MR
O'Connor	7	7	6	6	early mid	3	S	T	MS-S	
Sloop	6	7	5	6	mid	4	S	T	S/VS	MS
Yagan	9	6	6	4	v. early	5	S	T	S	-

*Source: Rob Wheeler, SA Field Crop Evaluation Program (SARDI)

Table 2.3: Site Details for 1999 field trials-Sowing date, fertilizer rates, harvest date, soil type and April-October rainfall

	Minnipa	Cooke Plains	Lowbank
Sowing Date	3rd June	17th June	4th June
Fertilizer Rate	75 kg ha^{-1} of 17:19 (5% Zn)	99 kg ha^{-1} of 9:17 (5% Zn)	143 kg ha^{-1} of 9:17 (5% Zn)
Harvest Date	19th November	15th December	29th November
Soil Type	Sandy loam (0-10 cm) over loam	Sandy loam (0-80 cm)	Sandy loam (0-80 cm)
April-October Rainfall	200	224	179

Table 2.4: Site Details for 2000 field trials-Sowing date, fertilizer rates, harvest date, soil type and April-October rainfall

	Minnipa	Cooke Plains	Lowbank
Sowing Date	2nd June	30th May	2nd June
Fertilizer Rate	75 kg ha^{-1} of 17:19 (5% Zn)	143 kg ha^{-1} of 9:17 (5% Zn)	99 kg ha^{-1} of 9:17 (5% Zn)
Harvest Date	24th November	29th November	15th November
Soil Type	Sand over sand over clay	Sandy loam (0-80 cm)	Sandy loam (0-80 cm)
April-October Rainfall	293.3	316	175.5

2.2.3 Establishment of Trials

2.2.3.1 1999

Trials were established as randomised complete block designs (RCBD) with each entry replicated 4 times. Plot areas at sowing were 10.5 m^2 at Minnipa and 18 m^2 at Lowbank and Cooke Plains. Sowing rates were adjusted for each species, plot area and germination percentage to attain the recommended plant densities of 145 plants m^{-2} for the barley varieties and Bevy rye, 170 plants m^{-2} for Frame wheat and 210 plants m^{-2} for Tahara triticale. At sowing, depth of seeding was approximately 2.5-3 cm. Plot area at harvest was 7.5 m^2 at Minnipa, and 15 m^2 at Lowbank and Cooke Plains.

2.2.3.2 2000

Trials were established as randomised complete block designs (RCBD) spatially randomised using SpaDes® (Coombes, 1999), with each entry replicated 8 times. Plot areas at sowing were 18m^2. Sowing rate was adjusted for each variety, based on seed weight and germination percentage, to attain a plant density of 145 plants m^{-2}. At sowing, depth of seeding was approximately 2.5-3cm. Plot area at harvest was 15 m^2.

2.2.4 Measurements

2.2.4.1 Coleoptile length

Seed of each variety was pre-germinated in a Petri dish with 2 moist Whatman No. 1 filter papers, in an incubator at 20°C for 5 days. Germinated seed was then laid out onto 32x46 cm (R6) filter paper, pre-soaked in reverse osmosis (R.O.) water. Four sheets (replicates) of 25 seeds per sheet were set up for each variety. Each filter paper was rolled up carefully, covered with aluminium foil and placed in a container with a small amount of R.O. water to ensure the filter paper remained moist. The samples were then incubated at 20°C for 7 days. After incubation the coleoptile length of each seed was measured with a ruler. The mean length for the 25 seeds per filter paper was then regarded as a replicate.

2.2.4.2 Soil cores

Five cores (four in 2000), to a maximum depth of 80 cm (limit of soil auger), were collected to obtain a detailed description of the soil profile at each site. Replicate 10 cm fractions were pooled, air dried and analysed for texture, colour, Colwell extractable phosphorus and potassium (Colwell, 1963), nitrogen (nitrate and ammonium), sulphur, organic carbon, iron, electrical conductivity and pH. At Minnipa in 1999, soil depth to the calcrete layer varied across the trial from 40 cm at the lower (northern) end to 80 cm at the top of the ridge, and 60-70 cm at the western and eastern ends.

2.2.4.3 Early Vigour Measurements

2.2.4.3.1 Dry matter production

At approximately 6 weeks post-sowing (8 weeks in 2000), and at a plant development stage of Z14/Z21-22 (early tillering), establishment (plants m^{-2}) was scored by counting the number of plants in a 0.25 m^2 quadrat at one (2000) or two locations in each plot. The plants were harvested, dried at 80°C for 48 hours and then weighed. At this developmental stage total weight of dry matter production (E_DMP) in a square metre, was used as a measure of early

vigour. Dry matter production has also been used as a measure of early vigour by various other authors (Brown *et al.*, 1987; Turner and Nicolas, 1987; Whan *et al.*, 1991). Whole shoots were analysed for a range of macro- and micro-nutrients using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICPAES) analysis (Zarcinas *et al.*, 1987). Total Phosphorus content (P, mg) was calculated from the tissue concentration determined by ICPAES, and total dry matter production. Phosphorus efficiency ratio[1] (PER, g mg^{-1} P) and Phosphorus utilisation efficiency[2] (PUE, g^2 mg^{-1} P) was estimated according to Siddiqi and Glass (1982).

[1]PER (g mg^{-1}) = Above ground dry matter (g) / Total content of P in dry matter (mg)

[2]PUE (g^2 mg^{-1}) = PER * Above ground dry matter (g)

2.2.4.3.2 Leaf Area Development

Five plants per plot were randomly harvested at Lowbank and Cooke Plains (2000 only), and leaf area (LA) measured using a planometer. From the LA measurements, leaf area index[3] (LAI, cm^2 m^{-2} soil area), specific leaf area[4] (SLA, cm^2 g^{-1} leaf) and leaf area ratio[5] (LAR, cm^2 g^{-1} whole shoot) were calculated. Both LAI and LAR were adjusted for plot area and plant density. SLA, LAR and LAI are all measures of leaf area development, and in turn early vigour (Lopez-Castaneda *et al.*, 1996; Rebetzke and Richards, 1996 & 1999; El Hafid *et al.*, 1998; Rebetzke *et al.*, 1999; Richards, 1991 & 2000). In addition, SLA and LAR can be used to infer the relative growth rate (RGR) of a plant (Poorter and Remkes, 1990); again a measure of early vigour. This is possible because RGR is the product of net assimilation rate and LAR, while LAR is a function of SLA and leaf weight ratio (fraction of total plant weight allocated to the leaves).

[3]LAI = total leaf area (cm^2) / ground area (m^2)

[4]SLA = leaf area (cm^2) / weight of leaf (g)

[5]LAR = leaf area (cm^2) / total plant weight (g)

2.2.4.4 Anthesis and Physiological Maturity Measurements

At anthesis and physiological maturity, plants in a $0.25m^2$ quadrat in each plot were harvested at ground level, dried at 80°C for 48 hours and then weighed to measure dry matter production.

2.2.4.5 Fructan and Ethanol soluble carbohydrate (ESC) analysis

Material for the analysis of ESC (fructose, sucrose and glucose) and fructan was sampled at Lowbank only in 2000. At anthesis, five main stems were sampled from each replicate of each variety by removing the stem at ground level. In each plot an additional 10 main stems, of equivalent maturity, were also tagged with coloured tape at this time for sampling at physiological maturity (Z92, Zadoks *et al.*, 1974). At maturity, as at anthesis, five main stems per replicate were sampled. For the sampling at anthesis and physiological maturity, the leaves (at the auricle) and the heads (at the collar) were discarded. Stems were placed in plastic bags and stored on dry ice during transport, and transferred to a –20°C cold room until required for analysis. Prior to analysis, samples were freeze dried and ground, to pass through a 2mm screen. ESC concentration was determined using a method based on that devised by Yemm and Willis (1958). Fructan content was measured using the Fructan analysis kit developed by Megazyme International Ireland Ltd. (McCleary *et al.*, 1997). ESC and fructan was converted to total content per stem (dry weight basis).

2.2.4.6 Agronomic Measurements

Scores for maturity were made in late September to early October. From the harvested material, grain yield (t ha^{-1}), screenings (proportion of grain below 2.5mm, %), and 1000 grain weight (g) were measured.

2.2.5 Statistical Analysis

All field trial data were analysed by a linear mixed model analysis using residual maximum likelihood (REML) (Patterson and Thompson, 1971). Analysis was performed using Genstat statistical software (Genstat® for Windows™ software, 5th edition, Lawes Agricultural Trust). REML produces Wald statistics to test the significance of fixed (treatment) effects. Early dry matter production (early vigour), 1000 grain weight, screenings and grain yield were also analysed using a MET (multi-environment trial) statistical analysis (Cullis *et al.*, 1998, Smith *et al.*, 2001). The analyses employ spatial techniques to adjust the means of data to accommodate variability across a field trial site (soil depth, fertility) and also variability due to cultural practices (*e.g.* harvesting in two directions). In addition, the MET analysis adjusts across site means to accommodate differences in genetic and environmental variance between sites, and provides information on the environmental stability of varieties and the heritability (h^2) of the traits analysed. The MET analysis was conducted using ASREML (Gilmour et al, 1999).

MET analysis generates loadings for each site that describes the weight of each sites contribution in calculating the performance ranking of varieties for any trait (*e.g.* grain yield) at the 'average' site. Calculation of the 'average' site is totally dependent on the sites used in the analysis. Site loadings also illustrate the correlation of each site with the 'average' site and the correlation between sites used in the analysis, and accommodates environmental variation between sites (*i.e.* the degree of correlation between sites) (Smith *et al.*, 2001). In addition, 'common effects' are produced for traits analysed (e.g. grain yield, E_DMP, 1000 grain weight, screenings percentage) and environment. The 'common effect' for traits (x-

axis) is effectively the ranking of varieties for the trait of interest. Varieties from left to right along the x-axis have subsequently higher overall performance. The 'common effect' (environment) (y-axis) describes the environmental stability or otherwise of the trait across the environments (field sites) used in the analysis. Varieties close to origin on y-axis are more stable in their ranking across environments for the trait of interest than varieties further away from the origin (above or below). Figures 2.1, 2.4, 2.13, 2.17 and 2.18 use 'common effects' to present data.

2.3 Results

2.3.1 Soil Properties

The soil types used in these experiments were characterised as a sandy loam texture (>50% sand), with the exception of Minnipa in 2000, which was a sand (>90% sand) over sand over clay (Tables 2.3 & 2.4). In terms of soil nutrition, all sites were typically low in nitrogen and phosphorus (Table 2.5 & 2.6). The mineral forms of nitrogen are pre-dominantly ammonium and nitrate. Adequate nitrogen is essential for good shoot and root growth and to achieve maximum yield potential. The level of nitrogen in the soil will determine the amount required to be applied as fertilizer to reach the target grain yield and protein content. This is also dependent on the type of variety (malt/feed) being grown. In addition previous crop history will have an effect on the level of soil nitrogen pre-sowing. The Nitrogen Calculator® (Payne and Ladd, 1994) is a useful tool for predicting fertilizer requirements.

The critical concentration for soil phosphorus, derived by Reuter *et al.* (1995) for barley in SA, is 18 mg P kg^{-1} of soil. At all sites, phosphorus was deficient in the soil fractions below 10 cm. The concentration in the 0-10 cm layer was usually higher than at depth, although only Cooke Plains in 1999 and 2000 exhibited adequate levels of phosphorus in this fraction.

The critical value for the adequacy of potassium in soil has been designated as 20 mg K kg^{-1} soil, based on studies conducted with wheat (Edwards, 1997). For all sites the concentration of potassium in the soil appeared to be adequate, based on the above critical value, although some variation was observed.

Table 2.5: Soil profile and analysis for the variety comparisons trials in 1999

Site	Soil depth (cm)	Texture	Colour	NO_3-N (mg/kg)	NH_4-N (mg/kg)	Colwell Phosphorus (mg/kg)	Colwell Potassium (mg/kg)	Organic carbon (%)	pH (H_2O)
Lowbank	0-10	Sandy loam	Light Brown	18	4	9	232	0.32	8.80
	10-20	Sandy loam	Light Brown	5	3	4	143	0.23	8.90
	20-30	Sandy loam	Light Brown	4	3	2	140	0.16	8.80
	30-40	Sandy loam	Light Brown	4	3	4	122	0.16	8.90
	40-50	Sandy loam	Light Brown	4	3	2	148	0.12	9.10
	50-60	Sandy loam	Light Brown	4	3	2	178	0.14	9.30
	60-70	Sandy loam	Light Brown	3	3	2	236	0.12	9.20
	70-80	Sandy loam	Light Brown	3	3	2	266	0.09	9.20
Cooke Plains	0-10	Sandy loam	Grey	30	9	26	99	0.51	6.30
	10-20	Sandy loam	Light Grey	13	4	14	110	0.24	7.00
	20-30	Sandy loam	Grey Brown	8	4	9	104	0.14	7.00
	30-40	Sandy loam	Light Brown	6	3	11	85	0.10	7.10
	40-50	Sandy loam	Light Brown	4	3	6	101	0.10	7.10
	50-60	Sandy loam	Light Brown	5	3	5	137	0.13	7.00
	60-70	Sandy loam	Light Brown	3	3	6	123	0.09	7.20
	70-80	Sandy loam	Light Brown	5	3	3	112	0.14	7.60
Minnipa	0-10	Sandy Loam	Brown	27	3	13	176	0.55	8.30
	10-20	Loam	Brown	13	3	7	134	0.26	8.50
	20-30	Loam	Brown	10	3	3	112	0.22	8.60
	30-40	Loam	Brown	10	3	8	92	0.24	8.60
	40-50	Loam	Brown	6	3	3	92	0.19	8.70
	50-60	Loam	Brown	5	3	3	91	0.23	8.70
	60-70	Loamy Clay	Light Brown	4	3	3	115	0.25	8.70
	70-80	Loamy Clay	Light Brown	4	3	3	154	0.24	8.70

Table 2.6: Soil profile and analysis for the variety comparisons trials in 2000

Site	Soil depth (cm)	Texture	Colour	NO_3-N (mg/kg)	NH_4-N (mg/kg)	Colwell Phosphorus (mg/kg)	Colwell Potassium (mg/kg)	Organic carbon (%)	pH (H_2O)
Lowbank	0-10	Sandy Loam	Light Brown	14	2	15	175	0.24	8.60
	10-20	Sandy Loam	Light Brown	17	1	7	159	0.10	8.80
	20-30	Sandy Loam	Light Brown	11	1	9	150	0.12	8.80
	30-40	Sandy Loam	Light Brown	9	3	3	140	0.10	8.80
	40-50	Sandy Loam	Light Brown	8	3	3	132	0.11	9.00
	50-60	Sandy Loam	Light Brown	8	3	2	121	0.09	8.90
	60-70	Sandy Loam	Light Brown	8	3	3	127	0.01	9.00
Cooke Plains	0-10	Sandy Loam	Grey Brown	21	9	30	75	0.46	6.20
	10-20	Sandy Loam	Grey	15	5	23	56	0.22	6.20
	20-30	Sandy Loam	Brown White	3	3	10	28	0.01	6.30
	30-40	Sandy Loam	Brown White	3	2	11	42	0.01	6.60
	40-50	Sandy Loam	Brown White	3	4	10	43	0.01	6.70
	50-60	Sandy Loam	Brown White	5	3	9	49	0.01	6.80
	60-70	Sandy Loam	Brown Yellow	4	2	7	60	0.01	6.8

As is typical for sandy soils, the level of soil organic carbon (S.O.C) was extremely low, especially in the deeper fractions of the soil profile. For a sandy loam, values less than 0.7% S.O.C are considered low, while a value less than 0.5% S.O.C is considered low for sand (Hughes *et al.*, 1996).

The pH of the soil has crucial implications for the environment around the roots, and hence the availability of nutrients (Slattery *et. al.*, 1999). The soil at Cooke Plains ranged from very slightly acidic at the surface to neutral down the soil profile. At Lowbank and Minnipa where the soils were of an alkaline to highly alkaline nature, micro-nutrient deficiencies are more common and boron toxicity may be evident. At an extremely high pH (>8.5), exchangeable sodium dominates. The presence of sodium carbonate can cause a further decline in nutritional status and soil structure.

2.3.2 Comparison of Cereal species on sandy soils

Measurement of coleoptile length highlighted differences between cereal species for this characteristic. Barley, in general, had the longest coleoptile length, although there was some variation between varieties (Table 2.7). Galleon had the longest coleoptile, while Sloop had the shortest of the barley varieties. Of the other cereal species Cereal rye (Bevy) had a coleoptile length similar to Sloop. Triticale (Tahara), and especially Frame (wheat) had the shortest coleoptile length of any of the cereals. Seed size did not define the variation in coleoptile length, since varietal differences remained statistically significant even when adjusting for seed size. The high coefficient of variation (CV) for each variety highlights that the range in coleoptile length within replicates was quite large. Nevertheless the average seed weight of barley (48.1 mg) was greater than for the other cereals, which suggests seed weight may still contribute to the variation in coleoptile length.

Table 2.7: Coleoptile length and seed size for 7 barley varieties, and Bevy (cereal rye), Frame (wheat) and Tahara (triticale). Values appended by the same superscript letter are not significantly different at $P \leq 0.05$.

	Coleoptile Length (cm)	Seed Size (mg)	Average CV (%)
Galleon	86.19^{a}	48.71	23.5
Barque	79.74^{a}	46.17	16.9
Forrest	68.83^{b}	50.95	16.7
Keel	67.86^{b}	48.42	18.2
Mundah	66.24^{b}	53.54	25.4
Clipper	63.82^{bc}	43.48	22.2
Bevy	58.41^{c}	16.88	37.0
Sloop	58.00^{c}	45.30	19.7
Tahara	50.82^{d}	33.62	44.5
Frame	38.05^{e}	30.64	50.7
LSD (0.05)	***5.72***		

To ensure that appropriate comparisons between cereal species could be achieved, sowing rates were adjusted according to average seed size and germination percentage to give the recommended plant density for each species. Bevy rye established better at all sites except

Minnipa, followed by barley, which was better than or equivalent (Minnipa) to Frame wheat (Table 2.8). Triticale had significantly low establishment counts at all sites. In all cases the barley varieties likely to perform well on sand (Mundah, Forrest and even Barque) expressed good early vigour, in terms of dry matter production, which was superior to Tahara and Frame, and equivalent to or better than Bevy (Table 2.8).

The range in dry matter production across barley varieties, regardless of adaptation was generally better than triticale and wheat, and equal to or better than cereal rye. Comparison between species for grain yield indicated that some interaction with environments existed. In general however, the barley varieties were lower yielding then cereal rye, except at Minnipa, and Mundah at Lowbank. In contrast, the barley varieties were generally higher yielding than triticale and wheat, except at Lowbank. However, Mundah, the highest yielding barley at this site, produced a grain yield equivalent to that of Frame wheat. The overall MET analysis (Figure 2.1) of the two seasons of data for grain yield indicated that cereal rye and triticale outperformed barley, while only Mundah yielded better than wheat. The common effect due to environment (y-axis) indicates that Mundah and Yagan were more environmentally stable than the other species.

Table 2.8: Comparison of barley with wheat, cereal rye and triticale for establishment, early vigour and grain yield at three sites in 1999. Data was analysed by REML analysis.

	Lowbank			Minnipa			Cooke Plains		
	Establishment (% plant density*)	Early Vigour ($g\ m^{-2}$)	Grain Yield ($t\ ha^{-1}$)	Establishment (% plant density)	Early Vigour ($g\ m^{-2}$)	Grain Yield ($t\ ha^{-1}$)	Establishment (% plant density)	Early Vigour ($g\ m^{-2}$)	Grain Yield ($t\ ha^{-1}$)
Barley#	77.8	19.20	0.40	101.3	54.06	1.35	75.7	18.63	1.86
(range)		*(15.81-26.93)*	*(0.32-0.53)*		*(49.75-64.11)*	*(1.17-1.70)*		*(14.05-24.14)*	*(1.56-2.19)*
Frame	62.6	12.83	0.55	101.3	45.13	0.97	75.7	14.78	1.63
Bevy	82.5	17.91	0.55	82.6	50.76	1.01	88.8	18.41	2.56
Tahara	24.2	11.33	0.61	60.4	43.49	1.12	38.4	13.94	1.77
LSD (0.05)		*4.10*	*0.08*		*15.59*	*0.24*		*7.34*	*0.26*

Mean of the 7 core barley varieties
* Target plant density at sowing = 145 plants m^{-2}

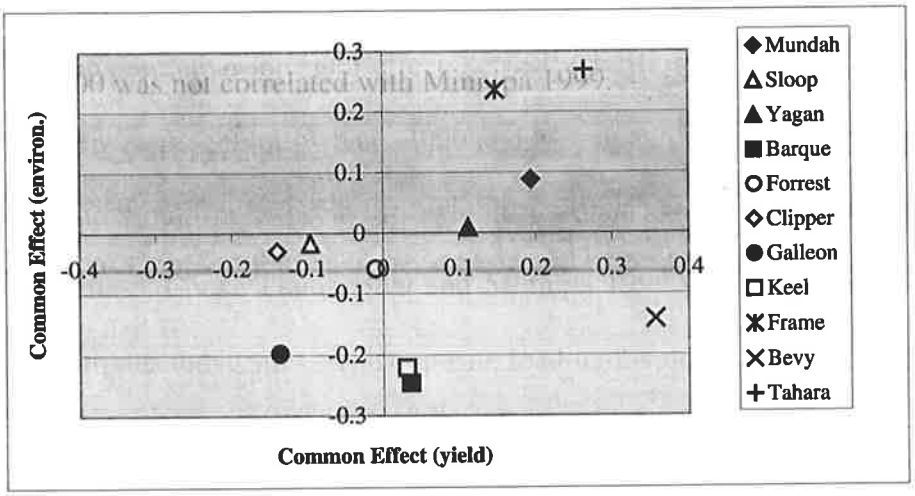


Figure 2.1: MET analysis of grain yield data for the 7 core barley varieties plus Yagan, and Bevy rye, Frame wheat and Tahara triticale in sand evaluation trials (1999-2000).

2.3.3 Barley

2.3.3.1 Sites

Figure 2.2 illustrates the calculated genetic correlations (MET analysis) between sites in terms of grain yield response. Loading 1 describes yield performance ranking across all sites. Loading 2 explains the environmental effects, effectively illustrating any lack of correlation between sites. Lowbank 1999, Lowbank 2000 and Minnipa 2000 were highly correlated, and therefore each site would be expected to exhibit a similar response in terms of grain yield rankings of varieties. Cooke Plains 1999 and Minnipa 1999 were also correlated, but were not correlated with the above sites. The opposite loading 2 values for these two sites suggests that the environmental response was distinctly different at these sites from Lowbank 1999, Lowbank 2000 and Minnipa 2000.

Cooke Plains 2000 had a low and negative loading 1, and was negatively correlated to most sites including Cooke Plains 1999. The poor relationship between this and all other sites was due mainly to a spring radiation frost event and considerable powdery mildew damage. Cooke Plains 2000 was not correlated with Minnipa 1999.

The loadings calculated by the MET analysis for each site based on grain yield also provide a guide to the amount of information used from each site in the overall analysis of the data. Data from Lowbank 1999, Lowbank 2000 and Minnipa 2000 displayed a ranking of varieties that was more indicative of sand adaptation as determined by long term grain yield in SARDI sand trials (Table 2.1). Cooke Plains 2000,Cooke Plains 1999 and Minnipa 1999, on the other hand, did not rank varieties in the expected way. Consequently the weighting of the grain yield data at these sites in the MET analysis was reduced.

In addition, MET analysis results also indicate that varieties with large positive common effects (yield) will perform well at sites with large positive loadings 1 (x-axis) and vice versa. Discussion in the section on grain yield below expands on this and provides full details on the best-performed varieties and those that were highly variable in terms of environmental response.

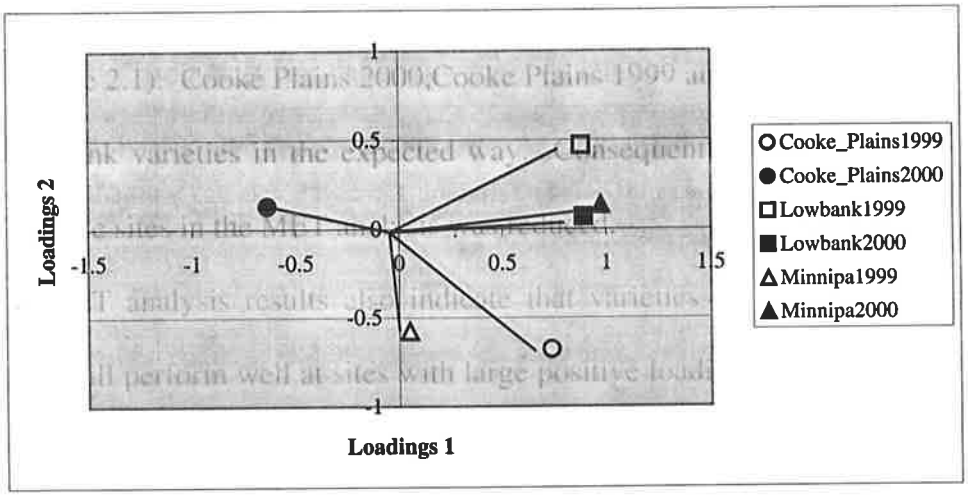


Figure 2.2: Plot of loadings calculated by MET analysis showing the relationship between all sites.

2.3.3.2 Establishment

Even with seeding rate being adjusted to accommodate seed weight and germination percentage differences, establishment varied between varieties (significance values listed in Tables 2.9a & b), sites and seasons (Table 2.10 & 2.11; Figure 2.3). Mundah displayed greater consistency across environments with establishment exceeding 90% of seeding density in 4 out of the 6 site x season trials. Even at Minnipa in 2000, where all varieties showed very poor establishment, Mundah was clearly superior with over 60% establishment. In 2000, the Western Australian varieties Yagan, O'Connor and Forrest also had better establishment than the SA varieties, except at Minnipa.

Table 2.9(a): Wald statistics from the REML analysis of various traits from the variety comparison trials, 1999.

Year	Trait	Site	Wald Statistic	d.f.	Significance
1999	Establishment	Minnipa	41.71	19	P=0.002
		Cooke Plains	63.47	19	P<0.001
		Lowbank	133.28	19	P<0.001
	E_DMP	Minnipa	41.37	19	P=0.002
		Cooke Plains	57.99	19	P<0.001
		Lowbank	151.40	19	P<0.001
	PER	Minnipa	100.24	19	P<0.001
		Cooke Plains	133.46	19	P<0.001
		Lowbank	141.57	19	P<0.001
	PUE	Minnipa	36.37	19	P=0.009
		Cooke Plains	89.66	19	P<0.001
		Lowbank	191.17	19	P<0.001
	Grain Yield	Minnipa	151.99	19	P<0.001
		Cooke Plains	650.39	35	P<0.001
		Lowbank	322.09	35	P<0.001

Table 2.9(b): Wald statistics from the REML analysis of various traits from the variety comparison trials, 2000.

Year	Trait	Site	Wald Statistic	d.f.	Significance*
2000	Establishment	Minnipa	15.13	8	P=0.057 ns
		Cooke Plains	19.52	8	P=0.012
		Lowbank	29.57	8	P<0.001
	E_DMP	Minnipa	10.24	8	P=0.248 ns
		Cooke Plains	20.88	8	P=0.007
		Lowbank	66.15	8	P<0.001
	PER	Minnipa	5.11	8	P=0.746 ns
		Cooke Plains	76.36	8	P<0.001
		Lowbank	11.76	8	P=0.162 ns
	PUE	Minnipa	9.08	8	P=0.336 ns
		Cooke Plains	27.65	8	P<0.001
		Lowbank	43.09	8	P<0.001
	Early Vigour	Minnipa	116.13	8	P<0.001
		Cooke Plains	338.37	8	P<0.001
		Lowbank	287.08	8	P<0.001
	SLA	Cooke Plains	19.10	8	P=0.014
		Lowbank	67.89	8	P<0.001
	LAR	Cooke Plains	29.59	8	P<0.001
		Lowbank	54.93	8	P<0.001
	LAI	Cooke Plains	74.57	8	P<0.001
		Lowbank	124.06	8	P<0.001
	A_DMP	Lowbank	42.12	8	P<0.001
	M_DMP	Lowbank	25.19	8	P<0.001
	Grain Yield	Minnipa	216.03	8	P<0.001
		Cooke Plains	38.97	8	P<0.001
		Lowbank	74.30	8	P<0.001
	1000 grain weight	Minnipa	1275.34	8	P<0.001
		Cooke Plains	16.13	8	P=0.041
		Lowbank	359.34	8	P<0.001
	Screenings	Minnipa	775.74	8	P<0.001
		Cooke Plains		8	
		Lowbank	1268.65	8	P<0.001

* ns = not significant

Table 2.10: Comparison of 7 barley varieties for establishment, early vigour and grain yield at three sites in 1999. Data was analysed by REML and MET.

	Lowbank			Minnipa			Cooke Plains		
	Establishment (% plant density*)	Early Vigour ($g\ m^{-2}$)	Grain Yield (t ha)	Establishment (% plant density)	Early Vigour ($g\ m^{-2}$)	Grain Yield ($t\ ha^{-1}$)	Establishment (% plant density)	Early Vigour ($g\ m^{-2}$)	Grain Yield ($t\ ha^{-1}$)
Barque	77	20.20	0.39	96	55.37	1.37	68	19.60	2.19
Clipper	64	15.93	0.36	100	49.75	1.17	66	15.63	1.56
Forrest	67	17.17	0.41	96	51.08	1.22	70	16.57	1.82
Galleon	77	18.1	0.32	100	53.36	1.31	76	19.31	1.82
Mundah	99	26.93	0.53	100	64.11	1.46	82	21.11	1.93
Keel	76	20.20	0.39	91	54.92	1.70	89	24.14	2.14
Sloop	84	15.81	0.38	100	49.84	1.26	78	14.06	1.61
LSD (0.05)		*4.10*	*0.08*		*15.59*	*0.24*		*7.34*	*0.26*

* Target plant density at sowing = 145 plants m^{-2}

Table 2.11: Comparison of 9 barley varieties for establishment, early vigour and grain yield at three sites in 2000. Data was analysed by REML and MET.

	Lowbank			Minnipa			Cooke Plains		
	Establishment (% plant density*)	Early Vigour ($g\ m^{-2}$)	Grain Yield ($t\ ha^{-1}$)	Establishment (% plant density)	Early Vigour ($g\ m^{-2}$)	Grain Yield ($t\ ha^{-1}$)	Establishment (% plant density)	Early Vigour ($g\ m^{-2}$)	Grain Yield ($t\ ha^{-1}$)
Barque	93	68.40	1.37	51	54.71	1.30	81	64.63	1.40
Clipper	92	57.86	1.22	53	49.61	1.07	86	68.79	1.67
Forrest	100	60.95	1.22	52	51.09	1.31	100	70.07	1.38
Galleon	92	63.37	1.20	50	52.28	1.00	92	68.99	1.61
Mundah	100	85.15	1.52	65	62.80	1.68	100	70.72	1.47
Keel	85	68.40	1.32	47	54.72	1.35	83	67.37	1.58
Sloop	88	57.63	1.29	49	49.48	1.11	84	58.33	1.59
O'Connor	100	58.25	1.31	50	49.79	1.45	94	61.48	1.53
Yagan	100	83.74	1.45	55	62.12	1.52	91	69.83	1.48
LSD (0.05)		*13.27*	*0.14*		*19.34*	*0.14*		*16.36*	*0.25*

* Target plant density at sowing = 145 plants m^{-2}

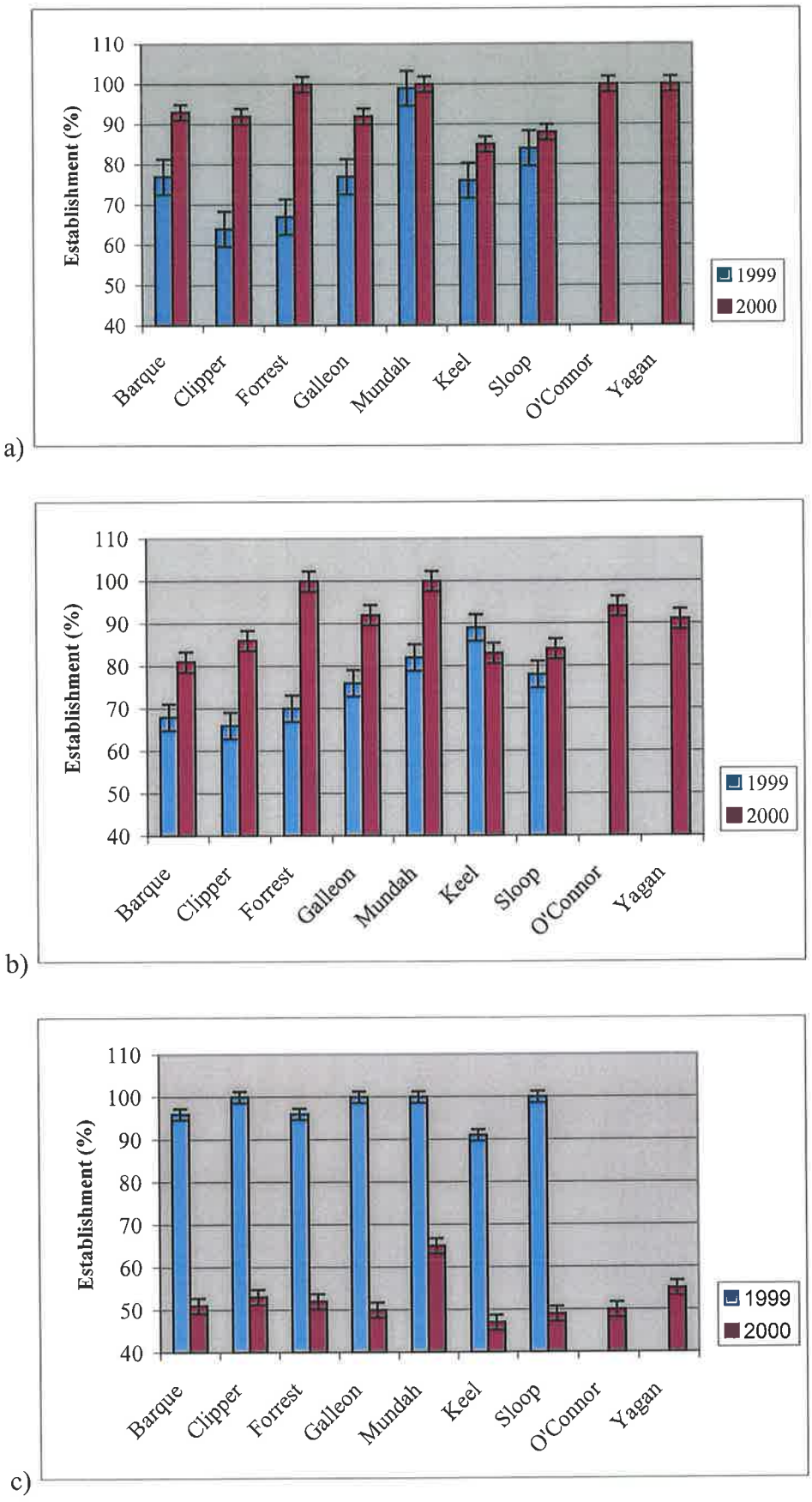


Figure 2.3: Establishment (percentage of seeding density) of 9 barley varieties at a) Lowbank, b) Cooke Plains and c) Minnipa. Data analysed by REML. Error bars are SED at $P \leq 0.05$

2.3.3.3 Early vigour

Improved grain yield potential of barley, and wheat, has been found to be associated with greater early vigour under moisture-limiting conditions (Brown *et al.*, 1987; Ceccarelli, 1987; Acevedo *et al.*, 1991) and in terms of drought tolerance on light textured soils (Turner and Nicolas, 1987). On sandy soils, improvement in the grain yield potential of barley may also be associated with superior early vigour (E_DMP and/or LAD), considering all the sand adapted varieties are exceptional for this trait (Table 2.2).

2.3.3.3.1 Early dry matter production

MET analysis of E_DMP highlighted early vigour as a stable trait across environments (Figure 2.4). This indicated that the ranking of varieties was unlikely to vary to any large extent, and that real genetic variance was evident, although small, and stable, on sandy soils. The h^2 of E_DMP was generally moderate to low (below 50%). This was due to low genetic variance and most likely, because the environmental variance was low, high experimental error. Lowbank 2000 was the exception, where the h^2 of E_DMP was 75.9%. Mundah and Yagan had significantly greater E_DMP (significance values listed in Tables 9a & b) than the other lines tested in the evaluation trials (Tables 2.10 & 2.11). The varieties with the poorest early vigour were Clipper and Sloop. Barque and Keel had intermediate early vigour. Forrest was inconsistent with its WA counterparts, and was even inferior to Galleon. Table 2.12 shows the overall means for the varieties from the MET analysis.

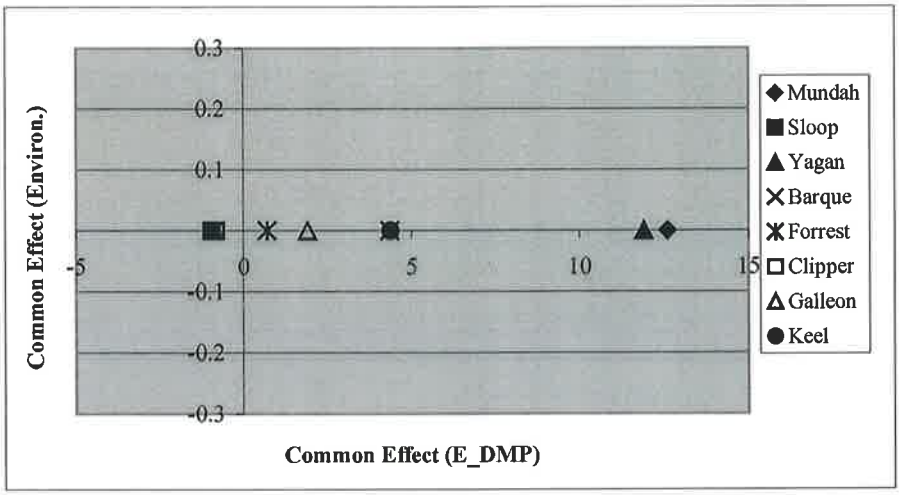


Figure 2.4: MET analysis of early vigour for the 7 core barley varieties and Yagan in sand evaluation trials (1999-2000).

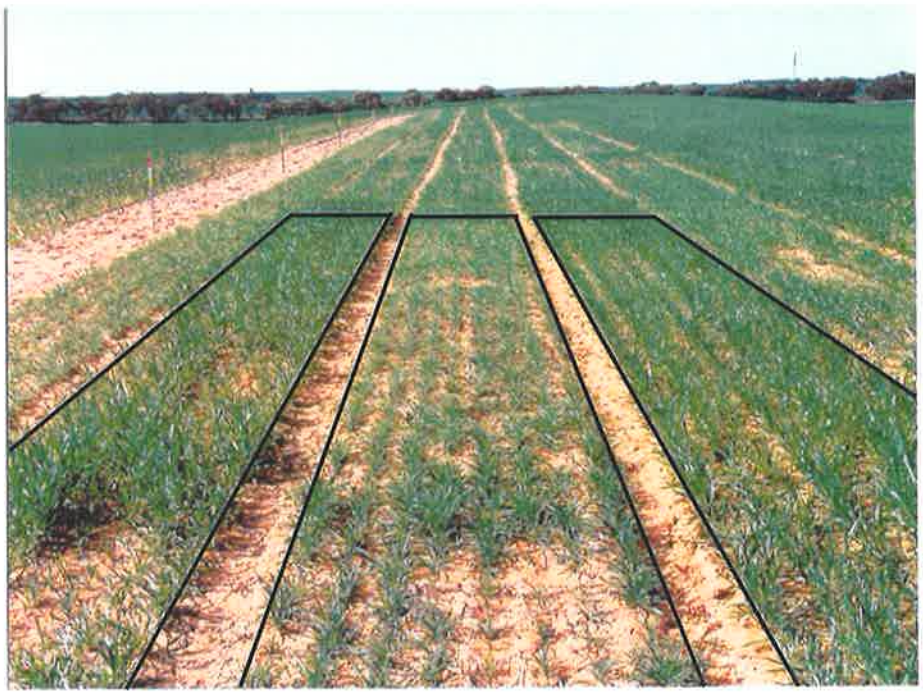


Plate 2.1: View of Yagan (left), Galleon (middle), and Mundah (right) showing differences in E_DMP, leaf area development and growth habit at Lowbank in 2000.

Table 2.12: Mean early vigour (E_DMP) of the 7 core barley varieties, and Yagan and O'Connor, at early tillering for all sites (1999-2000). Data analysed by MET.

Variety	Early Vigour ($g\ m^{-2}$)
Mundah	55.14
Yagan	54.58
Keel	48.29
Barque	47.15
Galleon	45.91
Forrest	44.49
Clipper	42.93
O'Connor	41.98
Sloop	40.86

2.3.3.3.2 Leaf Area

Mundah, Yagan and Forrest were superior in terms of SLA (Figure 2.5), LAR (Figure 2.6) and LAI (Table 2.13) at early tillering (significance values listed in Tables 9b). In contrast, Keel and Galleon displayed the lowest rankings for all three traits. For these five varieties SLA, LAR and LAI were stable between sites. The pattern was less concise, however, for Barque, Sloop, O'Connor and Clipper.

Covariate analysis (Table 2.14) indicates that the higher LAI of Mundah and Yagan contributed towards their high E_DMP at Lowbank ($P<0.001$; Figure 2.7a). However, a higher LAI, up to a value of 1, does not appear to significantly improve E_DMP. Covariate analysis also identified that even with adjusting for SLA and LAR, Mundah and Yagan had significantly greater E_DMP ($P<0.001$). In other words, their high SLA and LAR contributed to their superior early vigour. Although Keel, Galleon and Clipper displayed high E_DMP, similar to Mundah, Yagan and Forrest, despite having lower LAI (Figure 2.7b), the benefit of greater canopy development was also valid at Cooke Plains ($P<0.01$; Table 2.14).

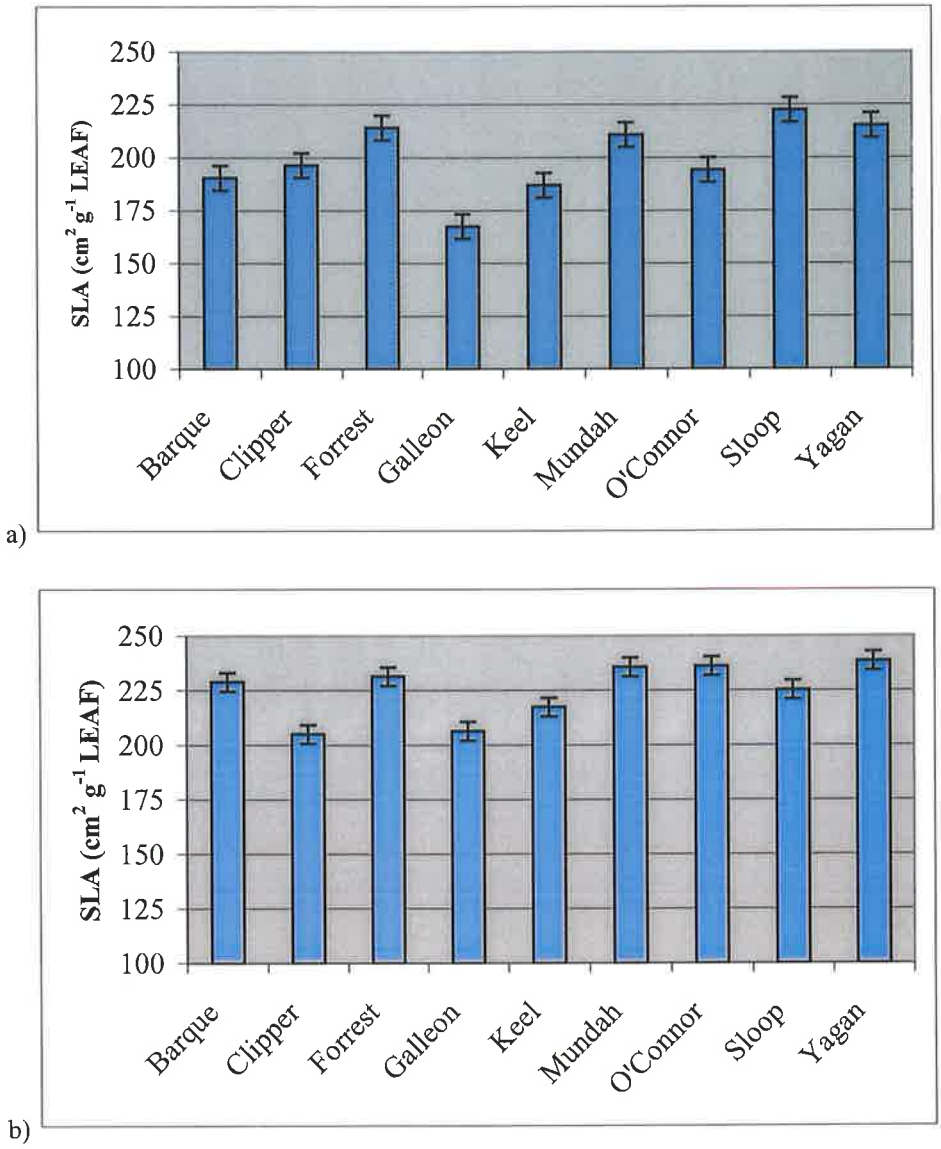


Figure 2.5: Specific leaf area (SLA) of 9 varieties at a) Lowbank & b) Cooke Plains in 2000. Data analysed by REML.

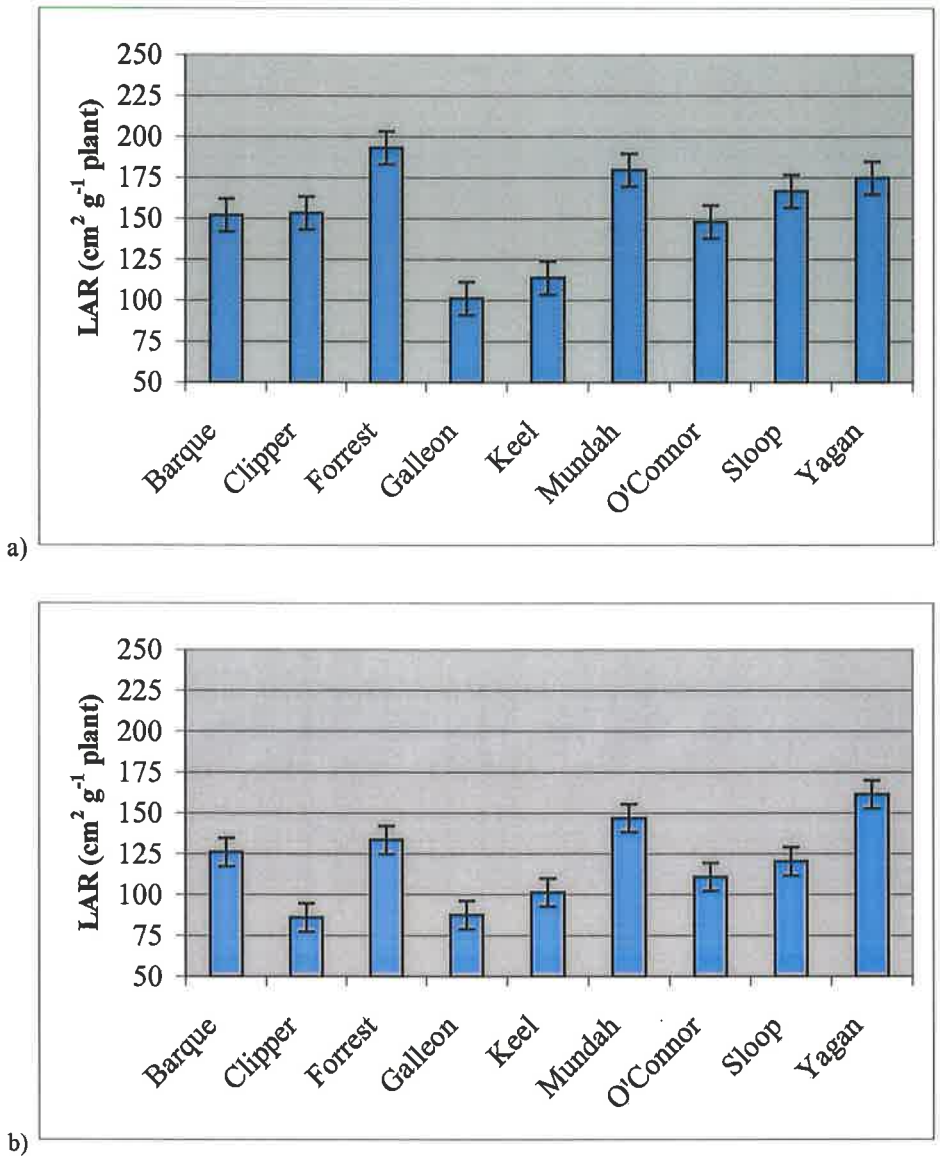


Figure 2.6: Leaf area ratio (LAR) of 9 varieties at a) Lowbank & b) Cooke Plains in 2000. Data analysed by REML.

Table 2.13: Leaf area index (LAI) of 9 varieties at Lowbank and Cooke Plains in 2000. Data analysed by REML.

Variety	LAI (cm^2 m^{-2})	
	Lowbank	Cooke Plains
Mundah	1.461a	1.051a
Yagan	1.362a	1.001a
Forrest	1.005b	0.946a
Sloop	0.932b	0.549b
O'Connor	0.833b	0.671b
Clipper	0.820b	0.679b
Barque	0.772bc	0.699b
Keel	0.529c	0.571b
Galleon	0.513c	0.576b
LSD(0.05)	*0.278*	*0.244*

Table 2.14: Wald statistics from the covariate analysis (REML) of early vigour (E_DMP) at Lowbank and Cooke Plains in 2000.

Site	Trait	Covariate	d.f.	Wald Statistic	Significance
Lowbank	Early vigour	LAI	1	53.24	P<0.001
Cooke Plains	Early vigour	LAI	1	8.84	P<0.01

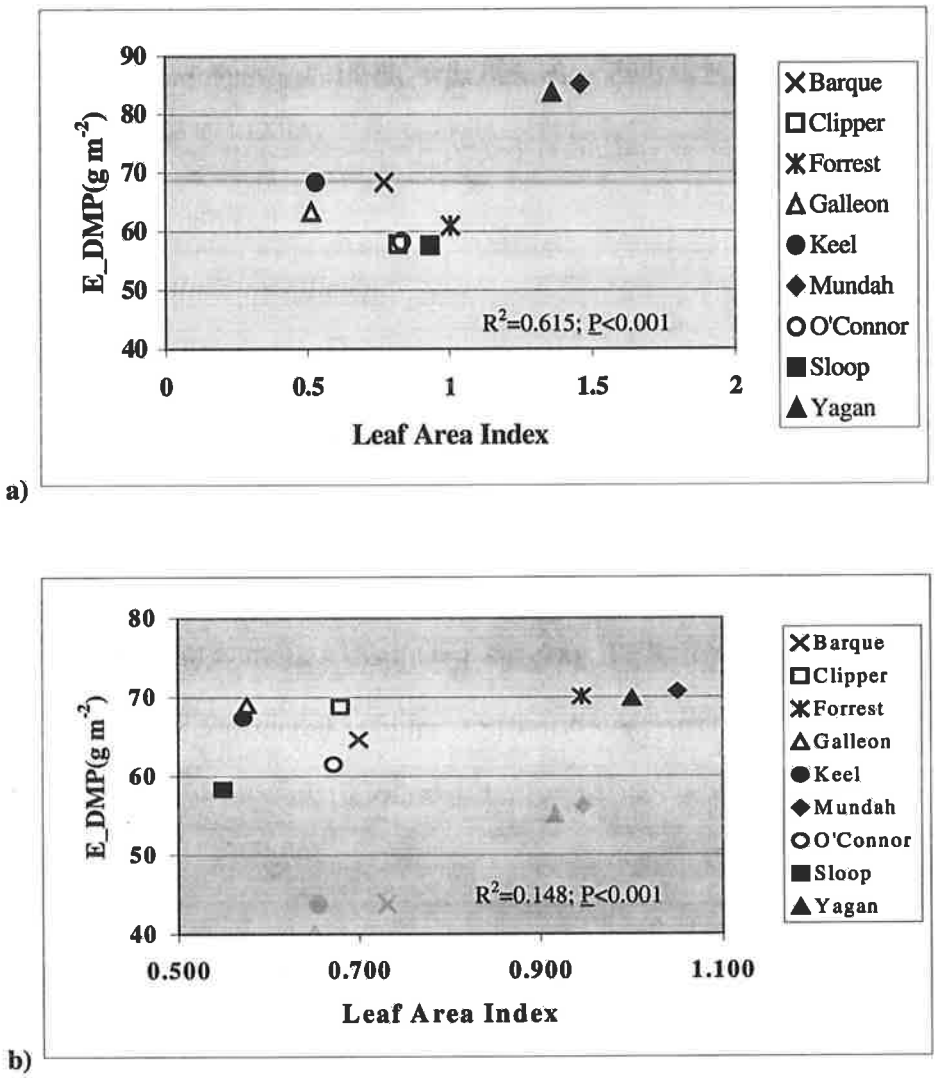


Figure 2.7: Relationship between E_DMP and leaf area index at a) Lowbank & b) Cooke Plains in 2000. Data analysed by REML.

2.3.3.3.3 Phosphorus utilisation efficiency

Overall means for both PER (Table 2.15) and PUE (Table 2.16) indicate that Mundah was the most efficient in utilising phosphorus (*i.e.* the utilisation of P for growth), and Sloop and Clipper the least efficient. In terms of PER Mundah was surpassed by Keel at Cooke Plains and Minnipa 1999, Galleon at Lowbank 2000, and Forrest at Minnipa 2000. With the exception of Galleon, all varieties displayed superior PER to Mundah at Minnipa 1999. However, when PUE is considered (Figure 2.8) only Keel at Cooke Plains 1999 exhibited

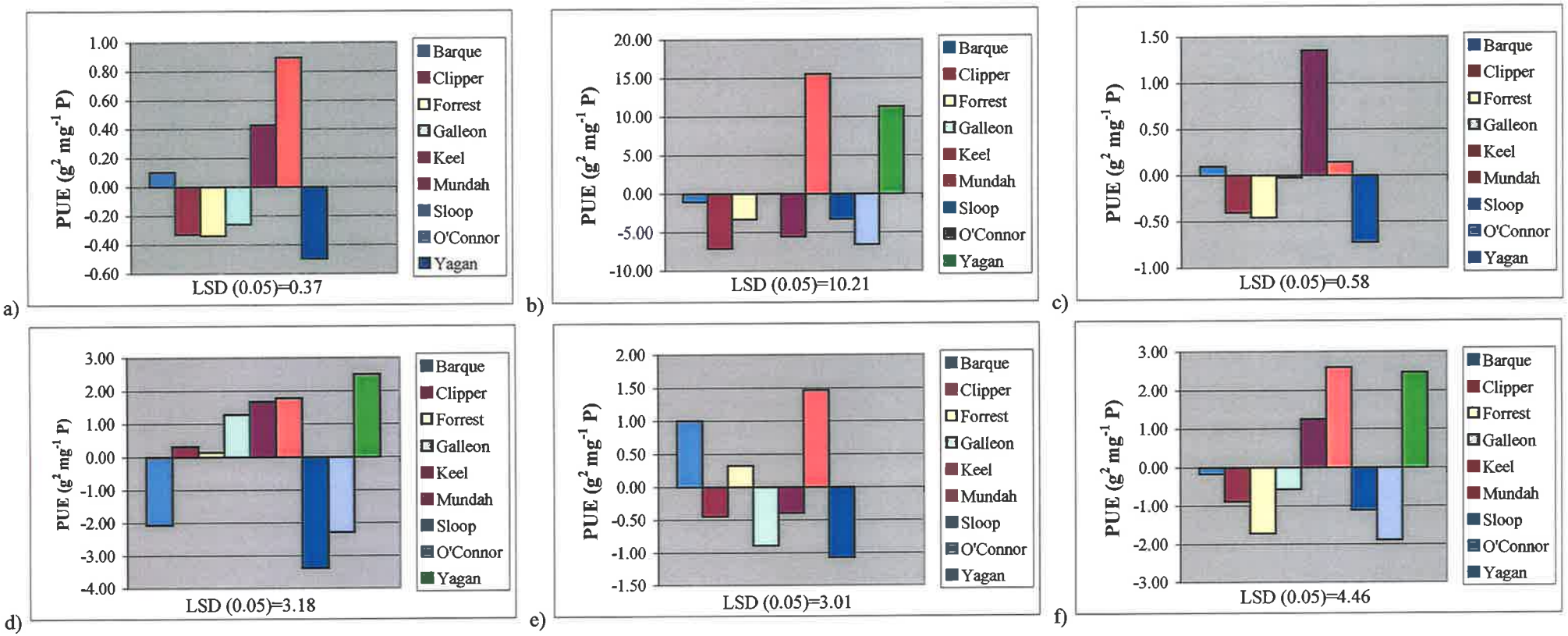


Figure 2.8: Deviation of PUE (g^2 mg^{-1} P) from site mean, of the core varieties sown at a) Lowbank 1999, b) Lowbank 2000, c) Cooke Plains 1999, d) Cooke Plains 2000, e) Minnipa 1999 and f) Minnipa 2000. Data analysed by MET analysis.

significantly better efficiency of utilisation than Mundah. The PUE for Mundah was greater than the site mean at all sites, Keel was below the site mean at two sites, and Sloop, the least efficient variety, was below the site mean at all sites. Clipper and Galleon displayed PUEs equal to, or below, the site means, while Forrest was lower than the site mean at 4 of the 6 sites. Barque was interesting in that, while its ranking suggested moderate utilisation efficiency, in relation to the varieties examined, it had PUEs greater than the site means in 1999, but inferior in 2000. Both Yagan and O'Connor were ranked the highest for PER and PUE, however, they were tested in 2000 only. The generally superior early growth of the varieties in 2000, compared to 1999, resulted in inflated means for Yagan and O'Connor. The results relating to the utilisation efficiency of O'Connor may be misleading, since its ranking in 2000 at each site suggests poor efficiency in terms of PER and PUE.

Table 2.15: PER* (g mg^{-1} P) of 9 varieties, 1999-2000. Data analysed by REML.

	Cooke Plains 1999	Lowbank 1999	Minnipa 1999	Cooke Plains 2000	Lowbank 2000	Minnipa 2000	Mean PER
Barque	0.079b	0.080b	0.179ab	0.170c	0.590ab	0.218a	0.219
Clipper	0.072cd	0.077b	0.187a	0.169c	0.555b	0.217a	0.213
Forrest	0.064d	0.075b	0.192a	0.170c	0.605ab	0.228a	0.222
Galleon	0.076bc	0.080b	0.134c	0.185bc	0.683a	0.210a	0.228
Keel	0.096a	0.099a	0.170ab	0.210a	0.544b	0.207a	0.221
Mundah	0.085b	0.095a	0.164b	0.193b	0.626ab	0.228a	0.232
Sloop	0.070cd	0.080b	0.171ab	0.178bc	0.603ab	0.210a	0.219
O'Connor	N/A	N/A	N/A	0.176c	0.552b	0.213a	0.314#
Yagan	N/A	N/A	N/A	0.212a	0.613ab	0.227a	0.351#
Mean	**0.077**	**0.084**	**0.171**	**0.185**	**0.597**	**0.218**	
LSD (0.05)	*0.010*	*0.010*	*0.022*	*0.016*	*0.102*	*0.030*	

N/A-not applicable

Overall mean for Yagan and O'Connor is for 3 sites only (2000)

Table 2.16: Mean PUE† (g^2 mg^{-1} P) of 9 varieties, 1999-2000. Data analysed by REML.

	Mean PUE
Barque	11.51
Clipper	10.39
Forrest	10.97
Galleon	11.79
Keel	11.65
Mundah	15.60
Sloop	10.18
O'Connor	15.83#
Yagan	24.86#

Three sites only (2000)

†PUE = PER * Above ground dry matter (g)

2.3.3.4 Anthesis and maturity dry matter production

Several authors have suggested that dry matter production at anthesis is associated with early vigour (Turner and Nicolas, 1987; Brown *et al.*, 1987; Ceccarelli, 1987; Acevedo *et al.*, 1991; Whan *et al.*, 1991; Annicchiarico and Pecetti, 1995; El Hafid *et al.*, 1998; Simane *et al.*, 1998). The analysis of dry matter at early tillering, anthesis and maturity at Lowbank, while indicating that dry matter production increased with developmental stage (Figure 2.9d), produced no discernible relationship between: dry matter production at maturity and anthesis; dry matter production at anthesis and early dry matter production; and dry matter production at maturity and early dry matter production (Figure 2.9a, b & c). In other words, the superior early vigour of Mundah and Yagan did not translate into superior biomass production at anthesis and maturity. Yagan, in particular had poor dry matter production at anthesis (significance values listed in Tables 9b), before recovering to be equivalent to Mundah at maturity (Figure 2.9d). In contrast Clipper, with poor early vigour, had the highest production of dry matter at anthesis, which remained steady through to maturity.

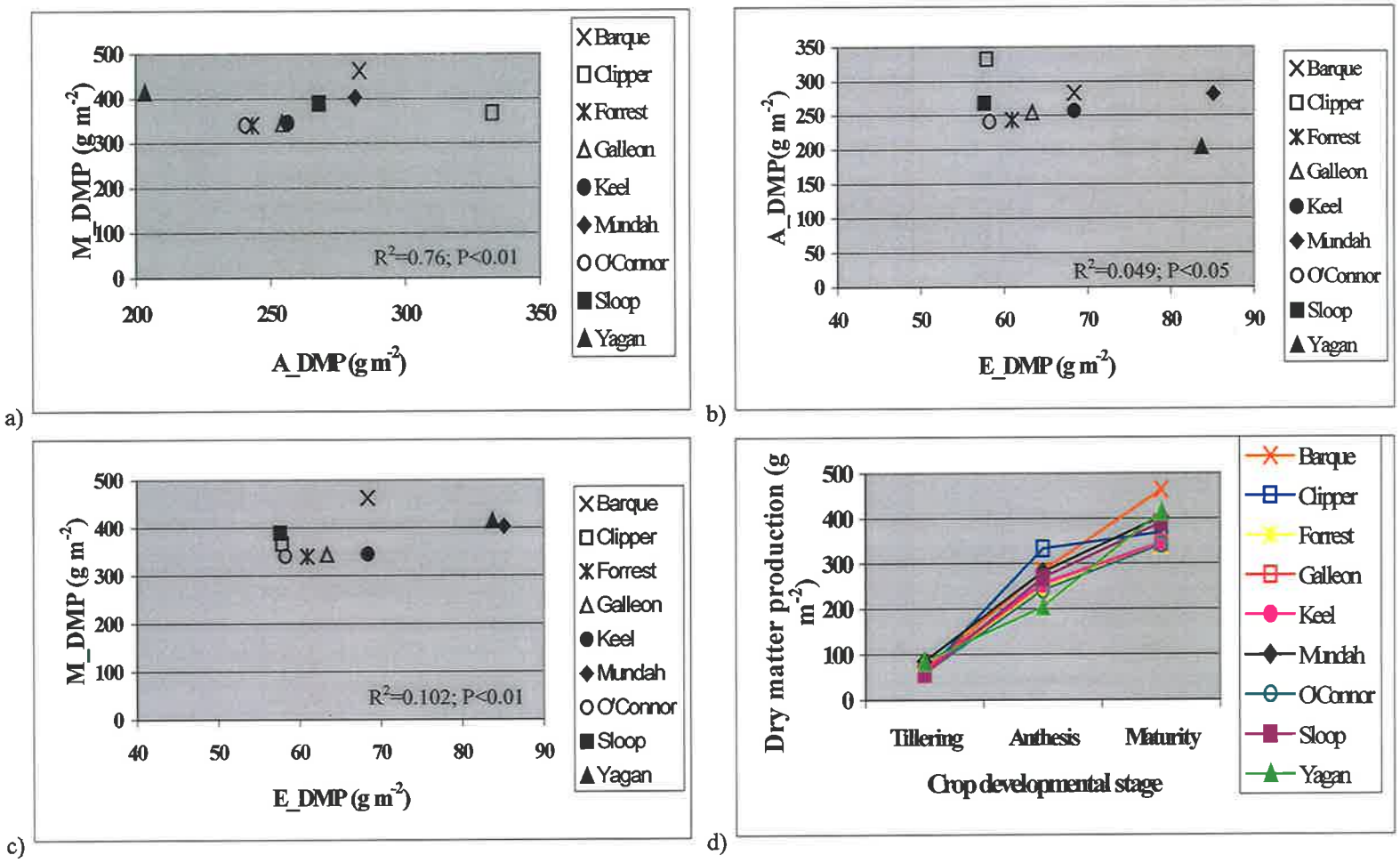


Figure 2.9: Relationship between a) maturity and anthesis, b) anthesis and early tillering, c) maturity and dry matter production early tillering, and d) dry matter production with crop developmental stage at Lowbank, 2000. Data analysed by REML.

Sampling was also conducted at Cooke Plains; however the incidence of frost and powdery mildew, which impacted on crop development, would mean that any relationship between dry matter production at anthesis, maturity and at early tillering is likely to be confounded by these factors, rather than a sand adaptation effect. Consequently, no data is presented for Cooke Plains.

2.3.3.5 Fructan and ESC

REML analysis indicated significant varietal differences ($P<0.001$) in fructan content at anthesis and in terms of the change in content between anthesis and maturity, and from this presumably utilization in grain filling (Figure 2.10). In addition, the ranking of varieties for utilisation of fructan was consistent with total content at anthesis, yet ranking did not necessarily reflect adaptation. Yagan ranked at the top, while Mundah was moderately low for both the content of fructan at anthesis and the change in amount between anthesis and maturity. Barque, which shows improved adaptation relative to other SA varieties, was very similar to Mundah. Sloop also ranked highly for fructan, although not considered adapted to sandy soils. Poorly adapted varieties such as Galleon and Clipper ranked mid-range for fructan content. Keel, also poorly adapted, had the lowest ranking of all the varieties evaluated.

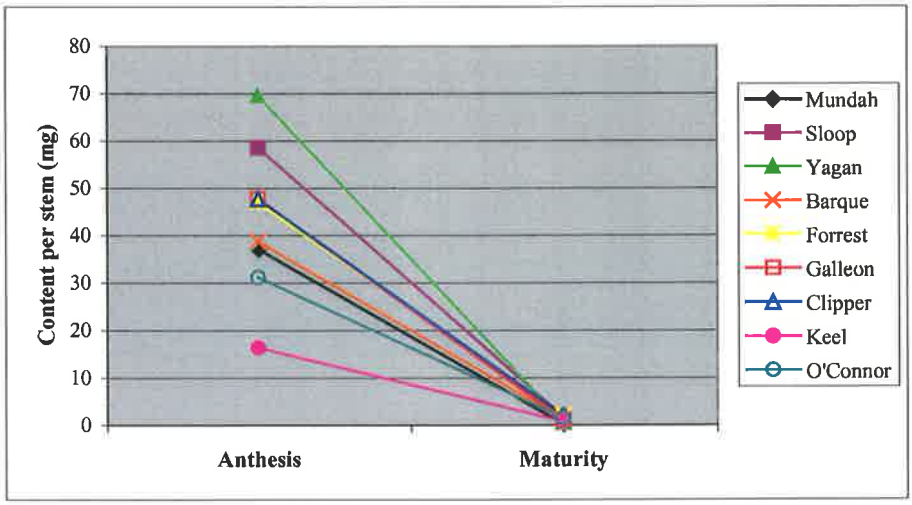


Figure 2.10: Total fructan content per stem of 9 varieties at anthesis and maturity. Site: Lowbank, 2000. Data analysed using REML analysis.

Figure 2.11 illustrates the content of the low molecular weight carbohydrates or ESC (sucrose, fructose & glucose) measured at anthesis and maturity. Varietal differences in content were significant at anthesis ($P<0.001$) and maturity ($P=0.016$, $P=0.002$ for glucose), as was the difference in content between anthesis and maturity (utilisation) ($P<0.001$). At anthesis the ranking of varieties was similar for each ESC, and they could be divided into three groups. The group with the highest amount of ESC at anthesis included Mundah, Yagan and Clipper. Sloop, Barque and Forrest made up the group with moderate levels of the ESC, relative to the top group, and Keel, O'Connor and Galleon had the lowest amount of ESC. ESC content at anthesis, and utilisation, did in general reflect the adaptation of the variety, although there were a couple of notable exceptions. The utilisation of ESC (Table 2.17) for both Sloop and Clipper was high, despite poor adaptation, while for Forrest utilisation of ESC was lower than Barque, but equivalent to Keel. Although Keel showed an improved utilisation of ESC over Galleon and O'Connor, it was still lower than Barque. Regardless of final ESC content per stem, within each variety, each ESC fraction contributed proportionally the same amount to the total ESC content at anthesis (Figure2.12) and maturity, and for the total amount utilised per stem.

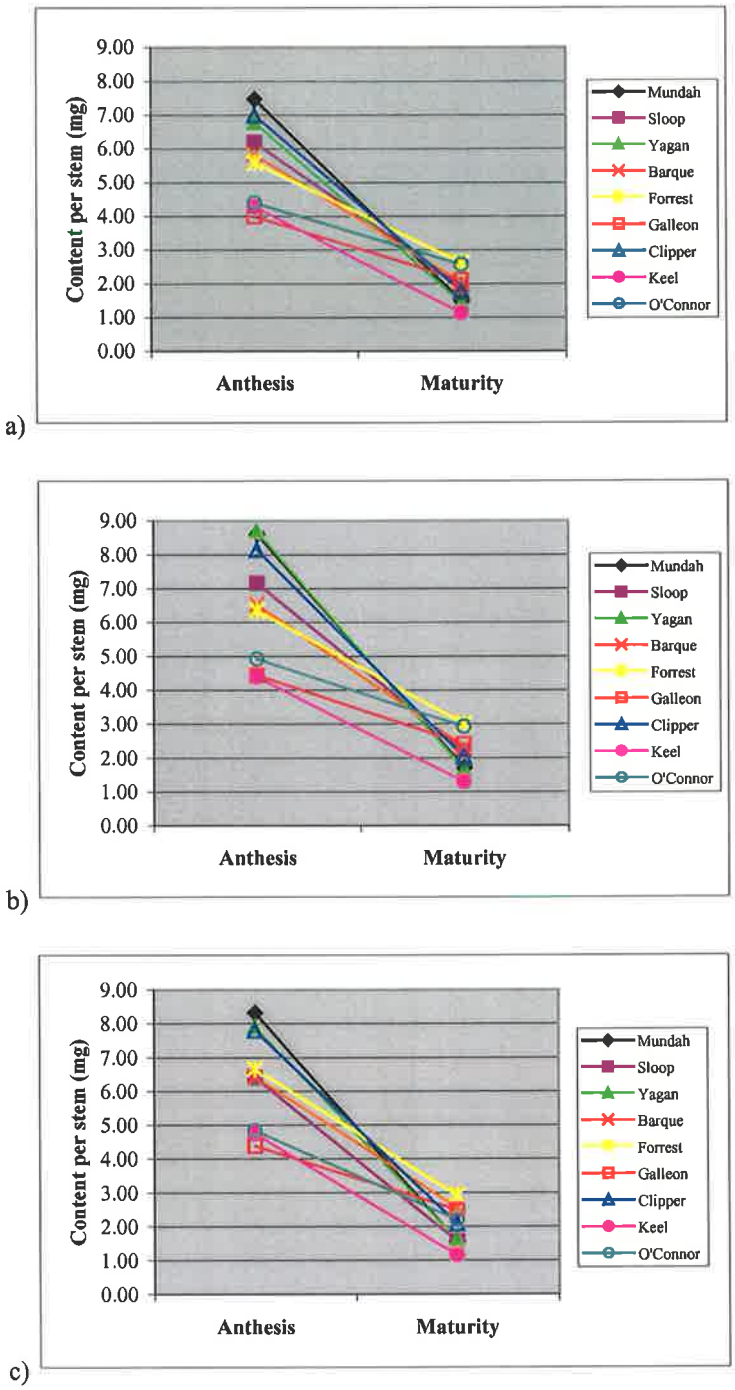


Figure 2.11: Content per stem of a) Sucrose, b) Fructose & c) Glucose for 9 varieties at anthesis and maturity. Site: Lowbank, 2000. Data analysed using REML.

Table 2.17: Utilisation of Sucrose, Fructose and Glucose (mg) for 9 varieties at Lowbank, 2000. Data analysed using REML.

Variety	Sucrose	Glucose	Fructose
Barque	3.600b	4.000bc	4.122bc
Clipper	5.006a	5.563ab	5.731ab
Forrest	3.063bcd	3.403cde	3.506cde
Galleon	1.508d	1.675e	1.726e
Keel	3.162bc	3.513cd	3.620cd
Mundah	6.068a	6.743a	6.948a
O'Connor	1.741cd	1.935de	1.993de
Sloop	4.630ab	5.146ab	5.302abc
Yagan	5.668a	6.298a	6.489a
LSD (0.05)	*1.595*	*1.772*	*1.826*

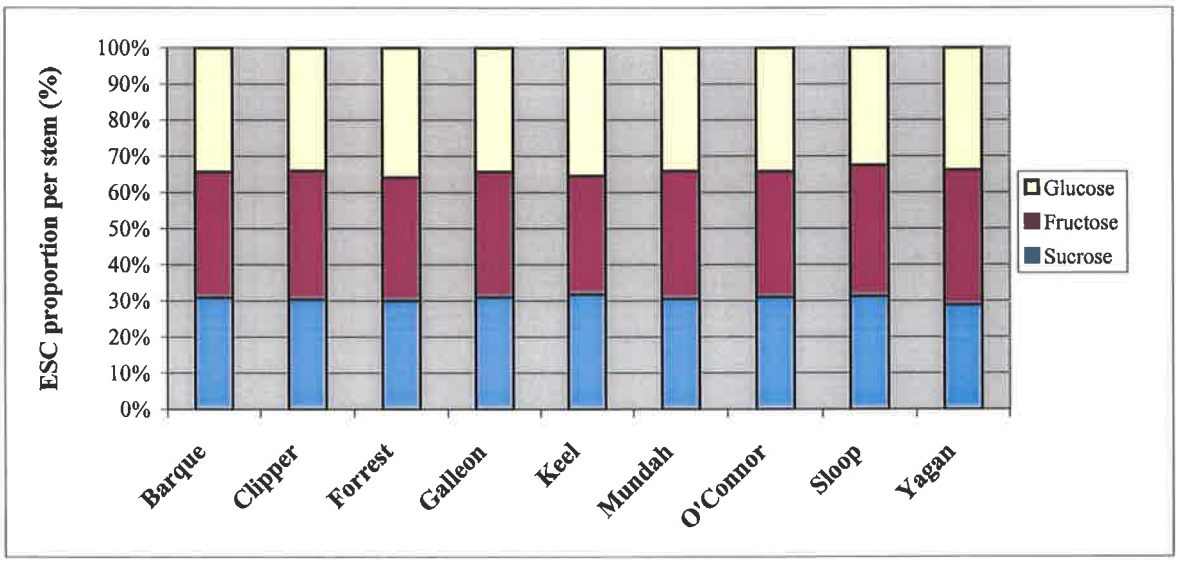


Figure 2.12: The contribution of sucrose, frutose and glucose to total ESC content per stem for 9 varieties at anthesis. Site: Lowbank, 2000. Data analysed using REML.

On combining fructan and ESC content, to calculate a total measure of non-structural carbohydrate at anthesis and maturity, a new picture emerged to distinguish varieties (Table 2.18). Yagan, Mundah and Forrest exhibited the greatest change in content between anthesis and maturity. Barque, Clipper and Galleon had a moderate shift in content, while Keel and O'Connor displayed the smallest change in total carbohydrate content. Sloop was an exception with the utilisation of total carbohydrate content slighly better than Mundah, but less than Yagan. From this, a general conclusion can be drawn to suggest that varieties with good adaptation displayed a higher utilisation of total non-structural carbohydrate ($\underline{P}<0.001$).

Table 2.18: Total non-structural carbohydrate content per stem of 9 varieties at anthesis, maturity and utilisation. Site: Lowbank, 2000. Data analysed using REML †.

Variety	Total non-structural carbohydrate (mg)		
	Anthesis	Maturity	'Utilisation'
Yagan	92.29a	5.48bcd	86.07a
Sloop	77.66ab	7.93bcd	73.31ab
Mundah	62.2bc	4.34cd	68.26b
Forrest	65.17bc	11.89a	63.67bc
Clipper	69.73bc	7.91bcd	58.59bc
Galleon	61.59bcd	8.47ab	50.14c
Barque	57.84cd	8.54ab	47.63cd
O'Connor	45.64de	8.27abc	31.12de
Keel	30.37e	4.27d	28.05e
LSD (0.05)	*16.34*	*3.94*	*17.80*

†Anthesis, maturity and 'utilisation' data analysed separately, therefore 'utilisation'≠anthesis-maturity

2.3.3.6 Grain Yield

The MET analysis of site data, based on grain yield (Table 2.1), indicated that Lowbank 1999 ($\underline{P}$<0.001), Lowbank 2000 ($\underline{P}$<0.001) and Minnipa 2000 ($\underline{P}$<0.001) displayed the typical grain yield response, for the varieties evaluated, for sandy soils (Table 2.19). Mundah and Yagan were the superior genotypes, overall, on sandy soils (Figure 2.13). Barque and Keel were characterised by a more medium grain yield, although the grain yield of these two varieties was distinctly more variable between sites compared to the WA lines, which were quite stable across environments. Sloop, Clipper and Galleon were the lowest yielding varieties. At sites where severe moisture stress encountered later in crop development (Minnipa & Cooke Plains 1999), Barque, Keel and Galleon ranked highly. Forrest ranked below Barque and Keel. However there was not the same degree of variability between sites as with the latter two varieties. The h^2 of grain yield was high at all sites, with the values at Lowbank (1999-2000) and Minnipa (2000) the only ones to be related to sand adaptation.

Table 2.19: Grain yield (t ha^{-1}) for the 7 core barley varieties, and Yagan and O'Connor, in sand evaluation trials (1999-2000). Data analysed by MET analysis.

	Lowbank1999	Lowbank2000	Minnipa2000
Mundah	0.534a	1.520a	1.682a
Yagan*	-	1.445ab	1.517b
O'Connor*	-	1.306cd	1.450bc
Forrest	0.409b	1.219cd	1.308d
Keel	0.389bc	1.318bcd	1.347cd
Barque	0.385bc	1.368bc	1.303d
Sloop	0.381bc	1.287cd	1.114e
Clipper	0.358bc	1.219d	1.069e
Galleon	0.321c	1.199d	0.997e
LSD(0.05)	*0.084*	*0.144*	*0.136*

* Yagan and O'Connor were not included in 1999 trials.

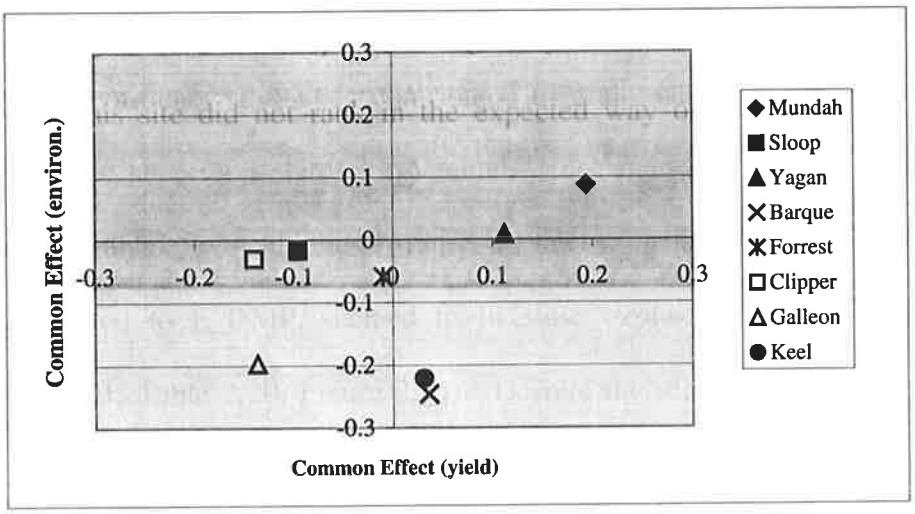


Figure 2.13: MET analysis of grain yield for the 7 core barley varieties and Yagan in sand evaluation trials (1999-2000).

At Lowbank (Figure 2.14a, b) and Cooke Plains in 1999 (Figure 2.14c) a small but significant ($P<0.001$) relationship between grain yield and E_DMP was apparent. The relationship between these two factors was not significant at Cooke Plains in 2000 (Figure 2.14d) and Minnipa (Figure 2.14e, f), although there was a trend towards increasing grain yield with higher E_DMP at Minnipa in both years (Figure 2.14e, f).

E_DMP was a significant ($P<0.001$) contributor to grain yield at Lowbank (Table 2.20), suggesting that the superior E_DMP of Mundah and Yagan conferred the improved grain yield potential of these two varieties (Figure 2.14a, b). The higher LAI of Mundah and Yagan, also related to E_DMP, seemed to likewise explain their higher grain yield at Lowbank ($P<0.001$, Table 2.20, Figure 2.15). Despite the relationship between grain yield and E_DMP at Minnipa (Figure 2.14e, f), E_DMP was not a contributing factor to grain yield (Table 2.20). In 1999, Keel out yielded Mundah despite lower E_DMP (Figure 2.14e). It is likely that some other factor contributed to Keel out yielding Mundah. In 2000 (Figure 2.14f), Forrest and O'Connor out yielded varieties with equivalent E_DMP (*e.g.* Sloop and Clipper). Although the relationship between grain yield and E_DMP held at Cooke Plains, the varieties at this site did not rank in the expected way on sand (Figure 2.14c). Keel

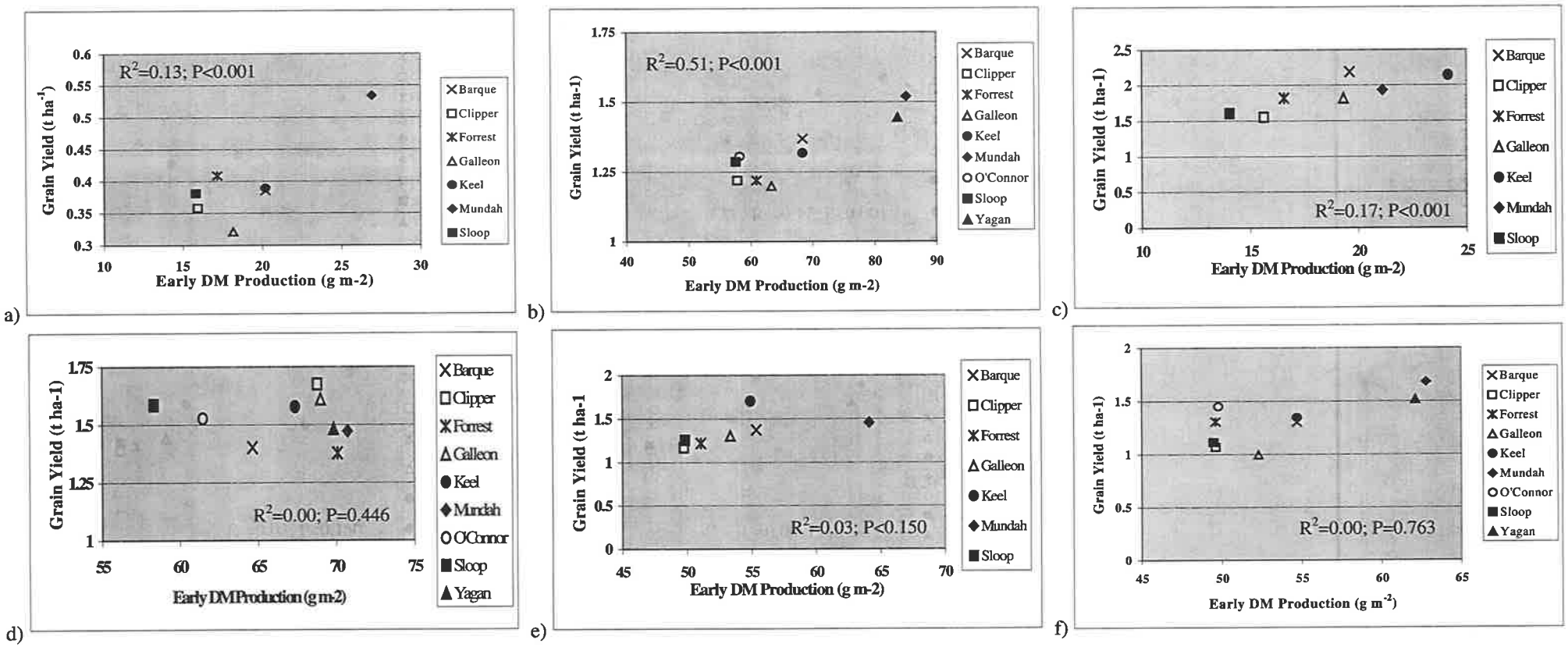


Figure 2.14: Relationship between grain yield and early dry matter production at a) Lowbank 1999, b) Lowbank 2000, c) Cooke Plains 1999, d) Cooke Plains 2000, e) Minnipa 1999 and f) Minnipa 2000. Data analysed by MET.

displayed superior E_DMP and grain yield, compared to Mundah, and Galleon performed similarly to Barque. As for Cooke Plains in 2000 (Figure 2.14d), powdery mildew and frost damage was an issue. Disease resistance and frost avoidance played a primary role in the response of varieties, and not early vigour

A small, but significant (P<0.001), relationship was evident between grain yield and dry matter production at anthesis and physiological maturity (Figure 2.16).

Table 2.20: Wald statistics from the covariate analysis (REML) of grain yield.

Site	Trait	Covariate	d.f.	Wald Statistic	Significance
Lowbank 1999	Grain yield	E_DMP	1	193.16	P<0.001
Cooke Plains 1999	Grain yield	E_DMP	1	0.47	P=0.495
Minnipa 1999	Grain yield	E_DMP	1	0.01	P=0.927
Lowbank 2000	Grain yield	E_DMP	1	21.07	P<0.001
	Grain yield	LAI	1	10.35	P<0.001
Cooke Plains 2000	Grain yield	E_DMP	1	0.01	P=0.905
	Grain yield	LAI	1	0.03	P=0.860
Minnipa 2000	Grain yield	E_DMP	1	2.33	P=0.127

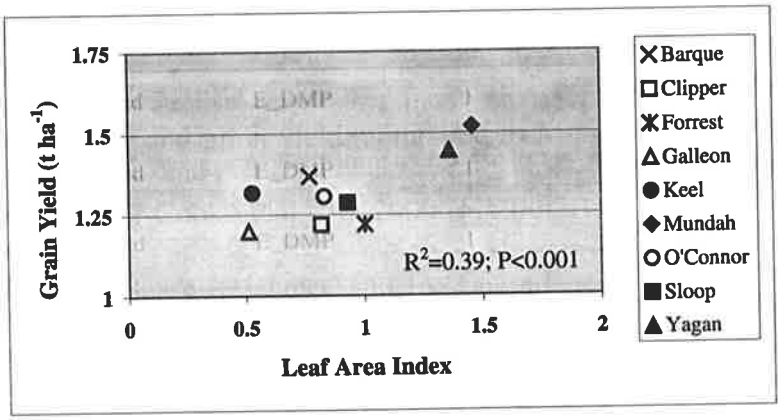


Figure 2.15: Relationship between grain yield and leaf area index (Lowbank, 2000). Data analysed by REML and MET analysis.

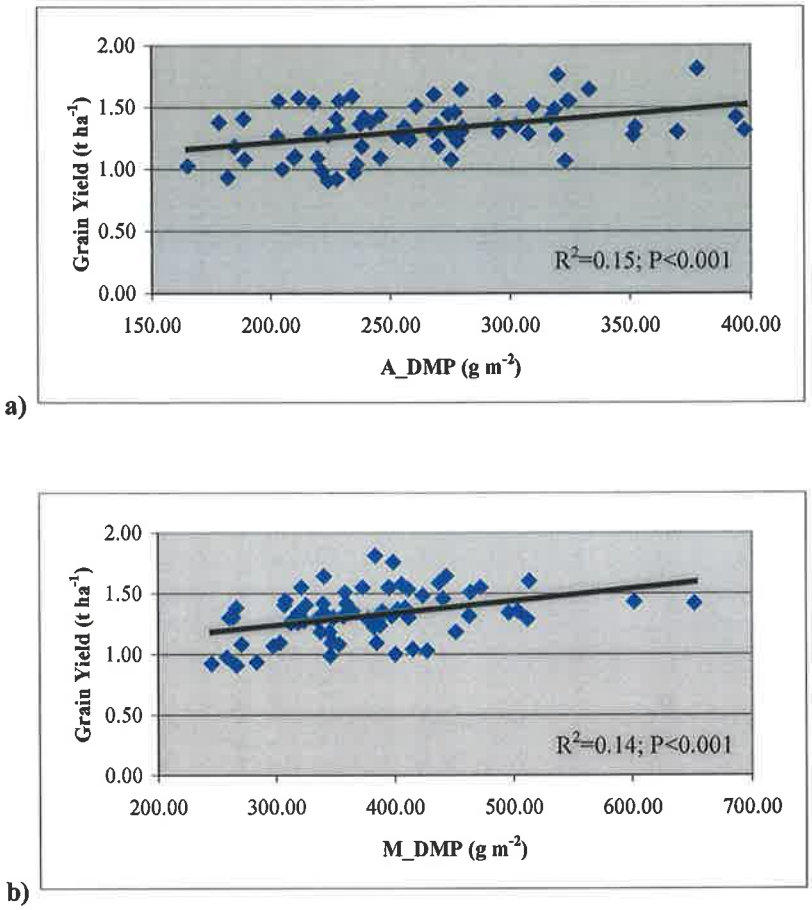


Figure 2.16: Relationship between grain yield, and a) anthesis dry matter and b) maturity dry matter (Lowbank, 2000). Data analysed by REML analysis.

2.3.3.7 1000 grain weight & Screenings

The ranking of varieties for 1000 grain weight and screenings (Figure 2.17) was stable between sites, and the differences significant between varieties ($\underline{P}<0.001$, $\underline{P}=0.019$, Table 2.9b). Galleon was clearly inferior to all the varieties evaluated, with an average 1000 grain weight of 41.43g and screenings of over 30%. Yagan, Mundah and Forrest produced the highest grain weight, 49.69g, 47.60g and 47.15g respectively, but in the case of Yagan and Mundah, had similar screenings percentage to Keel and Clipper (18-19%). Forrest on the other hand, maintained the lowest screenings percentage (11.69%). Sloop also had a lower screenings percentage (15.96%). The grain weight of Sloop however was 41.77g, only

slightly better than Galleon, suggesting that the shape of Sloop grain, which is more rounded than elongated, may the key factor when grain is sieved over slotted screens. In addition Barque had higher screenings (22.56%) then most of the varieties, except Galleon, despite its median grain weight (43.19g). Again this may be due to grain shape. The h^2 of both 1000 grain weight and screenings was high (88%) at Lowbank and Minnipa (2000).

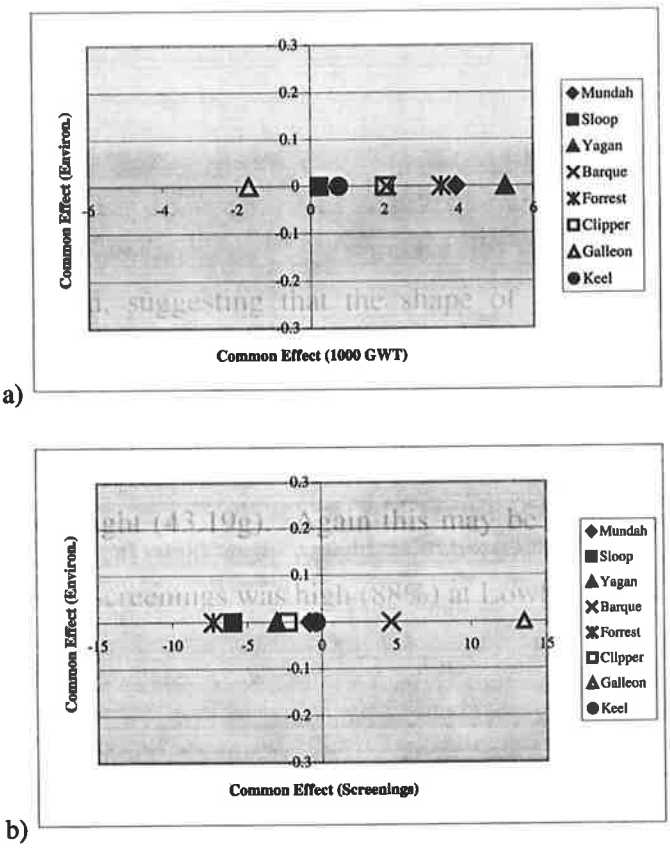


Figure 2.17: MET analysis of a) 1000 grain weight and b) screenings percentage for the 7 core barley varieties,and Yagan, in sand evaluation trials (1999-2000).

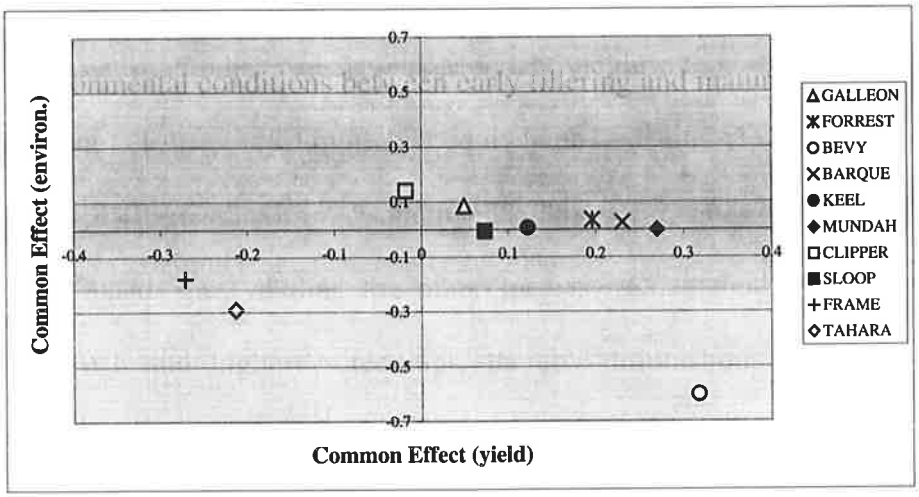
2.4 Discussion

2.4.1 Comparison of cereal species on sandy soils

Experiments to evaluate differences between cereal species in adaptation to sandy soils of low fertility were conducted in the first year of the project only (1999). The expectation for this component of the study was that adaptation on sandy soils would follow the general consensus; that the cereal species would rank in the order cereal rye, barley, triticale and wheat. Results of the MET analysis (Figure 2.1) however suggested that the overall ranking was Bevy rye, Tahara triticale, Frame wheat and barley, although the yield potential of Mundah was greater than Frame. This was likely to be a result of Frame and Tahara having higher responses at some sites than expected (*e.g.* Lowbank), and Bevy rye having a low response at Lowbank. The evaluation of different species was limited by the fact that assumptions on species adaptation on sand in this study was not appropriate, because they were based on a comparison between a single variety and several barley varieties of variable adaptation. In addition, the MET analysis indicated that the other cereals were not as stable as Mundah and Yagan across the environments. Two of the three sites used in these experiments (Lowbank and Cooke Plains) were a subset of the sites in SARDI long-term trials on sandy soils and represent the target environment of this study. However, the constraints imposed by the term and resources available for a Ph.D. study limited the comparative analysis of cereal species on sandy soils to one season and an appropriate genotype by year analysis could not be achieved. This factor is particularly important when considering the results, since long-term grain yield analysis of SARDI trials supports previous expectations (Figure 2.18). Grain yield is a complex interaction between genotype and environment (GxE), and long-term results remove the vagaries of specific GxE interactions that override the 'sand effect'. Frame wheat and Tahara triticale have a significantly lower grain yield potential than the core set of barley varieties. Cereal rye, over the 13 years these SARDI trials have been conducted, was

the highest yielding cereal on sandy soils, although in some environments (site x season) it exhibited very low grain yields. This is illustrated by the high negative value for common effect (environment); the y-axis in figure 2.18.

Several factors may explain the scenarios encountered in these experiments. One may relate to the soil profile. At Minnipa, a loam soil covered the soil profile to 80 cm under the initial 10cm of sandy loam. This may, in part, explain the observation of SA bred varieties out performing their WA counter parts. Typically, the SA varieties (*e.g.* Keel) have been bred on heavier soils under higher yielding conditions. The greater water holding capacity of the loams would have favoured all species and varieties, however, Keel may have better exploited the water resources. Another may be related to growing season (April-October) rainfall, and the degree and timing of moisture stress. At Cooke Plains and Minnipa, the total April-October (growing season) rainfall was approximately 40 mm below average. Keel was the highest yielding barley at both Cooke Plains and Minnipa, suggesting better drought tolerance mechanisms than the other barley varieties. Keel is a daylength sensitive genotype, such that the timing of phenological events is governed by daylength. A potential explanation for the performance of Keel at these sites may relate to daylength favouring Keel by increasing the length of BVP and stem elongation compared to Mundah, which is relatively insensitive to daylength, and thus allowing it to set more primordia (high maximum primodia number) (Miralles *et al.*, 2000). This, in addition to Keel possessing a higher fertile spikelet:primordia ratio (Coventry *pers. comm.*), favoured the grain yield potential of Keel, especially under soil water stress post-anthesis. Forrest was particularly disadvantaged under such conditions, while Mundah was ranked second at Minnipa and fourth at Cooke Plains. In general, barley was better adapted to the soil water limiting conditions.



*Source: Rob Wheeler, SA Field Crop Evaluation Program (SARDI)

Figure 2.18: MET analysis of long-term grain yield data for barley (7 varieties), wheat, cereal rye and triticale in SARDI sand evaluation trials (1988-2000).

The environmental circumstances at Lowbank in 1999 were in contrast to the other field sites. April-October rainfall was only slightly above average, but total rainfall for October was 40 mm above average. Because wheat, triticale and cereal rye are later maturing than barley, the prevailing conditions during October may well have favoured improving their grain weight and grain size compared to barley, which was already well into grain filling. The only barley line to compete with the other cereals was Mundah, yielding 0.534 t/ha, slightly lower than Tahara and Frame. Tahara and Frame had equivalent screenings to Mundah, although their 1000 GWT was 8-10g less. Bevy's superior adaptation on sandy soils was highlighted at Lowbank, significantly out yielding the other species. On the other hand, Bevy had the lowest 1000 GWT and highest screenings, thereby diminishing its performance. The unexpectedly superior grain yield of Frame and Tahara at Lowbank in 1999 is the only occasion since SARDI began evaluating cereals on sandy soils of low fertility, that this has been observed.

The variation in the performance of the barley varieties, across all sites in 1999, despite producing superior early dry matter production (early vigour), highlights the influence of the prevailing environmental conditions between early tillering and maturity. Certainly the below

average rainfall, at Minnipa and Cooke Plains, would have reduced the potential performance initially observed in terms of early vigour.

Length of the coleoptile has been associated with improvements, in general, in establishment and early vigour in cereals (Whan, 1976; Hughes and Mitchel, 1987; Radford, 1987; Radford and Wildermuth, 1987; Sharma, 1990; Bacaltchuk and Ulrich, 1990; Richards, 1992; Rebetzke and Richards, 1996; Schillinger *et al.*, 1998). It was noted that Frame and Tahara had considerably shorter coleoptile length using the filter paper test. Presumably Frame and Tahara also produced short coleoptiles in field experiments, and this, to an extent, would explain the lower establishment counts and lower early vigour of these cereals compared to barley and cereal rye.

2.4.2 Barley

Adaptation generally refers to grain yield potential and grain yield stability in a given environment. SARDI evaluation trials on sandy soils have suggested that WA varieties are better adapted than SA varieties (Table 2.1 & Figure 2.18). This ability to yield well and maintain stability occurs despite the lack of resistance to economically important diseases in SA. Results from the detailed study of growth and grain yield of a range of barley varieties on sandy soils of low fertility has corroborated the results of long term yield analysis of the SARDI trials. Mundah and Yagan not only exhibited significantly superior grain yield, but also demonstrated greater yield stability compared to the SA varieties. Grain weight, a measure of yield stability and a function of the rate and duration of grain filling, appeared to be a critical component of the adaptation of Mundah. Mundah seemed to be better able to develop large grain despite the poor moisture and nutrient relations of sandy soils. Logically, high screenings can also impact on potential grain yield and reflect the stability of yield in given environments. Galleon in particular was significantly inferior to the other cultivars for screenings (>30%), as well as grain weight. High screenings and low grain weight suggest that grain filling is unable to be sustained due to prevailing environmental (*e.g.* moisture

stress) and soil constraints and/or from the inadequate supply of carbohydrate to the developing grain. In addition, Galleon may also be unsuitable for sandy soils due to phenology, and over tillering, or the development of late tillers, where again grain filling is restricted because inadequate resources are available to support increased grain numbers.

Having ascertained that Mundah and Yagan, and to a lesser extent Forrest, are adapted to sandy soils, and are superior to most SA varieties, such as Barque, Keel and Galleon, what characteristics do they possess that improve their grain yield potential on this soil type?

The evidence from these variety evaluation trials is that early vigour is a major contributor to the improved adaptation of Mundah and Yagan on sandy soils. This was emphasised by a genuine relationship between grain yield and E_DMP (Figure 2.14). In addition, their superior E_DMP and grain yield also appears to be related to their high LAI, SLA and LAR. This agrees with the findings of Richards and Luckacs (2002) for wheat. Both varieties achieved a more rapid development of the canopy and appeared to place greater emphasis in investing energy/resources into leaf area development as a proportion of total above ground biomass production (LAR), thereby providing a higher interception of light at any point in time, and a reduction in time to reach full light interception (LAI=3.5). The advantage of rapid LAD is that crop photosynthesis is directly benefited, and the amount of radiant energy reaching the soil surface is limited, thereby enhancing water use efficiency through improved transpiration and reduced soil evaporation (Richards, 1991 & 2000). It is likely that the erect leaf posture and high canopy of Mundah and Yagan balances the need for moisture conservation through reducing transpiration loss and soil evaporation (increasing transpiration efficiency), and efficient light capture following full canopy closure to maintain adequate crop photosynthesis. Both canopy architecture and leaf area duration are considered important traits for maximising crop photosynthesis after closure of the canopy (Richards, 2000). For Galleon, which exhibits a prostrate growth habit, transpiration loss through the leaves is probably higher. However, combined with its poor biomass production on sandy soils, Galleon displayed inadequate water use efficiency critical for maximising early growth and

grain yield potential on soils with inherently poor moisture properties. Barque and Keel, on the other hand, displayed an intermediate growth habit (Plate 2.2 & 2.3). Other associated benefits of a faster developing ground cover is improved competitiveness with weeds, a reduction in erosion, and the possibility of reduced effects of sand "blasting".

The higher SLA of Mundah and Yagan during early plant development facilitates an increased net assimilation rate (NAR) for an equivalent leaf weight, because increased leaf area compensates for thinner leaves, and therefore less photosynthetically active cells through the leaf profile. Once full canopy closure occurs, shading effects increase and the volume of photosynthetically active cells acquiring light is reduced. For this reason a high SLA will reduce NAR (Richards, 2000). However, this may not be a problem in temperate cereals because there is a natural decline in SLA as time to anthesis approaches (Richards, 2000). Conversely, Sloop had a high SLA, which may be important in favourable conditions and environments, but does not facilitate good growth on sandy soils because of its inferior LAI and E_DMP compared to Mundah and Yagan.

Plate 2.2: Differences in growth habit between Mundah (left) and Keel (right) at Lowbank 2000.

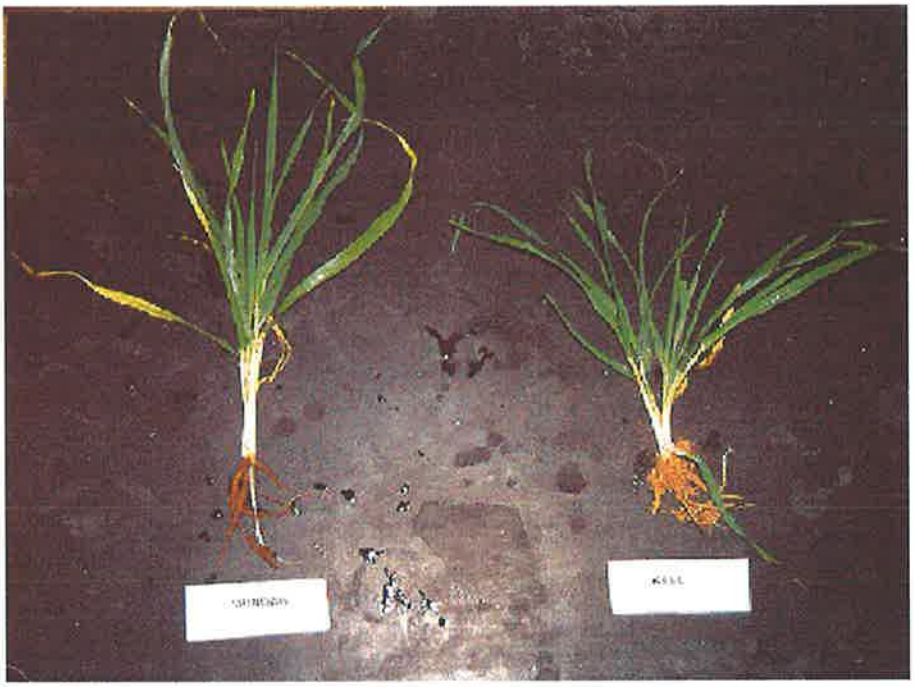


Plate 2.3: Differences in growth habit between Mundah (left) and Keel (right)

In interpreting how efficiently, or otherwise, the core set of nine barley varieties utilise phosphorus, two methods of expressing utilisation efficiency were considered. PER (g mg^{-1} P), alternatively referred to as the 'utilisation quotient', has been a standard value for comparing efficiencies among varieties and between species (Siddiqi and Glass, 1982). However, Siddiqi and Glass (1982) considered the 'utilisation quotient', while convenient and useful, to be an oversimplification both in a practical and theoretical sense. The concern was that this expression gave "little regard to growth, which is conceptually implicit in any consideration of utilisation". In practice, PER may consider one variety to be more efficient than another, however this may be due to lesser vegetative growth, and lower efficiency of absorption, and therefore lower total phosphorus in the plant. In other words, it is not due to a higher efficiency of utilisation (Siddiqi and Glass, 1982). This scenario led Siddiqi and Glass (1982) to produce a modified expression that took into account growth, in addition to PER, which they considered to be better able to convey differences in utilisation between varieties and species (*i.e.* PUE (g^2 mg^{-1} P)). A good example was the experiment at Minnipa in 1999 (Table 2.15 & Figure 2.8e). With the exception of Galleon, Mundah was inferior to the other varieties, in terms of PER. However, when considering PUE at the same site it was clear that Mundah exhibited superior utilisation efficiency. It would seem from the PUE results, in particular, but also from the PER data, that Mundah has a greater capacity to efficiently utilise available phosphorus. This is a critical issue considering all field sites were characterised by levels of phosphorus below the critical level for unimpeded crop growth (Table 2.5 & 2.6), a feature common to sandy soils in general. The varieties that exhibited poor adaptation, namely Clipper and Sloop, also displayed low phosphorus utilisation efficiency. Forrest was also defined as a poor utiliser of phosphorus in these trials, which agrees with Zhu *et al.* (2002). But what Zhu *et al.* (2002) also noted was that Forrest allocated phosphorus almost equivalently between the shoots and roots under low phosphorus availability, compared to a more favourable allocation to the shoots by Galleon, Clipper and Sloop. Accordingly, the ratio of root biomass to shoot biomass of Forrest was equal, whereas it was heavily weighted

in favour of the shoot for Galleon, Clipper and Sloop. Analysis of the roots was not attempted in these field experiments, because of the workload and error involved with sampling root material in the field. It would be of interest, in further developing the barley ideotype for sand adaptation, to determine whether Mundah also allocates phosphorus in equal proportions to the roots and the shoots, as does Forrest, in combination with an efficient utilisation of phosphorus in terms of shoot biomass production. This is because it is likely that root morphology, as well as early vigour (dry matter production and leaf area development), is a component of superior adaptation on sandy soils of low fertility.

Although the results presented here highlight a range in PUE for the barley varieties evaluated, barley, in general, has better PUE than Frame wheat, Bevy rye and Tahara triticale on sandy soils (Table 2.21). The results, although for only three sites in one season, also provisionally support the findings of Osborne and Rengel (2002), that rye and triticale are generally more efficient in utilizing phosphorus than wheat at deficient levels of phosphorus.

Table 2.21: PUE (g^2 mg^{-1} P) for Barley, Frame, Bevy and Tahara in 1999.

	Cooke Plains	Lowbank	Minnipa
Barley[#]	1.500	1.695	9.708
(range)	*(0.776-2.855)*	*(1.199-2.590)*	*(8.639-11.179)*
Frame	0.950	1.106	7.016
Bevy	1.228	1.787	5.986
Tahara	1.171	0.963	8.930
LSD(0.05)	*0.576*	*0.368*	*3.010*

[#]Mean of the 7 core barley varieties

Coleoptile length was a less important factor than one might have expected for sandy soils where seeding depth is generally uneven. A long coleoptile has been highlighted as an important contributor to superior establishment and early vigour in barley (Gul and Allan, 1976; Gorny and Patyna, 1981; Redona and Mackill, 1996; Rebetzke and Richards, 1996,

Rebetzke *et al.*, 1999; Richards, 1991 & 2000). It did clearly distinguish barley from the other cereals, with barley having improved establishment and early vigour due, atleast partly, to a longer coleoptile (Table 2.7). However, on comparison of the barley varieties, this relationship was confounded by Galleon, in particular, which was clearly inferior in early dry matter production and leaf area development to Mundah, although having a significantly longer coleoptile. It follows then that while a longer coleoptile can improve establishment potential, other factors during early growth, such as PUE, root morphology and WUE, also contibute to early plant development. In addition, seed size was not a factor in the variation in coleoptile length, either between barley varieties or between cereals. This implies that the effect of variety, and therefore genotype, on coleoptile length is greater than the effect of seed size, even though seed size may still be a contributor to the differences observed between cereals. Ceccarelli and Pegiati (1980), and Cornish and Hindmarsh (1988) and Botwright *et al.* (2001) have previously determined that genetic effects rather than seed size in barley and wheat, respectively, predominantly influence coleoptile length.

Poor moisture relations are a perennial problem of sandy soils throughout the growing season. Moisture stress during the vegetative growth phase is overcome, to some extent, by increased early vigour to reduce soil evaporation and increase transpiration efficiency, as discussed above. In addition a vigourous and deep root system will increase transpiration efficiency by improving access to available moisture. Regardless of this, increased moisture deficit during spring, when temperatures are increasing and rainfall events are decreasing, will impact significantly on grain filling by influencing the amount of current photosynthates available to the developing grain. Therefore the availability and contribution of non-structural carbohydrates (fructan, fructose, sucrose, glucose), stored in the stem prior to anthesis, to grain filling, as has been established as an important mechanism in drought tolerance (Austin *et al.*, 1980; Richards and Townley-Smith, 1987; Blum *et al.*, 1994; Blum, 1998; Gebbing *et al.*, 1999), may be a potential avenue for supplementing/sustaining grain filling to improve grain yield, yield stability and adaptation on sandy soils.

The content of non-structural carbohydrate at anthesis, and their utilisation, could not adequately explain the adaptation of varieties on it own, but could be an important contributing factor. Mundah and Yagan both achieved high levels of assimilate at anthesis, and displayed greater utilisation of the carbohydrate resource. Although, as a proportion of the total carbohydrate content at anthesis, Mundah contained double the amount of ESC of Yagan (Table 2.22). Equally important is the fact that both varieties are early flowering types, a trait essential in combination with increased early vigour in moisture limiting environments (Richards, 1991). Time of flowering appeared to be the key factor for Galleon, Clipper and Sloop. Although Sloop had a high utilisation of assimilate, that may reflect to a degree its improved malting quality (increased starch), its later flowering phenology, along with an inferior early vigour, makes it poorly adapted to sandy soils. Similarly, Galleon and Clipper are later flowering types, making them ill-suited to these environments despite equivalent assimilate content to Mundah at anthesis. The lower yield potential of Barque on sand, may reflect both its lower utilisation of assimilate and its slightly later maturity. Keel was the only variety in which the potential to sustain grain filling by the utilisation of assimilate, alone, seemed to be limited, and may account, in addition to its reduced early vigour, for its poorer adaptation on sandy soils.

Significant varietal differences were established for dry matter production at anthesis and maturity at Lowbank ($\underline{P}<0.001$), and a small, but significant ($\underline{P}<0.001$), relationship between grain yield and A_DMP, and grain yield and M_DMP, was evident. However, the ranking of varieties was not related to the expected adaptation response on sandy soils.

Table 2.22: Proportion of total assimilate as ESC and ESC:Fructan ratio at anthesis for the 7 core barley varieties, and Yagan and O'Connor at Lowbank 2000. Data analysed by MET.

Variety	Proportion of total assimilate at anthesis as ESC (%)	ESC:Fructan ratio
Barque	35.17bc	0.54bc
Clipper	30.66cd	0.49bcd
Forrest	29.71cd	0.41cd
Galleon	21.83d	0.28d
Keel	48.09a	0.92a
Mundah	40.34ab	0.68ab
O'Connor	34.82bc	0.51bcd
Sloop	26.01cd	0.37cd
Yagan	23.69d	0.35cd
LSD(0.05)	*9.58*	*0.242*

2.5 Conclusions

It has been intimated from the evaluation of Mundah on sandy soils (*e.g.* SARDI, Table 2.1), that its improved adaptation may be related to superior early vigour, good standing ability and early flowering phenology (Table 2.2). In these field experiments further progress has been made to understand which traits distinguish varieties with good adaptation from those with poor adaptation. It is postulated that Mundah's improved growth, grain yield and grain yield stability is related to an ability to support the development of larger grains (1000 grain weight). This is achieved through earlier flowering, and via the potential contribution of fructan and ESC, stored in the stem prior to flowering, to sustain grain filling as conditions become increasingly unfavourable for grain development (*e.g.* leaf senescence, moisture and

heat stress). In addition, the superiority of Mundah is laid on the foundation of greater early vigour (dry matter production and leaf area development), large seed size, which correlates to embryo size (Richards and Luckacs, 2002), high phosphorus utilization efficiency, and the potential for better establishment due to a longer than average coleoptile. The potential consequence of a concerted effort to improve early vigour is a reduction in the amount of water available after anthesis to sustain grain filling in dry finishes. Therefore to ensure adequate water is available during grain filling, an appropriate root morphology is likely to be an equally critical component of adaptation, so yields are not restricted by water limitations post-anthesis. The next chapter, detailing a controlled environment experiment, will focus, in addition to the early development of the shoot (biomass production and leaf area development), on aspects of the root system that compensate for the poor moisture and nutrient profile of sandy soils

Chapter 3. Traits associated with improved growth and grain yield of barley on sandy soils of low fertility: Controlled environment experiment

3.1 Introduction

In the first component of this study (chapter 2) field experiments were initiated to characterize traits likely to be associated with improved growth and grain yield of barley on sandy soils of low fertility. It was emphasized that traits potentially contributing to adaptation included early plant canopy development and biomass production, the efficient acquisition and conversion of phosphorus into dry matter, the storage and availability of pre-anthesis assimilate for grain filling, an erect growth habit and early flowering. Improved early vigour, both in terms of dry matter production and leaf area development (LAD), has been shown to be associated with superior performance under moisture limiting conditions, because of the associated improvement in water use efficiency and crop photosynthesis (Turner and Nicolas, 1987; Richards, 1987, 1991 & 2000; Richards and Townley-Smith, 1987). However the potential for grain yield to be limited by inadequate soil water reserves to sustain grain filling, due to greater pre-anthesis biomass production, means that an appropriate root morphology, especially a deep root system, is also considered a desirable trait (Turner and Nicolas, 1987). The poor moisture relations, and the content and availability of nutrients inherent in sandy soils suggests that an extensive and vigourous root system is likely to be a key component of adaptation to these environments. The practical limitations of field experiments, especially when extensive travel is required for routine monitoring, can mean the effective measurement of morphological traits must be restricted to a measurement of development at one point in time; as was the case for leaf area development and biomass production in the field

experiments described in chapter 2. In addition, while there are a substantial range of sampling methods available to characterise root morphology in field experiments, all methods are time consuming, labour intensive and the nature of the sampling procedures make them prone to a high degree of experimental error. Genotype x environment (GxE) interactions specific to that environment in which the experiment is conducted can also have a confounding effect on root measurements.

Experiments conducted in controlled conditions allow for the study of the morphology, physiology, and biochemistry of early plant development, and root morphology, in finer detail over time, and growing conditions can be uniformly replicated (Bőhm, 1979) and GxE interactions can be avoided. This is especially useful in characterising LAD, as a measure of early vigour between emergence and early tillering, particularly since this could not be adequately achieved in the field experiments described in chapter 2. The soil environment in a controlled situation is also of a finite size making it easier to handle, manipulate and sample. This permits the extraction of roots from the soil as an intact and complete system, allowing for the determination of individual root parameters that may influence overall root morphology in sandy soils (Bőhm, 1979). In addition, experiments in controlled environment growth rooms can be set up to mimic field conditions during the early growth period, but without the highly variable nutrient and moisture limitations that naturally impact on plant growth on sandy soils.

In his discussion of container experiments under controlled conditions Bőhm (1979) also makes clear the deficiencies of these types of experiments, in particular their relationship to performance under field conditions. Soil conditions in pots are unnatural. The uniformity (bulk density) of the soil will undoubtedly vary from the field because it has been disturbed during collection. Soil temperature is also substantially different from that in natural undisturbed soils because soils in pots do not exhibit the same buffering capacity to rapid changes in ambient temperatures. In terms of root development, the finite volume of soil in the pot inhibits the distribution and spread of roots. This is overcome to an extent by limiting

the study of plant and root development to the early vegetative period. Competition between the roots of different plants for soil water and nutrients can limit root elongation and distribution (Rahman, 1968), and this may be a significant factor in the field. However, the lack of root competition for resources in pot experiments, either as a result of the limited number of plants due to the restricted area of the pot and/or because the supply of both resources is adequately maintained, may be unavoidable. Consequently, root morphology may not be representative of field conditions.

Despite the limitations of container experiments under controlled conditions, it is a useful strategy to study traits likely to be influential to adaptation on sandy soils. With these limitations in mind the results should be interpreted with caution. In this controlled environment experiment the aim was to study in more detail the development of leaf area, as a component of early vigour, and root morphology between barley varieties and other cereal species, differing in sand adaptation.

3.2 Methods and Materials

The controlled environment experiment was conducted on three separate occasions from 1999-2001. Experiments 1 and 2 utilized soil amended with basal nutrients, while in experiment 3 no basal nutrients were supplied to the soil.

3.2.1 Varieties

3.2.1.1 Experiment 1

Nine barley varieties were used in the first experiment (Mundah, Forrest, Barque, Keel, Galleon, Sloop, Clipper, Chebec, Skiff), along with one wheat (Frame), cereal rye (Bevy) and triticale (Tahara) variety. As emphasized in chapter 2, the varieties considered 'good' performers on sand were Mundah and Forrest and the varieties considered 'intermediate' to

'poor' were Keel, Barque, Chebec, Galleon, Clipper, Skiff and Sloop. In general, barley is considered better 'adapted' on sandy soils than either wheat or triticale, but is inferior to cereal rye. Seed was screened (2.2-2.5mm) to ensure seed of equivalent size was used for all varieties.

3.2.1.2 Experiment 2

Only the barley varieties Mundah, Forrest, Barque, Keel, Sloop and Galleon were assessed.

3.2.1.3 Experiment 3

In order to establish whether basal nutrients were partly responsible for the performance of Galleon in experiments 1 and 2, a third experiment, excluding basal nutrients, but maintaining adequate moisture, was conducted. Mundah and Galleon were the only barley varieties assessed.

3.2.2 Design

3.2.2.1 Experiment 1

Soil was collected from a sand ridge in a paddock previously sown to canola (1999) at Cooke Plains in South Australia (Table 3.1). The soil was air dried and then passed through a No.15 (2.8mm) sieve to remove plant debris. Using a customised cement mixer with a plastic bucket and stainless steel components, calcium carbonate (0.33%) was thoroughly mixed through the soil. Various macro- and micro-nutrients were then applied separately in solution (mg kg^{-1} dry soil) (Table 3.2).

Table 3.1: Soil Analysis of the Cooke Plains and Lowbank soils.

	Cooke Plains soil	Lowbank soil
Texture	Sandy loam	Sandy loam
NO_3-N (mg/kg)	14	7
NH_4-N (mg/kg)	3	4
Colwell Phosphorus (mg/kg)	19	14
Colwell Potassium (mg/kg)	123	184
Organic Carbon (%)	0.45	0.25
pH (H_2O)	8.3	8.5

Table 3.2: Basal nutrients and amount applied to soils used in controlled environment experiments 1 and 2.

Base Nutrient	Amount (mg kg^{-1} dry soil)
NH_4NO_3	350
K_2PO_4	120
K_2SO_4	80
$MgSO_4.7H2O$	100
$CuSO_4.5H2O$	5
$MnSO_4.4H2O$	7
$CoSO_4.7H2O$	1
$FeSO_4.7H2O$	0.7
$NiSO_4.7H2O$	0.15
$ZnSO_4$	1.6
NaCl	4.2

The soil was transfered to 10 cm (diameter) x 29 cm (depth)(2.28 litre) PVC pots lined with a plastic bag to a bulk density of 1.36 g cm^{-3} (approx. 3 kg). The soil was watered with R.O. water to 75% field capacity (104.5 g kg^{-1} soil). A wetting agent (Wetta Soil®) was mixed with the water to ensure no repellency problems occurred. Six plants were sown per pot and culled to three seven days after sowing (DAS). Plants were harvested at 10 (first leaf fully

expanded), 17, 24 and 31 DAS. In total, there were 12 (variety) x 4 (sampling date) treatment combinations, with each combination replicated twice (blocks). There were a total of 96 pots in the experiment. The 12 varieties for each sampling were grouped in containers (sampling dates). The pots of each variety were randomised within each container and the position of each container was randomised within each block in the growth room. Pots were watered (R.O. water) to weight, to maintain the 75% field capacity, every two days at which time the pots and containers were 're-randomised'. Environmental conditions were set to 'simulate' field conditions during the first few weeks following seeding in South Australia (SA) in early June. Daylength was set at 10 hours day and 14 hours night. Air temperature was set in the range 16°C (day) to 10°C (night).

3.2.2.2 Experiment 2

Experiment 2 was similar in design to experiment 1. On this occasion a Lowbank sand (Table 3.1), collected from a paddock sown to wheat, was used. In addition, pots were not re-randomised following each harvest and the experiment was devised as a completely randomized design, grouped by time of sampling with 3 replicates (Plates 3.1-3.2). Daylength was of 8 hours duration, with 16 hours night. Air temperature was set at 16°C for day temperature (range: 16.4-17.9°C) and 10°C for night temperature (range: 10.5-11.5°C).

3.2.2.3 Experiment 3

The Lowbank soil employed in experiment 2 was also used in experiment 3. This experiment was arranged as a randomized complete block design of four replicates for each variety and sampling combination. In contrast to experiments 1 and 2, the soil was not amended with basal nutrients.

Daylength was set at 8 hours for the first 21 days and then adjusted to 9 hours for the duration of the experiment. Day air temperature was set at 16°C (range: 16.4-17.9°C) and night temperature was set at 10°C (range: 10.5-11.5°C).

Plate 3.1: Layout of experiment 2 of the controlled environment study.

Plate 3.2: PVC tubes containing 3 plants of equivalent growth stage. Pots were watered to weight, with the soil contained in plastic bags to prevent water loss through natural drainage. Perlite was use to cover the soil surface to reduce evaporation.

3.2.3 Sampling

At each harvest the soil was washed away from the plants (roots and shoots); then the roots were separated from the shoots. In addition, the leaves were removed from the stems and treated separately. The stem and leaf fractions were dried at 80°C for 48 hours, and the dry weights recorded. Roots were stored in a 70% ethanol solution.

3.2.4 Measurements

3.2.4.1 Biomass

Total above ground biomass (mg) was calculated at each sampling from the addition of leaf dry weight and stem dry weight. Crop growth rate[1] (CGR, mg day^{-1}) and relative growth rate[2] of the whole plant (RGR, mg mg^{-1} growing degree days $^{-1}$) were calculated from these values, as per Lopez-Castaneda *et al.* (1996).

$$^{1}CGR = (biomass_2 - biomass_1) / (t_2 - t_1)$$

$$^{2}RGR = [Ln (biomass_2) - Ln (biomass_1)] / (GDD_2 - GDD_1)$$

$$GDD = [((\text{day temp.} \times \text{No. hours}) + (\text{night temp.} \times \text{No. Hours}))/24] - T_b$$

GDD = growing degree days (°Cd)

T_b = base temperature (7.1°C, Goyne et al., 1996)

3.2.4.2 Phosphorus utilization efficiency

Absolute Phosphorus content (mg) was calculated from the tissue concentration determined by Inductively Coupled Plasma Atomic Emission (ICPAES) analysis (Zarcinas *et al.*, 1987), and total dry matter production. Phosphorus efficiency ratio[3] (PER, mg μg^{-1} P) and Phosphorus utilisation efficiency[4] (PUE, mg^2 μg^{-1} P), adjusted for phosphorus content in the seed, was estimated according to Siddiqi and Glass (1982).

[3]PER = Above ground dry matter (mg) / Total content of P in dry matter (μg)

[4]PUE = PER * Above ground dry matter (mg)

3.2.4.3 Leaf Area

Total leaf area per plant (LA) was determined at each harvest. In experiement 1 this was done either by measuring length and breadth using a ruler (10 and 31 DAS) or by photocopying the leaves (17 and 24 DAS) and scanning the copies using Sci-Scan image analysis system software, developed by Kirchhof (1992). In experiments 2 and 3 total leaf area per plant was measured using a planimeter. Additional characteristics calculated from total leaf area included specific leaf area[5] (SLA, ratio of leaf area to leaf dry weight, $cm^2\ g^{-1}$), leaf area ratio[6] (LAR, ratio of leaf area to plant dry weight, $cm^2\ g^{-1}$), leaf expansion rate[7] (LER, $cm^2\ day^{-1}$), and relative leaf expansion rate[8] (RLER, $cm^2\ cm^{-2}\ day^{-1}$) as per Rebetzke and Richards (1999) and Liang and Richards (1994).

[5]SLA = leaf area (cm^2) / weight of leaf (g)

[6]LAR = leaf area (cm^2) / above ground biomass (g)

[7]LER = (leaf area$_2$-leaf area$_1$) / (t_2-t_1)

[8]RLER= [LN (leaf area$_2$)-LN (leaf area$_1$)]/(t_2-t_1)

3.2.4.4 Root Morphology

Maximum rooting depth (cm) was measured, using a standard ruler, immediately following each harvest. Seminal root number was counted at harvest 1 (10 DAS) in experiment 1 only.

One to two millilitres of a 2% methyl-violet (organic dye) solution was added to the preserved roots (in 70% ethanol) and allowed to stain for 48 hours. Staining the roots with methyl violet provided sufficient contrast for the photocopying and scanning of the roots (Harris and Campbell, 1989). Preparation of the roots for photocopying and scanning followed the procedure of Goubran and Richards (1979) and Pederson *et al.* (1994). The root samples were floated in a water bath consisting of an upper perforated reservoir, lined with a sheet of blotting paper, and a lower reservoir with an inlet/outlet for water. The roots were floated over the blotting paper and, with the drainage of water from the lower reservoir, allowed to settle on the paper avoiding any overlap of roots. An overhead transparency sheet was placed over each sample and a high contrast image of the root system was attained using a commercial photocopier.

The images of each root system were scanned using a flatbed scanner at 300dpi resolution using Sci-Scan image analysis system software (Kirchhof 1992). The software allowed the computation of total rooting length (TRL, cm), total rooting volume (TRV, cm^3), and total root surface area (cm^2) within a range of mean diameter classes (0.2...2.0 mm). In addition root length density[9] (RLD, cm root cm^{-3} soil), a measure of rooting volume, was calculated as below.

$$^{9}\text{RLD} = \quad \text{Total root length (cm) / soil volume to maximum root depth } (cm^3)$$

Root samples were then dried at 80°C for 48 hours, and dry weight measured. Root:shoot ratio (R:S) was then determined.

3.2.5 Statistical analysis

Analyses of the data for all experiments were completed using standard analysis of variance (ANOVA) procedures within the Genstat statistical package (Genstat® for Windows™, 5th edition, Lawes Agricultural Trust).

3.3 Results

3.3.1 Experiment 1

3.3.1.1 Biomass (dry matter) production

No significant varietal differences or variety x sampling time interaction was evident for the parameters used to describe biomass production (Table 3.3). Frame wheat exhibited the lowest dry matter values at all samplings and as such had the lowest CGR, although its RGR was equivalent to all other varieties.

3.3.1.2 Leaf area development

The development of leaf area was fairly consistent and equivalent up to 24 DAS where a divergence between varieties became apparent (Figure 3.1a). Bevy rye produced the highest total leaf area, and Frame wheat and Tahara triticale the lowest. Galleon displayed the highest leaf area of the barley varieties. Sloop, Mundah and Barque were essentially equivalent, followed by Forrest, while Keel exhibited the lowest leaf area. Overall there was no significant difference between the barley varieties, but Bevy, and Frame and Tahara produced significantly higher and lower leaf area respectively ($\underline{P}<0.001$, Table 3.3). Varietal differences for leaf area between 24 and 31 DAS accounted almost entirely for the significant variety x sampling interaction ($\underline{P}<0.05$, Table 3.3), and the variation for LER ($\underline{P}<0.05$, Table 3.5). As with total leaf area, the barley varieties did not differ significantly for LER. Bevy attained the highest LER (5.36 cm^2 day^{-1}), and Frame (1.43 cm^2 day^{-1}) and Tahara (1.03 cm^2 day^{-1}) the lowest. While all barley varieties had moderate rates of leaf expansion (2.35-3.92 cm^2 day^{-1}), only Keel (2.35 cm^2 day^{-1}) was significantly lower than Bevy ($\underline{P}<0.05$). The LER of Tahara and Frame was significantly lower than Bevy ($\underline{P}<0.05$), but not relative to the barley varieties. While not significant, the leaf expansion rate of Frame was still substantially lower than barley. The period between 24 and 31 DAS was also where varietal differences

Table 3.3: Mean squares from analysis of variance of biomass, crop growth rate (CGR), relative crop growth rate (RGR), phosphorus efficiency ratio (PER), phosphorus utilization efficiency (PUE), and leaf area parameters (leaf area (LA), specific leaf area (SLA), leaf area ratio (LAR), leaf expansion rate (LER), relative leaf expansion rate (RLER)) of 9 barley varieties, one wheat variety, one cereal rye and one triticale variety in experiment 1.

Source of variation	df	Biomass	CGR	RGR	PER	PUE	LA	SLA	LAR	LER	RLER
Variety	11	0.08440	0.2537	$5.659x10^{-5}$	$4.525x10^{-3}$*	1.9494*	0.39677***	$0.950x10^{-4}$***	0.12105***	0.6717*	$6.805x10^{-4}$
Sampling	3	24.05738***	21.3029***	$7.101x10^{-4}$**	$3.293x10^{-2}$***	127.2514***	035.64107***	$0.166x10^{-2}$***	1.00213***	35.5483***	0.638***
Variety x Sampling	33	0.06051	0.3863	$8.782x10^{-5}$	$6.16x10^{-4}$	0.7031	0.15975*	$0.249x10^{-4}$***	0.07797***	0.4413	$9.533x10^{-4}$
Residual	47	0.05950	0.3630	$9.745x10^{-5}$	$2.16x10^{-3}$	0.9162	0.08477	$0.527x10^{-5}$	0.01525	0.3036	$6.038x10^{-4}$
CV (%)		*6.3*	*27.2*	*47.5*	*5.2*	*22.9*	*4.0*	*9.4*	*3.0*	*11.5*	*22.8*
Data transformation		Natural log	Square root		Square root	Square root	LOGe	Reciprocal	Natural log	Natural log	Square

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

for SLA and LAR were evident (Figures 3.1 b & c) and contributed largely to the highly significant variety x sampling interaction ($\underline{P}<0.001$, Table 3.3). There was no distinction between the barley lines for either SLA or LAR. Bevy clearly had the highest value for both traits, and was significantly different to all other varieties (Table 3.3). Tahara and Frame displayed the lowest SLA and LAR, which was significantly different to the majority of barley varieties. There was no significant varietal or variety x sampling interaction effects on RLER (Table 3.3).

3.3.1.3 Phosphorus utilisation

For both measures of phosphorus utilisation efficiency there was significant varietal differences ($\underline{P}<0.05$, Table 3.3) with Barque and Mundah ranked one and two respectively (Table 3.5). Keel and Forrest were the least efficient of the barley varieties, although only Forrest was significantly different from Barque and Mundah. Tahara and Bevy were very similar to Keel in their efficiencies and not significantly different to Mundah, while Frame had the poorest utilisation efficiency (PUE = 16.1 $mg^2\ \mu g^{-1}$).

3.3.1.4 Root morphology

ANOVA analysis of the various measures of root morphology identified significant varietal differences for seminal root number and total rooting volume, and significant variety x sampling interactions for maximum rooting depth and R:S ratio (Table 3.4). No significant variety or interaction effects were established for total rooting length.

3.3.1.4.1 Seminal root number

All barley varieties, and Tahara, exhibited between 5 and 6 seminal roots (Table 3.5). Bevy and Frame averaged 4 and 3 seminal roots per plant respectively.

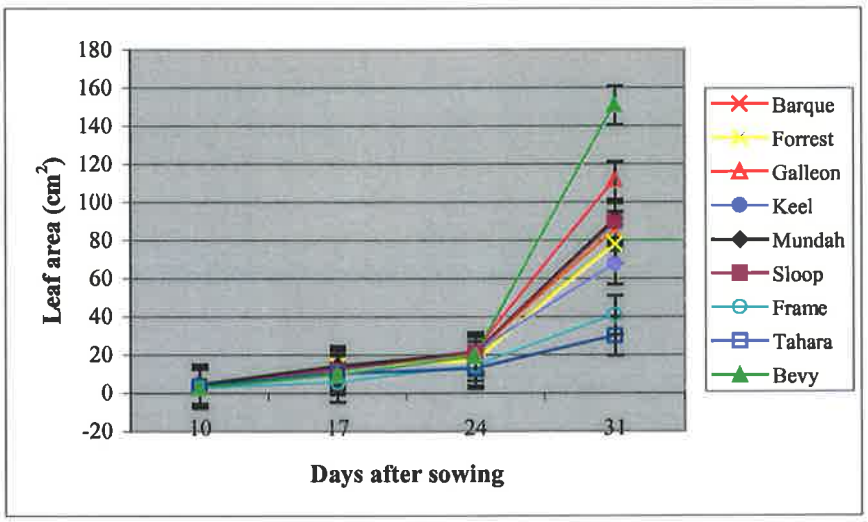
3.3.1.4.2 Maximum rooting depth

The significant variety x sampling interaction (P<0.01, Table 3.5) was particularly evident 31 DAS (Figure 3.2a). The varieties clustered into three groups with Barque and Galleon attaining the greatest depth of rooting by 31 DAS, followed by Forrest, Mundah and Bevy. Tahara, Keel, Sloop and Frame made up the third group whose depth of rooting was less than the other varieties. Sloop, Tahara and Frame had particularly shallow rooting depths, which were significantly different to all other varieties except Keel (Table 3.4). While the average rooting depth of Keel was not significantly different to varieties such Barque, Galleon, Mundah and Forrest (Table 3.4), it is clear from Figure 3.2a that the maximum rooting depth of Keel had reached a plateau, while the roots of these other varieties continued to extend down the soil 'profile'.

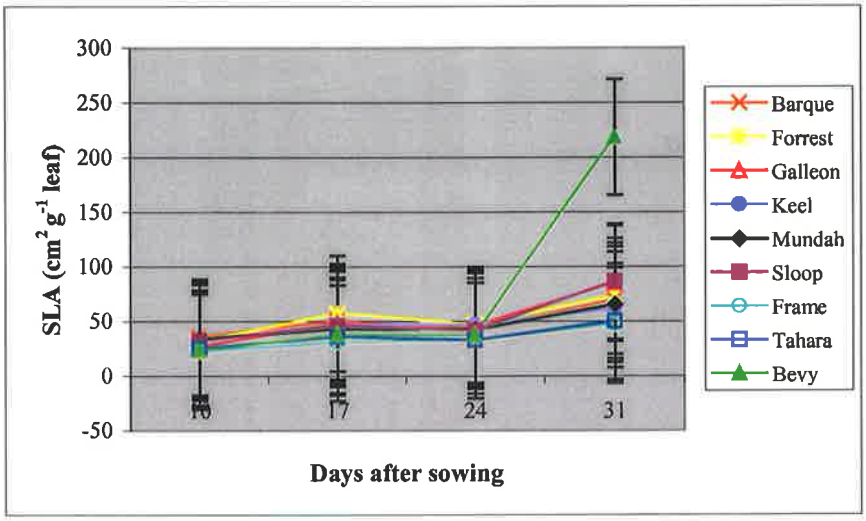
3.3.1.4.3 Total rooting volume, RLD and R:S ratio

ANOVA analysis of total rooting volume was characterised with a coefficient of variation (CV) of 32.8% (Table 3.3). The high CV value suggests a high degree of error from the measurement of total rooting volume and as such any statistically significant differences should be viewed with some caution. RLD was also characterised by a high CV (20.5%). The high root volume of Sloop, as determined by RLD, was high (0.773 cm cm^{-3}) under the conditions of this controlled environment experiment (Table 3.4). The range in RLD amongst the other varieties evaluated was quite large (0.408-0.610 cm cm^{-3}l), although the differences within this group of varieties were not estimated to be significant according to LSD values of the raw RLD results. In Figure 3.2b it is evident that the significant varietal differences, determined by ANOVA of the transformed data (reciprocal of RLD, Table 3.3), was related to RLD at 31 DAS. The RLD of Tahara remained relatively steady and low, and was the only variety not to exhibit an increase between 24 and 31 DAS. Consequently, Tahara achieved the lowest RLD of the varieties evaluated.

a)



b)



c)

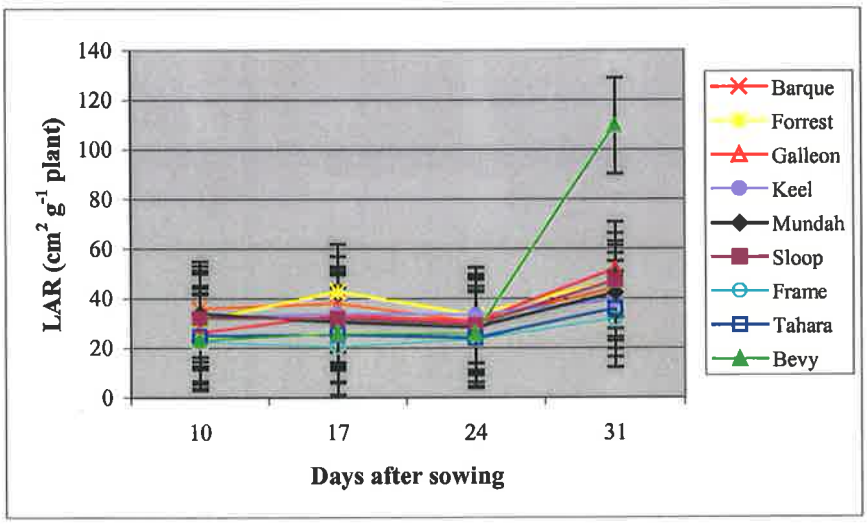


Figure 3.1: Leaf area development, measured as a) total leaf area, b) specific leaf area and c) leaf area ratio, of six barley varieties, one wheat variety, one cereal rye variety, and one tritcale variety in experiment 1.

The ratio of root dry weight to shoot dry weight displayed a declining trend to 24 DAS, at which point Bevy, Sloop, Galleon, Frame and Keel showed an increase in R:S ratio to 31 DAS, Mundah remained steady, and Tahara and Barque continued to decline to varying degrees (Figure 3.2c). Figure 3.2c illustrates that R:S ratio was quite variable between sampling dates, although Sloop was generally one of the higher ranked varieties, and Tahara had consistently the lowest R:S ratio (Table 3.4).

Table 3.4: Mean squares from analysis of variance of seminal root number, maximum rooting depth, total rooting length, total rooting volume, root length density (RLD), root:shoot (R:S) ratio of 9 barley varieties, one wheat variety, one cereal rye and one triticale variety in experiment 1.

Source of variation	df	Seminal root number	Max. rooting depth	Total rooting length	Total rooting volume	RLD	R:S ratio
Variety	11	4.9442***	19.329**	0.2479	1.8887*	1.1922***	0.014056**
Sampling	3	NA	1008.454***	19.1615***	101.4467**	31.7994***	0.129161***
Variety x Sampling	33	NA	14.999**	0.1872	0.9629	0.5081	0.009489**
Residual	47	0.1258 *(df=11)*	6.258	0.1278	0.8494	0.3160	0.004215
CV (%)		*6.7*	*11.4*	*4.7*	*32.8*	*20.5*	*6.8*
Data transformation				Natural log	Reciprocal	Reciprocal	Square root

***P<0.001, ** P<0.01, * P<0.05 NA = not applicable

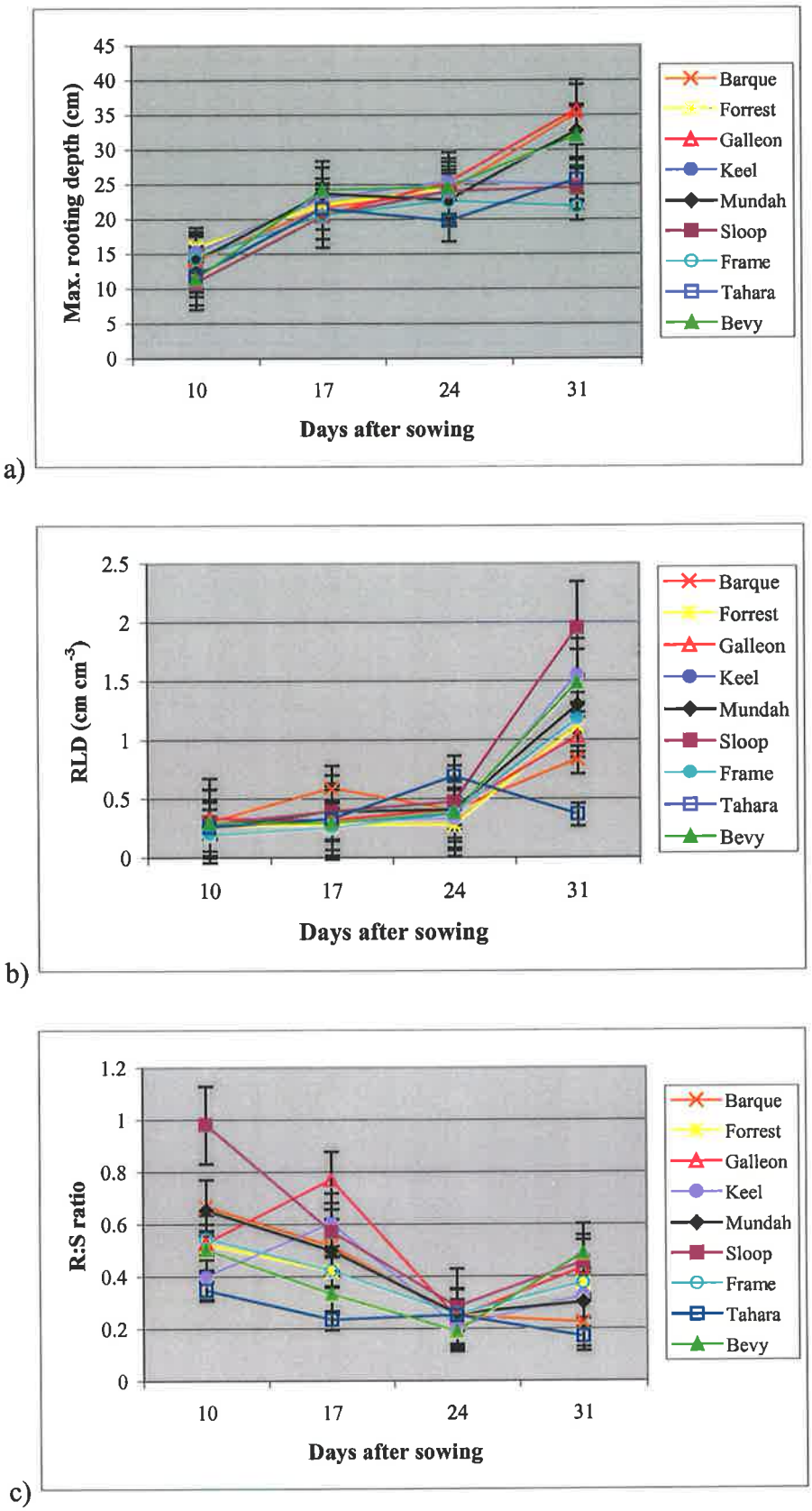


Figure 3.2: Root morphology measurements of six barley varieties, one wheat variety, one cereal rye variety, and one tritcale variety in experiment 1. a) maximum rooting depth, b) root length density, and c) root:shoot ratio.

Table 3.5: Mean values of leaf expansion rate (LER, cm^2 day^{-1}), phosphorus efficiency ratio (PER, mg μg^{-1}), phosphorus utilization efficiency (PUE, mg^2 μg^{-1}), seminal root number, maximum rooting depth (cm), total rooting volume (cm^3), root length density (RLD, cm cm^{-3}) and Root:Shoot (R:S) ratio of 6 barley varieties, 1 wheat variety, 1 cereal rye and 1 triticale variety in experiment 1. LSDs are related to the ANOVA of non-transformed data.

Variety	LER	PER	PUE	Seminal root number	Max. rooting depth	Total rooting volume	RLD	R:S ratio
Barque	2.98abc	0.378a	33.3a	5.82a	23.61a	0.96b	0.525ab	0.412ab
Forrest	2.74abc	0.255b	16.7b	5.66a	23.62a	1.40b	0.479ab	0.391abc
Galleon	3.92ab	0.347ab	27.4ab	5.50ab	23.91a	1.97ab	0.516ab	0.491a
Keel	2.35bc	0.276ab	20.0ab	5.66a	22.09ab	1.94ab	0.610ab	0.382abc
Mundah	3.21abc	0.376a	29.3ab	5.17b	23.20a	1.46b	0.588ab	0.428ab
Sloop	3.16abc	0.300ab	25.1ab	5.50ab	19.94b	5.32a	0.773a	0.450ab
Frame wheat	1.43bc	0.283ab	16.1b	3.21d	19.77b	0.76b	0.491ab	0.327bc
Bevy rye	5.36a	0.262b	19.5ab	4.35c	22.98a	4.22ab	0.607ab	0.376abc
Tahara triticale	1.03c	0.247b	19.8ab	5.50ab	19.67b	0.55b	0.408b	0.249c
LSD (0.05)	*2.64*	*0.110*	*15.9*	*0.39*	*2.52*	*3.69*	*0.316*	*0.152*

3.3.2 Experiment 2

3.3.2.1 Biomass (dry matter) production

The period of significant biomass production ($P<0.001$), and differentiation between varieties, was between 24 and 31 DAS (Table 3.6, Figure 3.3a). Galleon established the greatest increase in biomass over this period, as determined by CGR (Table 3.6, Figure 3.3b) and RGR (Table 3.8). Although Mundah exhibited a higher CGR and RGR than Barque and Keel between 24 and 31 DAS, final biomass production of these three varieties was equivalent. The low biomass of Sloop and Forrest at 31 DAS resulted from a low CGR and RGR (Table 3.8, Figure 3.3b). The CVs for CGR (45.4%) and RGR (39.9%) were high.

3.3.2.2 Leaf area development

A significant variety effect was established for total leaf area ($P<0.001$, Table 3.6) with the majority of varieties displaying an exponential pattern in leaf area development (Figure 3.3c). Sloop was the exception with leaf area development maintaining an almost linear trend from 17 DAS. Galleon attained the greatest leaf area by 31 DAS. Barque, Keel, Mundah, Sloop and Forrest ranked accordingly, but the differences were not significant. Only Barque was not significantly different to Galleon. There was no significant varietal differences or variety x sampling interaction for SLA, LAR, LER or RLER (Table 3.6). That the SLA and LAR of Galleon (290 $cm^2\ g^{-1}$, 178.1 $cm^2\ g^{-1}$) was not statistically different to Barque (303 $cm^2\ g^{-1}$, 189.3 $cm^2\ g^{-1}$), Mundah (317 $cm^2\ g^{-1}$, 179.4 $cm^2\ g^{-1}$), or Keel (340 $cm^2\ g^{-1}$, 179.2 $cm^2\ g^{-1}$) (Table 3.6), suggests that the biomass accumulation and leaf area development of Galleon was proportionately greater than these other varieties (Table 3.8). Sloop had the lowest LAR (167.6 $cm^2\ g^{-1}$), accumulated biomass (Figure 3.3a) and total leaf area (Figure 3.3b) at 31 DAS. Interestingly, Forrest also had the lowest total leaf area even though its LAR (194.4 $cm^2\ g^{-1}$) was greater, although not significant, to Sloop, and the other varieties.

Table 3.6: Mean squares from analysis of variance of biomass, crop growth rate (CGR), relative crop growth rate (RGR), and leaf areaparameters (leaf area (LA), specific leaf area (SLA), leaf area ratio (LAR), leaf expansion rate (LER), relative leaf expansion rate (RLER) of 6 barley varieties in experiment 2.

Source of variation	df	Biomass	CGR	RGR	LA	SLA	LAR	LER	RLER
Variety	5	0.0109*	$9.76x10^{-4}$***	$1.47x10^{-5}$**	2.9878***	0.0184	0.0105	0.1562	0.0011
Sampling	3	0.3996***	$2.05x10^{-2}$***	$2.65x10^{-4}$***	224.7874***	5.3801***	2.6654***	11.1395***	0.0420**
Variety x Sampling	15	0.0053***	$5.92x10^{-4}$***	$4.59x10^{-6}$	0.4879	0.0183	0.0229	0.0288	0.0034
Residual	48	0.0045	$1.57x10^{-4}$	$3.09x10^{-5}$	0.4974	0.0309	0.0218	0.0809	0.0056
CV (%)		*7.7*	*45.4*	*39.9*	*11.4*	*3.1*	*2.9*	*20.9*	*4.1*
Data transformation		Square root			Square root	Natural log	Natural log	Natural log	Square root

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

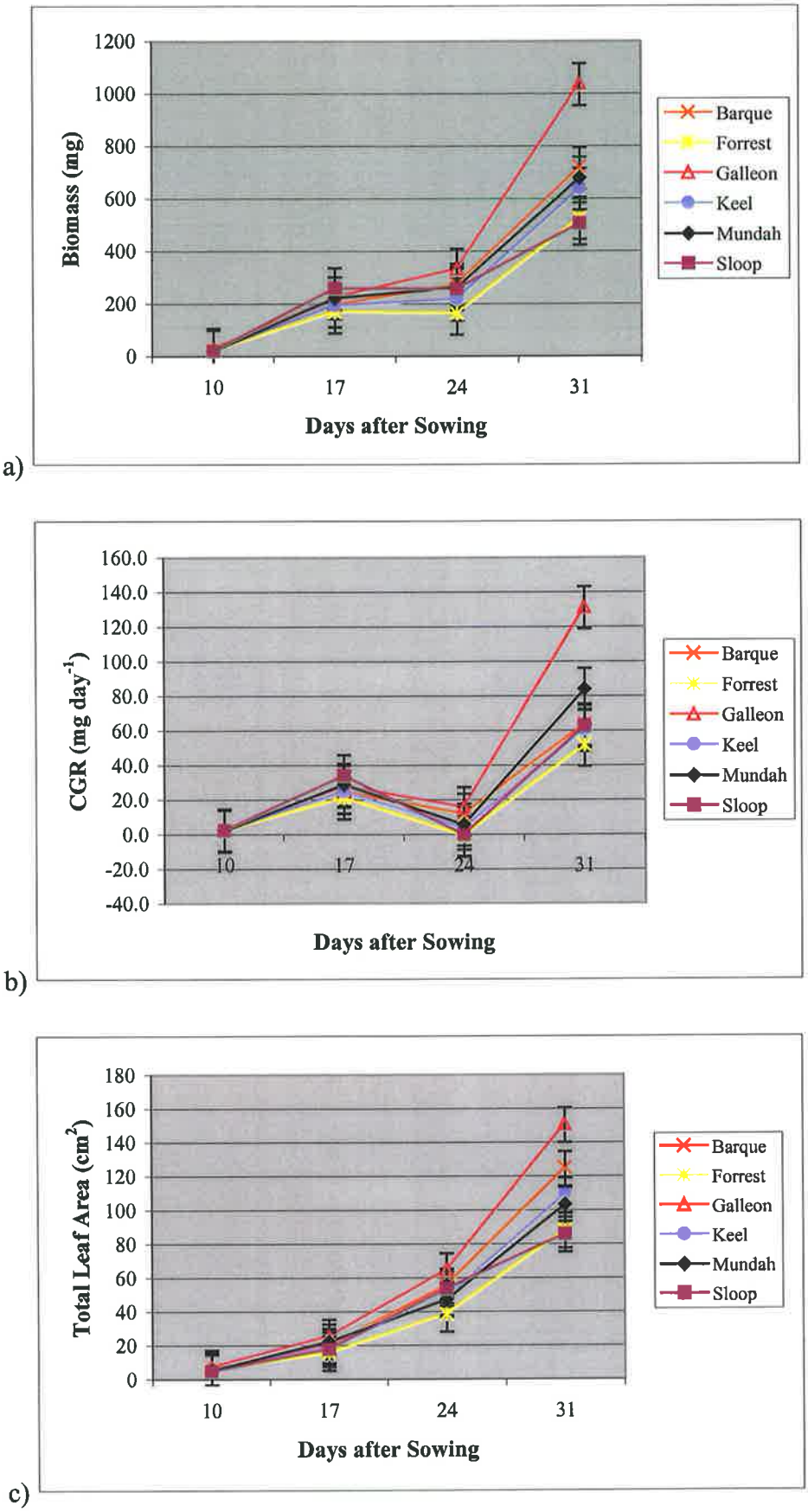


Figure 3.3: Biomass and leaf area development, measured as a) biomass production, b) crop growth rate and c) total leaf area, of six barley varieties in experiment 2.

3.3.2.3 Root morphology

3.3.2.3.1 Seminal root number

Results from the ANOVA showed a small but significant ($P<0.05$) varietal effect on seminal root number (Table 3.7), although this did not correlate to adaptation on sandy soils. The highest average number of 6.5 was recorded for Keel and Barque, and the lowest of 5.9 for Sloop.

3.3.2.3.2 Maximum rooting depth

No significant variety x sampling interaction was calculated for maximum rooting depth, but there was a significant main effect of variety and sampling date (Table 3.7). Barque attained the greatest average rooting depth (30.18 cm). Mundah, Galleon, Sloop and Keel were not statistically different to Barque (Table 3.8). The maximum rooting depth of Forrest however was significantly different to Barque, Mundah and Galleon, only extending 25 cm in to the soil profile. Figure 3.4a confirms the ranking of varieties for maximum rooting depth in Table 3.8. Between 24 and 31 DAS, Barque, in particular, but also Mundah, continued to exhibit an increase in the extension of the root system down the soil profile, while the other varieties seemed to be beginning to plateau in their rate of elongation.

3.3.2.3.3 Total rooting length, Total rooting volume, RLD and R:S ratio

Image analysis of the roots extracted from the soil at each time of sampling found that Galleon and Sloop had the most extensive rooting system, both in terms of total rooting length and total rooting volume (Table 3.8). Keel exhibited a low value for total rooting length (246.2 cm) and the lowest for total rooting volume (1851 cm^3). Mundah and Forrest also had low total rooting lengths, although their total rooting volume was more in parallel to Galleon and Barque than Keel. The differences established for total rooting length were also illustrated in the RLD values of the varieties (Table 3.8). This was not unexpected, since RLD is simply the ratio of total root length per volume of soil. Galleon and Sloop were

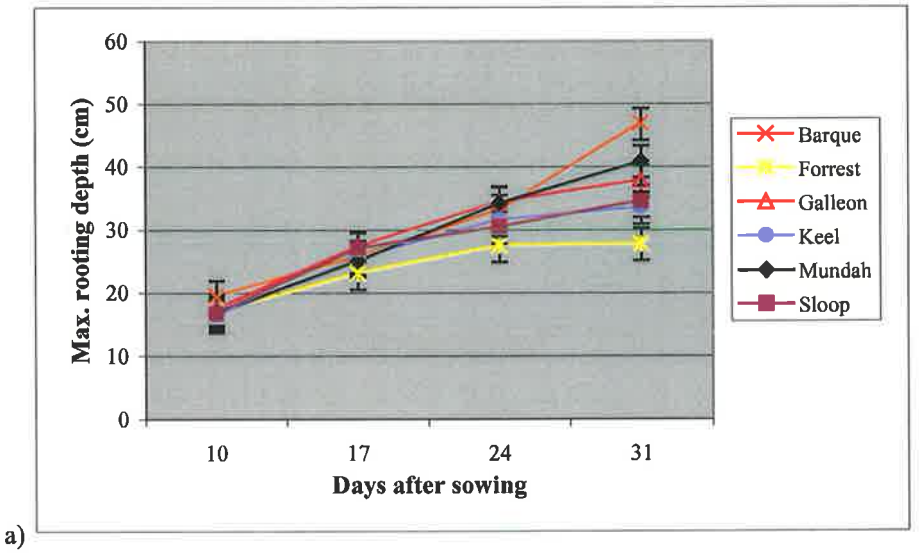
clearly superior to the other varieties. Mundah had the lowest RLD, although it was not significantly different to Forrest, Keel and Barque. The low RLD of Mundah came as a direct result of a substantial decline in the ratio between 24 and 31 DAS (Figure 3.4b). Up to this point the RLD of Mundah was equivalent to Barque and Keel. Over this same period the RLD of Forrest increased from the lowest at 24 DAS to the highest at 31 DAS. Sloop and Galleon maintained the highest RLD from 17 DAS, and were only exceeded by Forrest at 31 DAS.

As with experiment 1, R:S ratio was quite variable between sampling dates (Figure 3.4c). By 31 DAS Galleon had the highest R:S ratio of 0.613. Forrest, Mundah and Keel had a moderate ratio relative to Galleon. As for Barque and Keel, the dry weight of roots was only one quarter to one third the dry weight of the whole shoot. The coefficient of variation for R:S ratio was very large (32.9%), so while trends in the ratio between varieties can be surmised, the statistical significance of the data is unreliable.

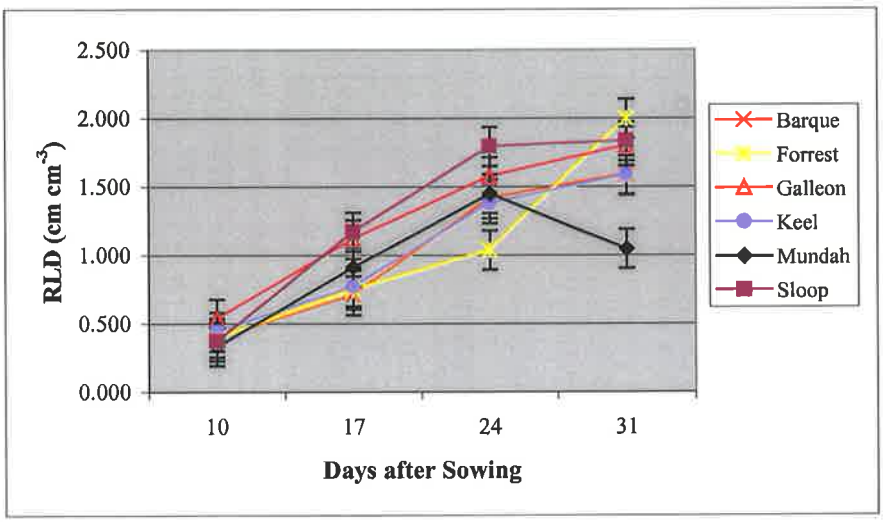
Table 3.7: Mean squares from analysis of variance of seminal root number, maximum rooting depth, total rooting length, total rooting volume, root length density (RLD), root:shoot (R:S) ratio of 6 barley varietiesin experiment 2

Source of variation	df	Seminal root number	Max. rooting depth	Total rooting length (as SQRT)	Total rooting volume (as LN)	RLD (as SQRT)	R:S ratio
Variety	5	0.7424*	65.25*	165.98**	0.5516**	0.0357**	0.0363**
Sampling	3	NA	1216.31***	6765.18***	4.1253***	0.9264***	0.3115***
Variety x Sampling	15	NA	17.55	61.59	0.2633	0.2285*	0.0186*
Residual	48	0.1914 *(df=5)*	19.28	33.82	0.1486	0.0085	0.0088
CV (%)		*7.1*	*15.8*	*12.1*	*5.1*	*7.4*	*32.9*
Data transformation				Square root	Natural log	Square root	

***P<0.001, ** P<0.01, * P<0.05 NA = not applicable



a)



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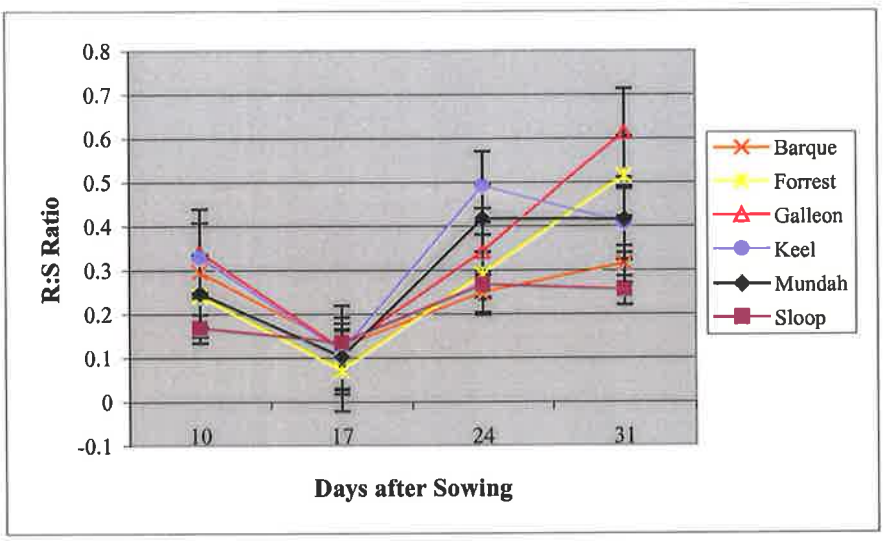


c)

Figure 3.4: Root morphology measurements of six barley varieties in experiment 2. a) maximum rooting depth, b) root length density, and c) root:shoot ratio.

Table 3.8: Mean values of biomass (m), crop growth rate (CGR, mg day^{-1}), relative growth rate (RGR, mg mg^{-1} GDD^{-1}), total leaf area (cm^2), seminal root number, maximum rooting depth (cm), total rooting length (cm), total rooting volume (cm^3), root length density (RLD, cm cm^{-3}) and root:shoot (R:S) ratio of 6 barley varieties in experiment 2. LSDs are related to the ANOVA of non-transformed data.

Variety	Biomass	CGR	RGR	Total leaf area	Seminal root number	Max. rooting depth	Total rooting length	Total rooting volume	RLD	R:S ratio
Barque	300ab	25.3ab	3.89ab	51.3ab	6.5a	30.18a	259.7bc	2100a	1.026b	0.244bc
Forrest	219b	18.5b	3.33ab	36.8b	6.0b	25.25b	216.2c	2022a	1.044b	0.317ab
Galleon	404a	36.7a	5.44a	61.7a	6.2ab	29.21a	308.1a	2314a	1.255a	0.353a
Keel	266b	22.5ab	3.78ab	45.2b	6.5a	26.67ab	246.2c	1851a	1.041b	0.334ab
Mundah	296ab	24.0ab	4.00ab	44.6b	6.2ab	29.31a	237.8c	2271a	0.936b	0.296abc
Sloop	259b	17.8b	3.00b	40.3b	5.9b	27.32ab	300.3ab	2769a	1.291a	0.205c
LSD (0.05)	*114*	*17.2*	*2.26*	*14.5*	*0.389*	*3.94*	*44.5*	*1035*	*0.203*	*0.103*

3.3.3 Experiment 3

3.3.3.1 Biomass (dry matter) production

Galleon maintained a higher biomass from 17 DAS (Figure 3.5). While the biomass of Mundah was lower than Galleon, there was not significant difference between the varieties at any stage (Table 3.9). The CV values for CGR and RGR were extremely high and as such no data or ANOVA information is presented for either measurement of growth rate, since the results cannot be fully or confidently interpreted due to the high extraneous experimental variation.

3.3.3.2 Leaf area development

The pattern of total leaf area development (Figure 3.5b) throughout the experiment was clearly influenced by the significant variety x sampling interaction for both LER ($\underline{P}<0.01$) and RLER ($\underline{P}<0.05$) (Table 3.9, Figure 3.6a & b), even though no significant difference between

Table 3.9: Mean squares from analysis of variance of biomass, leaf area parameters (leaf area (LA), specific leaf area (SLA), leaf area ratio (LAR), leaf expansion rate (LER), relative leaf expansion rate (RLER)) and root morphology parameters (maximum rooting depth, total rooting length, total rooting volume, root length density (RLD), and root:shoot (R:S) ratio) of Mundah and Galleon in experiment 3.

Source of variation	df	Biomass	LA	SLA	LAR	LER	RLER	Max. rooting depth	Total rooting length	Total rooting volume	RLD	R:S ratio
Variety	1	0.0006	0.2357	2206.6#	374.8	0.0926	0.0042	0.1136	$7.27x10^{6}$***	$2.68x10^{6}$***	0.07482**	$1x10^{-6}$
Sampling	3	0.0308***	33.5039***	13369.2***	5863.5***	1.2873***	0.0082*	10.3496***	$6.24x10^{7}$***	$5.51x10^{6}$***	2.9583***	$9.13x10^{-3}$
Variety x Sampling	3	0.0002	0.2756	32.9	44.3	0.4437**	0.0069*	0.1237	$1.46x10^{6}$**	$8.99x10^{5}$***	0.1780*	$1.53x10^{-3}$
Residual	21	0.0007	0.3313	557.8	402.1	0.0754	0.0018	0.0690	$2.61x10^{5}$	96788	0.0563	$8.53x10^{-3}$
CV (%)		*3.4*	*12.6*	*6.9*	*8.6*	*18.9*	*36.1*	*4.3*	*13.9*	*15.3*	*21.3*	*21.1*
Data transformation		Square root	Square root			Square root						

***P<0.001, ** P<0.01, * P<0.05, # P<0.10

varieties was determined for total leaf area by the analysis of variance. The expansion rate of leaf area for both Mundah and Galleon was similar up to 17 DAS, and so was the total leaf area. At 24 DAS the higher leaf area of Galleon seems to be linked to an increase LER, and the decline in RLER of Mundah. From 24 DAS the LER and RLER of Galleon declined to such an extent that the leaf area of Mundah exceeded Galleon. Variety was a moderately significant source of variation for SLA (P=0.10, Table 3.9). On average Galleon maintained a higher SLA (348.2 cm^2 g^{-1}) than Mundah (331.6 cm^2 g^{-1}) throughout the course of the experiment. No significant source of variation was determined for LAR (Table 3.9).

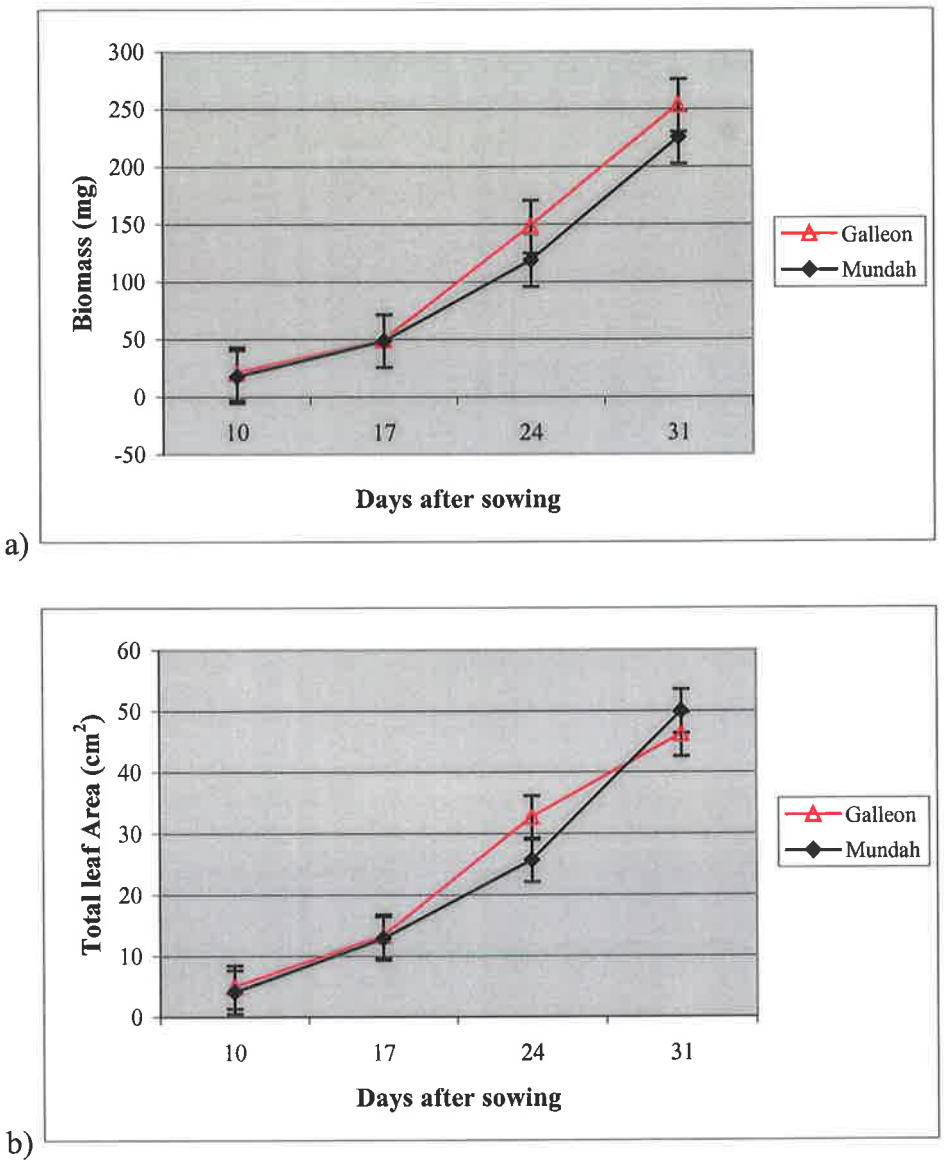
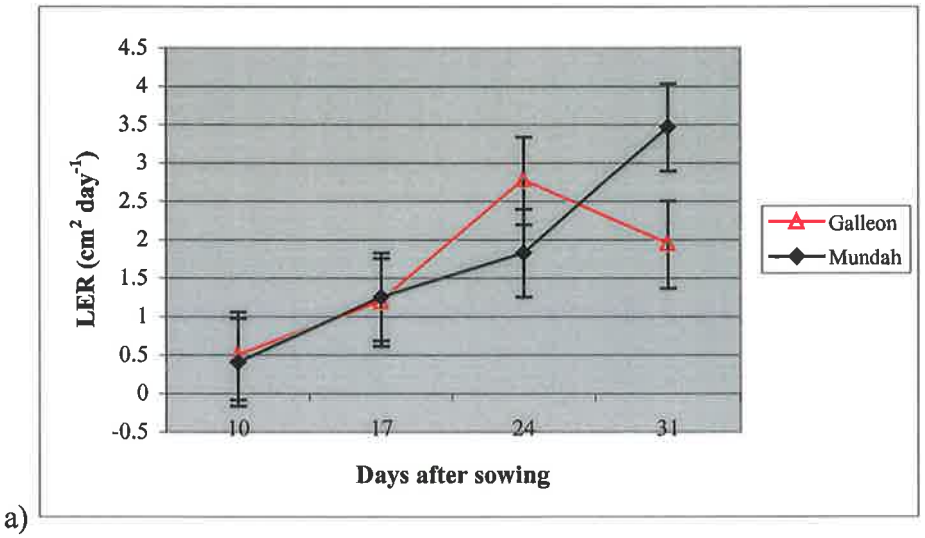
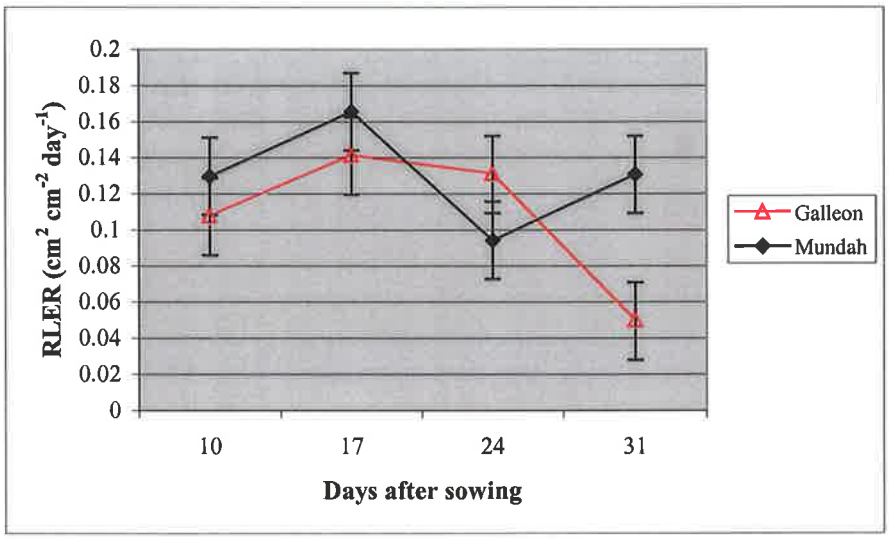


Figure 3.5: Biomass and leaf area development, measured as a) biomass production and b) total leaf area of, Galleon and Mundah in experiment 3.



a)



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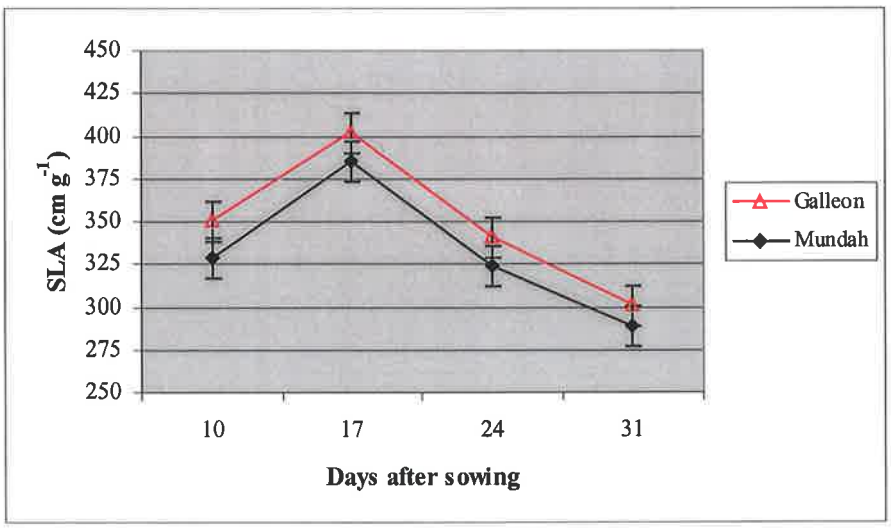


c)

Figure 3.6: Leaf area development, measured as a)leaf expansion rate, b) relative leaf expansion rate and c) specific leaf area, of Galleon and Mundah in experiment 3.

3.3.3.3 Root morphology

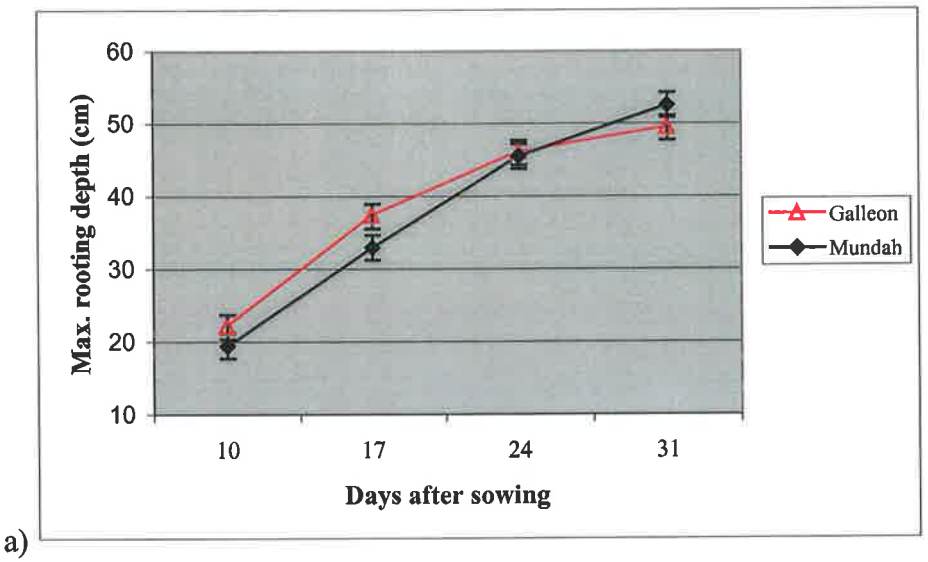
3.3.3.3.1 Maximum rooting depth

The extension of the seminal roots into the soil profile essentially followed the pattern exhibited by Mundah and Galleon in experiment 2. Galleon had a deeper rooting profile up to 17 DAS, from where there was a convergence until both varieties attained almost the same rooting depth at 24 DAS (Figure 3.7a). By 31 DAS Mundah had the deeper rooting profile, although neither variety nor the interaction between variety and sampling date were significant sources of variation (Table 3.9).

3.3.3.3.2 RLD, Total rooting length, Total rooting volume and R:S ratio

For both Mundah and Galleon, RLD increased with time, unlike in experiment 1 where RLD maintained a stable value to 24 DAS before rapidly rising. While Galleon maintained a higher RLD at each sampling, the difference was only significant for harvests after 17 DAS ($\underline{P}<0.05$, Figure 3.7b). By the final harvest (31 DAS), Galleon had reached a RLD of 2.15 cm cm^{-3} and Mundah a value of 1.44 cm cm^{-3}. In experiment 2, Galleon also attained a higher RLD value, however this was due to a decline in the RLD of Mundah from 24 DAS, rather than a difference in the rate of increase observed in experiment 3. The pattern of development for RLD and total rooting length (Table 3.7c) was essentially the same. Again this was not surprising considering RLD is a function of root length.

The average total rooting volume of Mundah (2328 cm^3) was significantly ($\underline{P}<0.001$) greater than that for Galleon (1749 cm^3). ANOVA also determined the interaction between variety and sampling to be highly significant ($\underline{P}<0.001$, Table 3.8). The significance of the interaction term was clearly a function of the increase in total rooting volume between 17 and 24 DAS of Mundah (Figure 3.8a). Overall, however, total rooting volume was quite variable between harvests, and may not account for the differences in adaptation between the two varieties.



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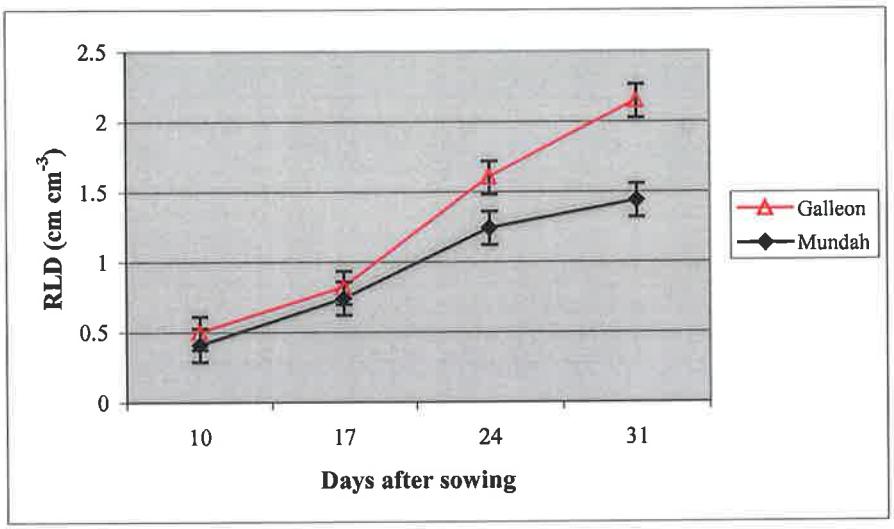

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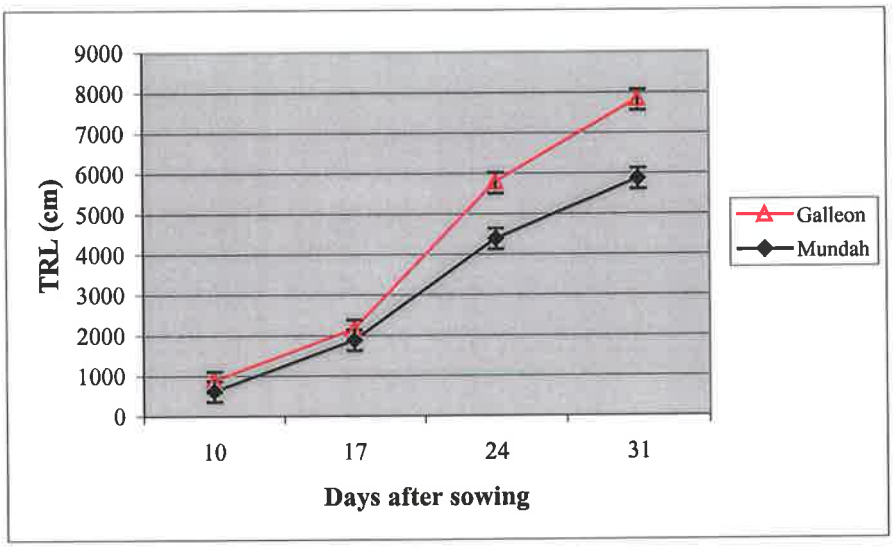
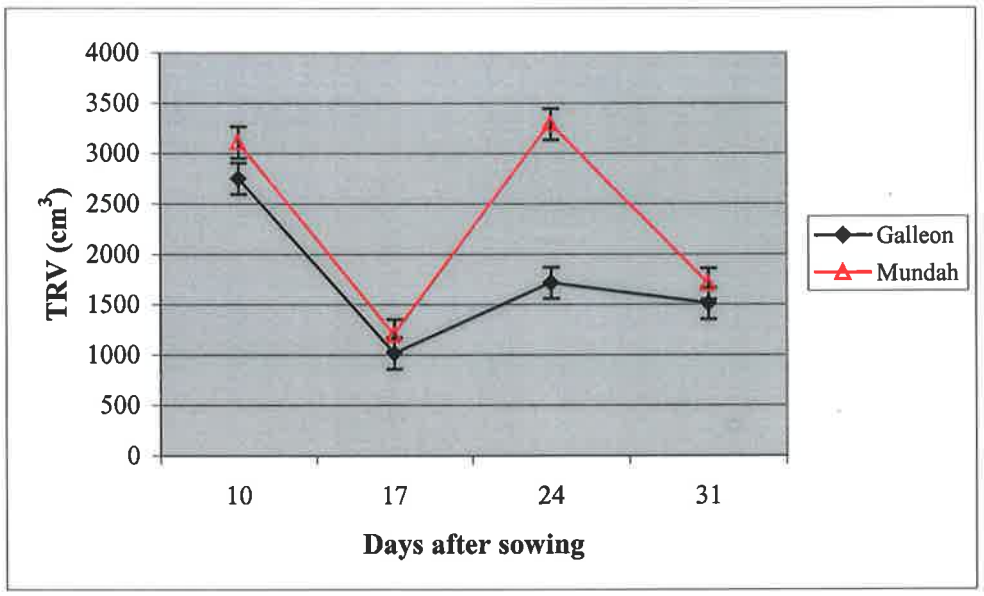


c)

Figure 3.7: Root morphology measurements of Galleon and Mundah in experiment 3 a) maximum rooting depth, b) root length density, and c) total rooting length.

No significant source of variation for R:S was determined by ANOVA (Table 3.9). R:S declined over the first 17 days after sowing, and continued to do so for Mundah to 24 DAS. For Galleon, however, R:S remained constant between 17 and 24 DAS (Figure 3.8b). After 24 DAS, R:S increased for both varieties.



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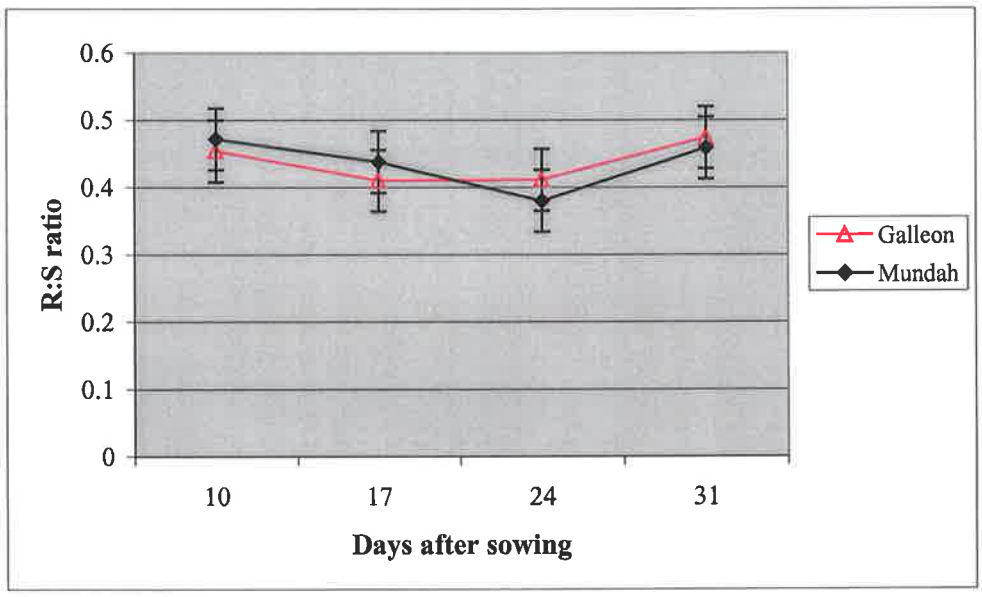


b)

Figure 3.8: Root morphology measurements of Galleon and Mundah in experiment 3 a) total rooting volume and b) root:shoot ratio.

3.4 Discussion

From the field component of this study (Chapter 2) it was proposed that specific traits defined the adaptation of barley, cereal rye, wheat and triticale on sandy soils. It was emphasized that traits potentially contributing to adaptation included early plant canopy development and biomass production, the efficient acquisition and conversion of phosphorus into dry matter, the storage and availability of pre-anthesis assimilate for grain filling, and an erect growth habit and early flowering. That study, plus the long term results from evaluation of barley varieties on sandy soils by SARDI, rank the relative adaptation of the control set of varieties in the order illustrated in Table 3.10 ("Sand" field rating).

Table 3.10: Relative adaptation of barley, Bevy rye, Frame wheat and Tahara triticale from field evaluation and ratings for specific traits measure in the controlled environment experiment.

Species	Variety	"Sand" Field Rating*	Controlled Environment Experiment						
			Biomass	Total leaf area	SLA	LAR	Maximum rooting depth	RLD	PUE
Cereal Rye	Bevy	1	Mod.	High	High	High	High	Mod. High	Low
Barley	Mundah	2	Mod.	Mod.	Mod.	Mod.	High	Mod.	High
	Barque	3	Mod.	Mod.	Mod.	Mod.	High	Mod.	High
	Forrest	4	Low	Mod.	Mod.	Mod.	High/Low	Mod. High	Low
	Keel	5	Mod.	Mod.	Mod.	Mod.	Mod.	Mod. High	Mod.
	Sloop	6	Low	Mod.	Mod.	Mod.	Mod.	High	Mod.
	Galleon	7	Mod. High	High	Mod.	Mod.	High	High	Mod.
Triticale	Tahara	8	Mod.	Low	Low	Low	Mod.	Low	Low
Wheat	Frame	9	Low	Low	Low	Low	Low	Mod.	Low

*based on long term yield analysis of SARDI sand evaluation trials (Source: R. Wheeler)

3.4.1 Response of different cereal species on a sandy soil under controlled environmental conditions (Experiment 1)

For many of the traits considered important for adaptation on sandy soils, such as biomass production, leaf area development, utilization of phosphorus and maximum rooting depth, Frame wheat and Tahara triticale were found to be inferior to barley and Bevy rye (Table 3.10). While Tahara was comparable or better than the barley varieties for biomass, its rating for other key sand adaptation traits was low (Table 3.10). Both Frame and Tahara had poor leaf area development, and SLA and LAR. The importance of these traits has already been discussed in chapter 2. A more rapid development of the canopy provides a higher interception of light at any one time, and a reduction in time to full light interception. In addition crop photosynthesis is likely to directly benefit, and the amount of radiant energy reaching the soil surface would be restricted, thereby enhancing water use efficiency through improved transpiration and reduced evaporation of moisture from the soil (Richards, 1991 & 2000). The low SLA and LAR of Frame and Tahara indicated that the investment in plant development in these varieties was substantially in favour of total dry matter production rather than leaf area development. The implications of this are that during early developmental stages the net assimilation rate is reduced, especially in contrast to a plant with an equivalent leaf weight but higher leaf area (Richards, 2000). The poorer adaptation of these two cereal species may also be due to their relatively inefficient use of phosphorus and shallow maximum rooting depth (Table 3.5 and Figure 3.2a). In contrast to the wheat and triticale cultivars the cereal rye variety (Bevy) displayed many features that may contribute to its known adaptation on sandy soils (Table 3.10). Bevy exhibited a high LER, which converted in to the high total leaf area attained by 31 DAS (Figure 3.1a). Bevy clearly had the superior SLA and LAR (Figure 3.1b & c), a maximum rooting depth equivalent to Mundah (Figure 3.2a) and a high total rooting volume (Table 3.5). The ability of cereal rye to satisfactorily perform on sandy soils is likely to reflect its good early vigour; its ability to use the available soil moisture supply; and the fact that it has a vigourous and extensive root system (Lovett,

1987). The results from experiment 1 lend some weight to these characteristics as important traits for sand adaptation. Lovett (1987) also suggested rye has a lower soil nutrient requirement. A better utilization efficiency for both macro- and micro-nutrients may be a critical factor in defining the superior canopy development of Bevy at 31 DAS. The results of experiment 1 did not support this conclusion. Bevy was characterized by a low PUE (≡Tahara), although the majority of varieties were determined not to be significantly different. The results of the field variety trials in 1999,discussed in chapter 2, also point to Bevy having a low PUE. The phosphorus content of the Cooke Plains soil (19 mg/kg) was marginally higher than the critical value (18 mg/kg, Reuter *et al.*, 1995). With the prior application of phosphorus, soil phosphorus content was unlikely to be limiting in the early stages of the experiment. The soil was not analysed for soils nutrients at each harvest, so it is not known whether phosphorus was limiting by 31 DAS. If soil phosphorus was not limiting for the duration of the experiment, PUE would not have been a critical factor. Zinc and manganese utilization efficiency values were not conclusive and could not account for the superior canopy of Bevy at 31 DAS (data not shown).

3.4.2 Response of barley varieties of varying adaptation on a sandy soil under controlled environmental conditions (Experiments 1 and 2)

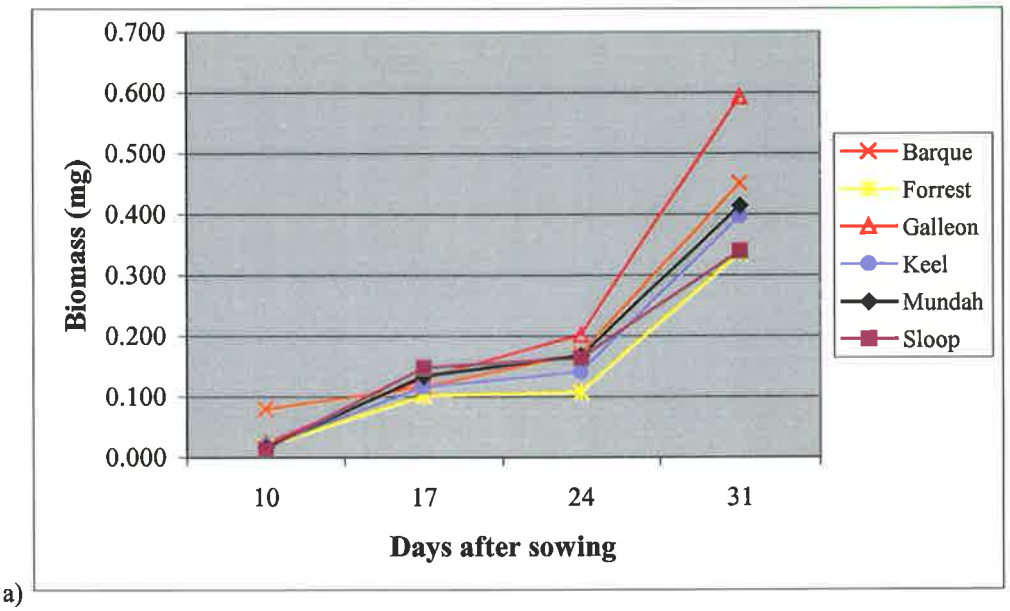
In both experiments 1 and 2, a sandy soil, low in inherent fertility, from Lowbank in South Australia was supplemented with basal nutrients and supplied with adequate moisture (75% field capacity) to remove them as limiting factors. In the sandy soil cropping regions of South Australia characterized, low soil nutrition and moisture are critical environmental constraints to adequate crop growth.

Under controlled environmental conditions, Mundah and Barque displayed features common to those shown in the field experiments and highlighted their superior adaptation on sandy soils of low fertility (Table 3.10). Both exhibited good biomass production, with Mundah also having a high crop growth rate (Figure 3.9a). Total leaf area was well developed by 31

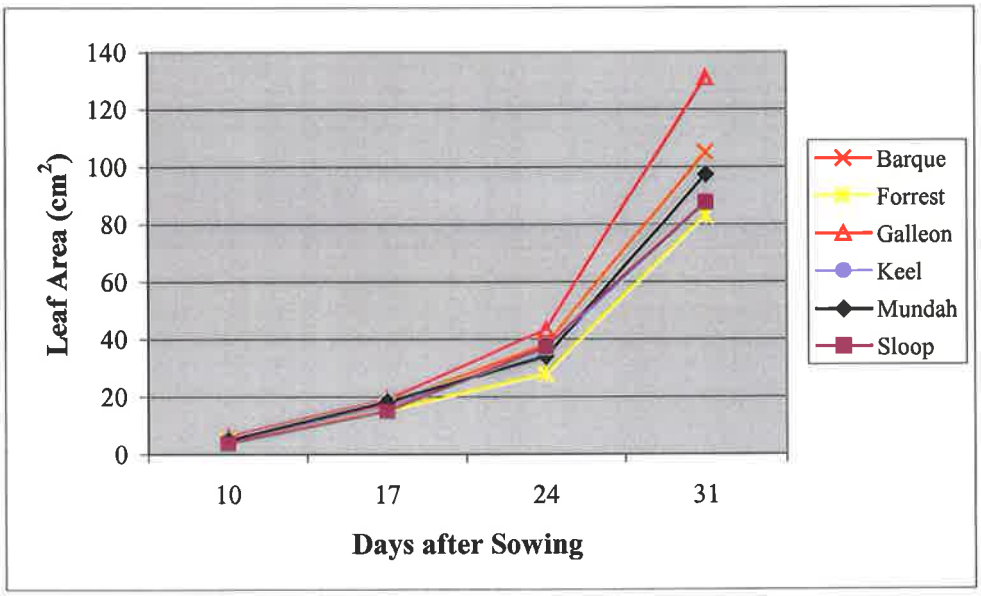
DAS, even though the rate of leaf area development (LER) was not a distinguishing feature between varieties (Figure 3.9b). Keel showed a similar biomass to Mundah, but its leaf area was lower, suggesting that, although there was no significant difference between these varieties for SLA and LAR, biomass production, as a proportion of total crop growth, was the primary component of the early development of Keel. This may represent a key reason for the poorer adaptation of Keel on sandy soils. The lower leaf area to biomass production ratio of Keel may be inadequate to sustain an equivalent biomass compared with Mundah because photosynthetic capacity is reduced. Sloop displayed a low biomass and leaf area despite showing a high leaf area in field experiments. Despite the supply of basal nutrients to off set any limitation on growth, Mundah and Barque still displayed a superior ability to efficiently convert phosphorus into biomass. The higher PUE of Mundah and Barque in the controlled environment experiment equates to their response in the field evaluation trials where phosphorus was deficient.

The detailed study of the roots highlighted morphological features likely to play a role in adaptation to this environment. Mundah and Barque had greater capacity to extend in to the soil profile than Sloop, Keel and Forrest (Figure 3.10a, Table 3.10). On the other hand Sloop, Keel and Forrest had a higher density of roots within the soil profile (Figure 3.10b, Table 3.10). It would appear from these results, and the characteristics of sandy soils, that a high RLD is not a desired trait on sandy soils. For Sloop and Keel the high RLD would most likely reflect their better adaptation on heavier soils where moisture tends to be retained in the upper soil profile, and a deep rooting system is not as essential to accessing available moisture. In contrast, sandy soils have a propensity for poor moisture distribution and rapid drainage down the soil profile. Consequently, a greater emphasis is likely to be placed on a deep rooting morphology to 'chase' moisture, and nutrients, at depth in the soil, rather than a proliferation of branching roots; and this is revealed in the rooting morphology of Mundah and Barque. This reduced emphasis on root branching (low RLD) typifies the response to

moisture stress observed by Gregory *et al.* (1978a), Eghball and Maranville (1993) and Proffitt *et al.* (1985).

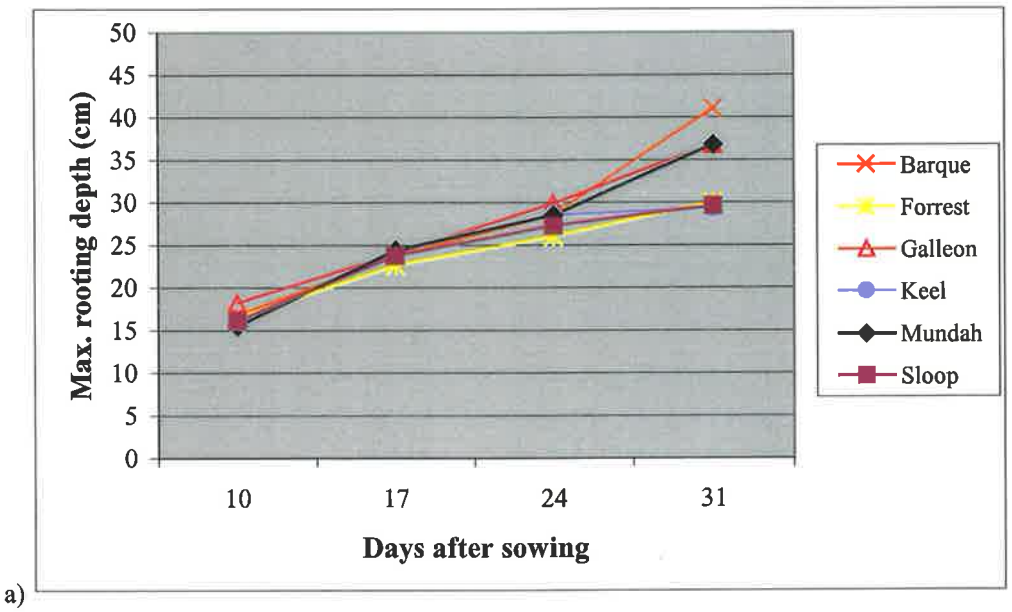


a)

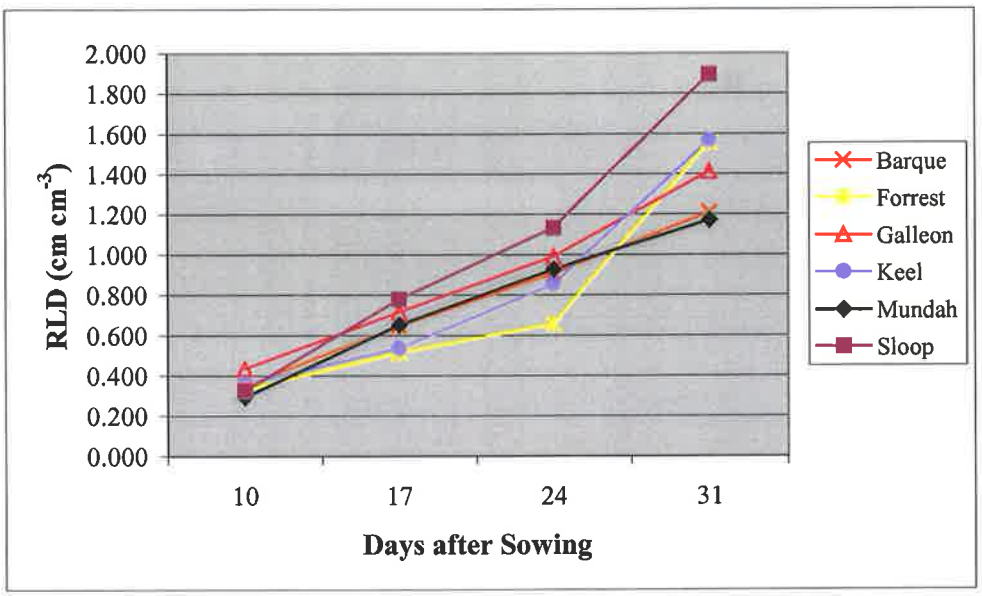


b)

Figure 3.9: Mean biomass production and total leaf area of six barley varieties in experiments 1 and 2.



a)



b)

Figure 3.10: Mean maximum rooting depth and RLD of six barley varieties in experiments 1 and 2.

Conversely, Forrest, which historically is considered adapted to sandy soils, had a high RLD. Together with the fact that in these experiments Forrest was distinctive for its poor biomass and low crop growth rate, poor leaf area development, a shallow maximum rooting depth and a low PUE, suggests that the mechanism by which Forrest adapts to sandy soils is likely to be different to that of Mundah and Barque. Having said that, in the field evaluation trials Forrest

displayed high leaf area development, only exceeded by Mundah and Yagan, but its biomass production was inferior to Keel. In addition, Forrest had a high utilization of assimilate between anthesis and physiological maturity, although it is unlikely that pre-anthesis assimilate would alone explain the adaptation of Forrest.

R:S ratio was not a good indicator of adaptation in these experiments. Both Barque and Keel had a lower R:S ratio than Mundah, despite differences in the adaptation of these two varieties. A major flaw with calculating the ratio of root dry weight to biomass (as a dry weight) to determine genotypic differences for root morphology, is that root weight is dominated by the larger (primary) roots, and reduces or ignores the contribution of smaller roots and root hairs. This is an important issue considering secondary roots (laterals) and root hairs serve more to the acquisition of nutrients and moisture than the larger roots (seminal and nodal roots), from which root mass is composed (Barley, 1970). Therefore root weight does not correlate with water and nutrient uptake (Bőhm, 1979). Neither total rooting volume nor total rooting length effectively explained genotypic variation of root morphology in terms of adaptation on sandy soils. In experiment 1 there was no significant difference between barley varieties for total rooting volume and total rooting length. Whereas in experiment 2, despite the fact that the ANOVA calculated variety to be a significant source of variation for both root morphological traits, there was no strong correlation with adaptation since varieties with poorer adaptation, namely Galleon, Sloop and Keel, displayed quite contrasting results. Galleon and Sloop both exhibited high values for total rooting length and volume, whereas Keel produced low values for each root growth trait.

The response of Galleon in experiments 1 and 2 was intriguing to say the least. Galleon exhibited traits likely to be associated with adaptation on sandy soils. Certainly for many of the traits measured in the controlled environment study Galleon was equivalent to, or superior to, Mundah and Barque (Table 3.10). Galleon displayed the highest biomass production and total leaf area by 31 DAS (Figure 3.9a & b), although in terms of LER, SLA and LAR, Galleon was not significantly different to the other barley varieties examined. As for root

morphology, Galleon attained an equivalent maximum rooting depth to Mundah (Figure 3.10a), but was more extensive in total root length and total root volume. Despite a high total root length, Galleon had a moderate RLD, which was between Keel, and Barque and Mundah. In addition, Galleon only displayed a moderate PUE of 27.4 $mg^2\ \mu g^{-1}$, slightly less than Mundah (29.3 $mg^2\ \mu g^{-1}$). These results tend to contradict those discussed in chapter 2 (Field study). In the field experiments Galleon clearly exhibited very mediocre growth and grain yield on sandy soils of low fertility. This was manifested in restricted biomass production, low leaf area development and poor PUE compared with Mundah at early tillering. Furthermore, a low contribution of pre-anthesis assimilates to the developing grain and a later flowering phenology compounds the unsuitability of Galleon for cropping on sandy soils. From these conflicting results it is possible to ascertain that the ability of Galleon to achieve equivalent or superior responses to Mundah under controlled conditions suggests that the application of basal nutrients to an otherwise low fertile soil, in addition to the supply of adequate moisture, may have assisted the development of Galleon, despite the sandy nature of the soil.

3.4.3 Response of Mundah and Galleon on a sandy soil without basal nutrients under controlled environmental conditions (Experiments 3)

Despite excluding basal nutrients, experiment 3 provided essentially the same outcomes as experiments 1 and 2. Galleon maintained a higher biomass production, SLA, RLD and TRL than Mundah throughout the experiment. However, a picture began to emerge by 31 DAS in terms of total leaf area and maximum rooting depth that suggested Galleon was inhibited by the lack of adequate soil nutrition. By this stage Mundah displayed a slightly greater leaf area and a significantly deeper root system (Figures 5b & 7a respectively). Since the harvesting of material ceased at 31 DAS, it can only be hypothesized that the superior leaf area and rooting depth displayed by Mundah was maintained or increased beyond this point. The fact that Galleon was consistently better than Mundah for biomass production and SLA, suggests that

the moisture status of the soil, for the reason that a sufficient amount was supplied and the soil was of finite depth (*i.e.* no free draining of water beyond the root zone), in these experiments also contributed to Galleon performing in a different way to the field experiments discussed in chapter 2. The higher RLD of Galleon indicates that the root system of Galleon differentially responds to adequate and consistent moisture supply than the root system of Mundah. In spite of a good supply of water, a deeper root system rather than a dense morphology was a characteristic of Mundah. It does seem as though the root system can display advantageous growth depending on the supply, demand and distribution of water (Proffitt *et al.*, 1985; Gregory, 1987; Eghball and Maranville, 1993)). In this context, it is likely that the larger root system of Galleon improved the availability of water, and directly supported greater biomass development. Whether moisture stress would alter the behaviour of root development in Galleon was not considered in these experiments. If root development in Galleon was similar between adequate and stressed moisture regimes, the disparate emphasis towards root development would not maintain an equivalent biomass under moisture limiting conditions. Accordingly the superior biomass of Galleon in these experiments reflects a larger root system better able to exploit the good supply of water.

3.4.4 Evaluation of controlled environment experiments in screening barley for traits associated with improved adaptation on sandy soils

In employing a controlled environment system to screen barley for traits associated with sand adaptation it is important to acknowledge the advantages and disadvantages of such a system. The issues were briefly discussed in the introduction of the chapter. In designing the controlled environment experiment, due care was taken to closely resemble environmental conditions during the early growth of barley immediately following emergence, and remove factors likely to confound trait response on sand. Issues surrounding the impact of seed size (embryo size) and nutrient content on establishment and early vigour (Wood *et al.*, 1977; Bolland and Baker, 1989; Longnecker *et al.*, 1991; Rengel and Graham, 1995a,b; Genc *et al.*,

2000) were removed by screening the seed, of all varieties evaluated in these experiments, to the same size. The spatial variability of early vigour and dry matter production within a crop in the field is high, and this is likely to result from patchy establishment and variable seedling vigour; a consequence of inconsistent seeding depth. In the controlled experiments there was no confounding effect of establishment on early vigour. Seed was pre-germinated, and six sown to equivalent depth in each pot, to ensure at least three healthy and even plants emerged per pot for evaluation. While sandy soils have particularly poor nutrient and soil water relations, basal nutrients were applied to the soil prior to filling the pots to limit this as a factor, and the soil was watered to 75% field capacity to avoid soil water stress, but also to prevent over watering. The adequate supply of water and nutrients in these experiments was proposed as a contributing factor to the contrasting response of Galleon from that observed in the field studies (Chapter 2). The convention in controlled pot studies, to water to 75% field capacity, should be questioned because the distribution of water through the soil profile has been observed to be non-uniform (Kramer and Boyer, 1995). Because of the hydrophobic nature of sandy soils, a wetting agent was used to reduce the possibility of water repellency down the soil profile, and this is likely to have improved the distribution of water. Certainly, the visual observation of the soil prior to recovery of the root system suggested there was no significant problem with non-wetting zones of the soil profile.

Typically, soils have a good buffering capacity to temperature. That is, the temperature of the soil is not significantly altered by sudden changes in ambient temperature. In pot experiments, where there is no medium to buffer the soil from changes in temperature, the temperature of the soil in pots is likely to be very similar to the surrounding environment. This dynamic of pot experiments needs to be addressed in any discussion of results because of the effect of soil temperature on root morphology, and moisture and nutrient uptake by the roots (Bowen, 1991; Kasper and Bland, 1992; McMichael and Burke, 1998; Sharratt, 1991). In the field, during early plant growth, soil temperature tends to hover around 10 to 15°C (Bowen, 1991). In addition, with typical night and day temperatures in SA around 6-9°C and

15-18°C respectively, during May and June (30 year average, 1961-1990), presetting the growth room to 10°C at night and 16°C at day will maintain the temperature of the soil fairly close to natural soil conditions during early growth to negate any significant confounding influence on root growth.

Despite the fact that the methodology employed for these controlled environment studies aimed to reduce the contribution of environmental and seed factors in confounding responses to sand, the viability of pot testing to identify genetic variability for both maximum rooting depth and sand adaptation *per se* may be questionable because of the anomalies exhibited by Galleon and Forrest in contrast to their field adaptation. Having said that, maximum rooting depth did distinguish Mundah and Keel based on their adapatation. The sizable errors, in some instances, determined by statistical analysis of traits measured may also limit the usefulness of pot experiments in identifying varieties with superior adaptation. The high CVs are likely to be associated with sampling procedures, and for root measurements, because there tends to be an unnatural proliferation of roots at the bottom of container (a minor factor in these experiments). One metre length PVC pots were employed in experiment 2 to overcome this problem, and to measure leaf area, biomass and root development beyond 31 days. However problems arose with the use of these pots. Because the pots were not free draining, water collected at the bottom of the pots leaving the majority of the soil profile dry and water was not available to the plants. This also complicated the watering of pots by weight. While the top soil was clearly dry, and plant growth was restricted, the pot weight indicated that the moisture content was at 75% field capacity. As a result plant growth in the one metre pots was well behind that of the smaller pots at the first four harvests, and therefore not comparable. Because of this the measurements of plant growth beyond 31 days could not have been a logical progression from the harvests up to 31 DAS, and were therefore ignored.

Although it is virtually impossible to accurately mimic the natural soil conditions and environment, such experimentation could still provide clues to identifying certain important traits associated with sand adaptation, and the relative differences between varieties of

contrasting adaptation. This is especially so for traits difficult to measure in field studies (*e.g.* root morphology).

3.5 Conclusions

In spite of the 'incongruous' responses of Galleon and Forrest in the pot experiments to their adaptation in the field, these controlled studies have provided further indications of the adaptation responses of contrasting barley varieties on sandy soils. In chapter 2 it was postulated, from field evidence, that Mundah's superior adaptation was, amongst other traits, laid on the foundation of greater early vigour. A better early biomass production and rapid leaf area development set up the improved grain yield potential of Mundah on sandy soils. Clearly though, in order to sustain the yield potential, it is critical for there to be mechanism(s) to ensure moisture and nutrients are not severely limiting to grain yield later in crop development. Consequently, an appropriate root morphology is essential to maintain an adequate supply of moisture and nutrients. For sandy soils, the free draining of moisture and the loss of nutrients down the soil profile, beyond the root zone, suggests a deep rooting profile is an important component of the criteria for adaptation. This certainly formed part of the hypothesis of Turner and Nicolas (1987) for improved grain yield on light textured soils. Furthermore they noted, and similarly suggested by Richards *et al.* (2002), that improved early vigour enabled greater root development, so yield was less restricted by water limitations. Steingrobe *et al.* (2001) similarly found in sandy soils that net root length (root density) was lower than in loamier soil types.

Evidence from this series of controlled environment experiments indicated that maximum rooting depth, in conjunction with a moderate to low RLD, was a feature which differentiated varieties based on their 'adaptation'. Mundah and Barque exhibited both these characteristics,

illustrating that a deep rooting system, rather than a dense rooting system, is a requirement of growth on sandy soils. The evidence presented, suggesting that a deep rooting system confers improved adaptation, seems to be further supported by recent papers by Asseng *et al.* (2002) and Dreccer *et al.* (2002), who predicted through crop simulation models, that fast early root growth and an increase in the maximum rooting depth had a large impact on wheat yields on light textured (sandy) soils. No other measures of root morphology (R:S ratio, TRL and TRV) were found to be associated with sand adaptation in these experiments.

As a final point, it is necessary to acknowledge that adaptation to any environment is a complex inter-relationship or combination of traits, and it is highly unlikely that any one variety will possess the optimum value for any trait in every situation. This is relevant given that the results for Galleon were inconsistent with its adaptation. More importantly, it is the overall package of traits that are associated with adaptation that is likely to be the key to improved growth and grain yield on sandy soils. The experimentation outlined in chapters 2 and 3, has identified those barley varieties that contain a balanced package of traits (*e.g.* Mundah) versus those with a poor combination of traits (*e.g.* Sloop, Galleon) for sand adaptation.

Chapter 4. Traits associated with improved growth and grain yield of barley on sandy soils of low fertility: Mundah seed size experiment

4.1 Introduction

In cereals, seed size is a crucial determinant of early plant development and in turn yield potential. Seed size is closely associated with embryo size (Ogilvie and Kaltsikes, 1977; Lopez-Castaneda *et al.*, 1996; Richards and Lukacs, 2002), and both can influence the rate of seedling emergence and establishment (Wood *et al.*, 1977; Roebuck and Trenerry, 1978; Richards and Lukacs, 2002), and the length of the coleoptile (Cornish and Hindmarsh, 1988; Botwright *et al.*, 2001). In turn a long coleoptile can improve establishment (Whan, 1976; Bacaltchuk and Ulrich, 1990; Hughes and Mitchel, 1987; Radford, 1987; Radford and Wildermuth, 1987; Sharma, 1990) and early vigour (Gul and Allan, 1976; Gorny and Patyna, 1981; Redona and Mackill, 1996; Rebetzke and Richards, 1996; Rebetzke *et al.*, 1999), especially in conditions where establishment is difficult. In contrast Giles (1990) presented evidence that half seeds (*i.e.* small seeds) germinated faster, but had reduced vigour compared with whole seed counterparts of wild barley. It is likely that the reduced capacity of the endosperm inhibited adequate growth following germination. Ceccarelli and Pegiati (1980) studied the effects of seed size and concluded that there was no effect of size or weight of intact seed, for a given cultivar, on coleoptile dimensions; rather coleoptile length was primarily dependent on genotype.

Superior early vigour (Roebuck and Trenerry, 1978; Djisbar and Gradner, 1989; Peterson *et al.*, 1989; Giles, 1990; Lopez-Castaneda *et al.*, 1996; Richards, 2000; Richards and Lukacs, 2002) and grain yield potential (Spilde, 1989; Giles, 1990) have also been shown to be

attributable to larger seed size. Richards and Lukacs (2002) showed that the components of early vigour such as leaf area, early dry matter production, and leaf number, length and width improved, albeit modestly, with larger seed weight. Small seed has also been shown to account for between 4 and 6% reductions in grain yield in barley (Spilde, 1989). This may be related to the production of fewer spikes, and hence fewer seeds, as observed with plants from half seeds in the study by Giles (1990). In contrast, the effect of seed size in the study by Roebuck and Trenerry (1978) was not significant, but there was a trend towards improved establishment, vigour and grain yield with larger seed, particularly with deeper seed placement.

It is suggested then, that the impact of seed size on plant growth and grain yield is equally, or more, relevant to improving the performance of barley on sandy soils. This is because the unreliability of crop production on sandy soils stems from their inherently poor properties for plant production, *viz.* poor plant establishment and growth, difficulty with controlling seeding depth and the effects of sand blasting. In addition, rapid establishment and development of ground cover (early vigour) would maximise water use efficiency and set up the potential for adequate and stable productivity.

Seed size and variation in seed size is determined by a complex interplay between genetic effects, which sets potential and also influences 'realised' grain size (via disease resistance, flowering type, lodging resistance, spike geometry), and environmental effects (rainfall, temperature, soil evaporation, soil fertility). Environmental effects, in particular, can have a large impact on the variation in the proportion of seed in size classes, mean seed size, and seed nutrient content.

However there is also a natural distribution of seed sizes because of a fixed natural size variation within the spike, not related to genetic and environmental factors (Giles, 1990). This is associated with the innate variation in flowering time within the inflorescence leading to differences in grain filling duration (see Giles, 1990), and is also likely to result from competition for assimilates.

This chapter describes an experiment devised to estimate the effect of seed size on growth and grain yield on sand, independent of genotypic and environmental variation in seed size. Seed of Mundah was selected from various breeders' experiments within the SA Barley Improvement Program (SABIP), and the natural variation within every seed sample was exploited to screen samples into different size fractions. Mundah has a comprehensive package of traits that confer superior adaptation on sandy soils (*e.g.* larger seed size, longer coleoptile, better establishment and good early vigour), rather than possessing the optimal value for any one trait. The rationale for employing Mundah in this experiment is to determine whether seed size effects can influence inherent sand adaptation.

4.2 Methods and Materials

4.2.1 Treatments

Seed of Mundah was obtained from Stage 2 breeders' trials at Weetulta (1999, 2000), Geranium (1999), Callington (1999, 2000), Charlick (1999), Clinton (2000), Tuckey (2000) and Yeelanna (2000). The soil type at Geranium, Callington and Tuckey is characteristically sandy, whereas the other sites have, to varying degrees, heavier soil types. Within these experiments Mundah was sown as a control genotype in a grid pattern. Seed from each plot of Mundah, within a trial, was pooled and screened into <2.2mm, 2.2-2.5mm, 2.5-2.8mm, >2.8mm size fractions by sorting over slotted screens. In all, there were 16 (4 source sites x 4 seed size categories) and 20 (5 source sites x 4 seed size categories) treatments for the 2000 and 2001 field experiments respectively (Table 4.1).

4.2.2 Establishment and design of field trials

Treatments were sown as a randomized complete block design with three replicates in 2000 and four replicates in 2001. Randomization of the treatments was devised using the SpaDes® software (Coombes, 1999) to spatially arrange the treatments so that they were not repeated within columns. In addition sowing rate for each seed fraction was adjusted, according to seed weight and germination percentage, to sow exactly the same number of germinable seeds per unit area (145 seeds/m^2). Table 4.1 shows the average seed weight, extrapolated from the weight of 1000 seeds, for each seed source x seed size combination.

Table 4.1: Average seed weight for each seed fraction from each site

Trial year	Source Site and year	Seed Size (mm)	Average Seed Weight (mg)*	Trial year	Source Site and year	Seed Size (mm)	Average Seed Weight (mg)
2000	Weetulta 1999	<2.2	-	2001	Weetulta 2000	<2.2	26.8
		2.2-2.5	35.0			2.2-2.5	39.2
		2.5-2.8	47.3			2.5-2.8	52.6
		>2.8	55.4			>2.8	59.8
	Geranium 1999	<2.2	27.0		Clinton 2000	<2.2	24.8
		2.2-2.5	36.6			2.2-2.5	37.2
		2.5-2.8	46.2			2.5-2.8	47.9
		>2.8	55.3			>2.8	56.3
	Callington 1999	<2.2	24.0		Callington 2000	<2.2	24.8
		2.2-2.5	37.2			2.2-2.5	36.3
		2.5-2.8	46.5			2.5-2.8	46.1
		>2.8	53.0			>2.8	54.6
	Charlick 1999	<2.2	22.9		Tuckey 2000	<2.2	26.2
		2.2-2.5	34.4			2.2-2.5	35.4
		2.5-2.8	48.8			2.5-2.8	43.6
		>2.8	55.4			>2.8	51.6
					Yeelanna 2000	<2.2	30.4
						2.2-2.5	38.3
						2.5-2.8	45.7
						>2.8	51.7

* No seed of Weetulta <2.2 was available for field experiments in 2000

4.2.3 Trial Sites

Experiments were located at Geranium (2000, 2001), Tuckey (2000), Darke Peake (2001) and Lowbank (2001) on sandy textured soils, and at Charlick (2001) and Brinkworth (2001) on heavier textured soils (Appendix 1). The conduct of trials on heavier soils allowed a

comparison to evaluate whether seed size was an important determinant of establishment, early growth and grain yield independent of soil type. Site details are provided in table 4.2. The trials at Lowbank and Darke Peak were sown into plots of area 10.5m^2. Plots were scaled back to 7.5m^2 for harvest. The remaining trials were sown into 4.1m^2 plots and harvested from 3.9m^2 plots.

Table 4.2: Site Details-Sowing date, fertilizer rates, harvest date, soil type and April-October rainfall

	2000		2001				
	Geranium	**Tuckey**	**Geranium**	**Lowbank**	**Darke Peake**	**Charlick**	**Brinkworth**
Sowing Date	14th June	25th May	15th June	18th June	7th June	27th June	29th June
Fertilizer Rate	190 kg/ha of 13:15	130 kg/ha of 13:15	100 kg/ha of 22:15	98 kg/ha of 17:19	100 kg/ha of 17:19	100 kg/ha of 22:15	100 kg/ha of 22:15
Harvest Date	14th December	17th December	21st December	8th January 2002	10th December	5th December	19th December
Soil Type	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sand (57cm) over calcareous clayey sand	Loam	Loam
April-October Rainfall (mm)	332	302	310	215	270	306	389

4.2.4 Measurements

4.2.4.1 Coleoptile length

Seed for each variety was pre-germinated (Petri dish with 2 moist Whatman No. 1 filter papers) in an incubator at 20°C for 5 days. Germinated seed was then laid out onto 32 x 46 cm (R6) filter paper, pre-soaked in R.O. water, so there were 25 seeds per sheet. Four sheets (replicates) of 25 seeds per sheet were set up for each variety. Each filter paper was rolled up

carefully, covered with aluminium foil and placed upright in a container. The samples were incubated at 20°C for 7 days. After incubation the length of the coleoptile was measured for each seedling with a ruler. The mean length for the 25 seeds per filter paper was then regarded as a replicate.

4.2.4.2 Soil samples

Six samples from the 0-10 cm soil fraction were collected to acquire a representative soil sample of each site in 2001. The replicate samples were pooled, air dried and analysed for texture, colour, Colwell extractable phosphorus and potassium (Colwell, 1963), nitrogen (nitrate and ammonium), sulphur, organic carbon, iron, electrical conductivity and pH. No soil samples were collected for analysis in 2000.

4.2.4.3 Seed nutrient status

Seed from each seed fraction was analysed using Inductively Coupled Plasma Atomic Emission (ICPAES) analysis (Zarcinas *et al.*, 1987) for a range of macro- and micro-nutrients.

4.2.4.4 Early Vigour Measurements

Approximately 8 weeks post-sowing and at a decimal growth stage of Z14/Z23 (early tillering) (Zadoks *et al.*, 1974) establishment was scored by counting the number of plants in a 0.25 m^2 quadrat in each plot. The plants in these quadrats were harvested, dried at 80°C for 48 hours and then weighed. Dry matter production (g m^{-2}) was used as a measure of early vigour (E_DMP). Visual early vigour scores (1-poor to 9-excellent) were also recorded at each trial. Dry matter cuts were not collected at Tuckey. Dry matter cuts and establishment counts were collected at Darke Peak at a relative growth stage of Z12 (two leaf stage).

4.2.4.5 Agronomic Measurements

Grain yield (t ha^{-1}), screenings (proportion of grain below 2.5mm, %) and 1000 grain weight (grams, g) were measured.

4.2.5 Statistical Analysis

All field trial data was analysed by a linear mixed model analysis using residual maximum likelihood (REML) (Patterson and Thompson, 1971). Analysis was performed using Genstat statistical software (Genstat® for Windows™, 5th edition, Lawes Agricultural Trust). REML produces Wald statistics to test the significance of fixed (treatment) effects. Early dry matter production (early vigour), 1000 grain weight, screenings and grain yield were also analysed using a MET (multi-environment trial) statistical analysis (Cullis *et al.*, 1998, Smith *et al.*, 2001). The analyses employ spatial techniques to adjust the means of data to accommodate variability across a field trial site (soil depth, fertility) and also variability due to cultural practices (*e.g.* harvesting in two directions). In addition, the MET analysis adjusts across site means to accommodate differences in genetic and environmental variance between sites, and provide information on the environmental stability of varieties and the heritability (h^2) of the traits analysed. The MET analysis was conducted using ASREML (Gilmour et al., 1999). Coleoptile length was analysed using the ANOVA directive in Genstat (Genstat® for Windows, 5th edition, Lawes Agricultural Trust).

4.3 Results

4.3.1 Soil analysis

Soil analyses of the 2001 sites are presented in Table 4.3. The sandy soil sites (Darke Peak, Geranium and Lowbank) were lower in nitrogen content than the two heavier soil types at Charlick and Brinkworth. Differences in nitrate concentration particularly distinguished soil types. The analyses only provided data on the available forms of nitrogen, although it is generally well recognized that soil containing total nitrogen levels less than 0.08-0.10% require fertilizer application for adequate cereal cropping (Strong and Mason, 1999). All sites were characterised by sufficient levels of Colwell phosphorus and potassium. The organic content (as carbon) of the soils at Charlick and Brinkworth were regarded as within normal range for a loam soil type (Hughes *et al.*, 1996). For the sandy soils, organic content was very high at Darke Peak, and moderate and low at Geranium and Lowbank respectively. The pH at Darke Peak was essentially neutral and slightly acidic at Geranium. The other sites were alkaline in nature.

Table 4.3: Soil analysis at sites where the Mundah seed size trials were conducted in 2001.

Site	NO_3-N (mg/kg)	NH_4-N (mg/kg)	Colwell Phosphorus (mg/kg)	Colwell Potassium (mg/kg)	Organic carbon (%)	pH (H_2O)
Darke Peak	9	5	28	123	1.62	6.8
Geranium	23	5	37	250	0.74	6.2
Lowbank	11	7	23	250	0.35	8.6
Charlick	33	10	83	484	1.46	8.1
Brinkworth	28	10	34	702	1.51	8.2

4.3.2 Seed nutrient content

Tables 4.4 and 4.5 illustrate the average seed weight and analytical data for each seed size interval. While seed weight increased with seed size, mean seed weight was essentially equivalent between sources of seed, although the Tuckey site produced marginally smaller average seed weight, and seed from Weetulta was generally larger. With a couple of exceptions, there was a general trend towards higher seed content (concentration multiplied by seed weight) of phosphorus, zinc and manganese with greater seed size. Despite this, for the majority of sites, nutrient contents for each seed size category were not considered to be within deficiency ranges according to Reuter *et al.* (1997). However, seed phosphorus contents at Weetulta (both years), and seed manganese at Yeelanna were in the deficient range for each seed size fraction (Reuter *et al.*, 1997).

Because of the overall adequacy of nutrients for seed in each seed size category, improvements in coleoptile length, establishment and, to an extent, early vigour with seed size are not likely to reflect seed nutrient status, but rather the variation in size of the embryo and the reserves of starch in the endosperm.

Table 4.4: Analytical data for the Mundah seed size trials in 2000.

Source of seed	Seed Size (mm)	Average Seed Weight (mg)	Seed Phosphorus content (µg/grain)	Seed Zinc content (µg/grain)	Seed Manganese content (µg/grain)
Callington	<2.2	24.0	88.80	0.74	0.75
	2.2-2.5	37.2	133.92	1.06	1.05
	2.5-2.8	46.5	148.80	1.23	1.22
	>2.8	53.0	159.00	1.33	1.31
	Mean	**40.2**	**132.63**	**1.09**	**1.08**
Charlick	<2.2	22.9	77.86	0.38	0.28
	2.2-2.5	34.4	110.08	0.45	0.40
	2.5-2.8	48.8	141.52	0.72	0.50
	>2.8	55.4	138.50	0.65	0.58
	Mean	**40.4**	**116.99**	**0.55**	**0.44**
Geranium	<2.2	27.0	113.40	0.79	0.43
	2.2-2.5	36.6	139.08	0.91	0.51
	2.5-2.8	46.2	152.46	0.97	0.54
	>2.8	55.3	176.96	1.15	0.64
	Mean	**41.3**	**145.48**	**0.95**	**0.53**
Weetulta	<2.2*	-	-	-	-
	2.2-2.5	35.0	61.25	0.63	0.55
	2.5-2.8	47.3	87.03	0.87	0.69
	>2.8	55.4	110.80	1.13	0.84
	Mean	**45.9**	**86.36**	**0.87**	**0.69**

* Not enough seed available for ICPAES analysis

Table 4.5: Analytical data for the Mundah seed size trials in 2001.

Source of seed	Seed Size (mm)	Average Seed Weight (mg)	Seed Phosphorus content (µg/grain)	Seed Zinc content (µg/grain)	Seed Manganese content (µg/grain)
Callington	<2.2	24.79	91.72	0.43	0.71
	2.2-2.5	36.28	119.72	0.57	0.90
	2.5-2.8	46.13	133.78	0.68	1.08
	>2.8	54.61	147.45	0.78	1.27
	Mean	**40.45**	**123.17**	**0.61**	**0.99**
Clinton	<2.2	24.83	89.39	0.39	0.46
	2.2-2.5	37.20	126.48	0.62	0.67
	2.5-2.8	47.93	172.55	0.83	0.84
	>2.8	56.27	174.44	0.78	0.94
	Mean	**41.56**	**140.71**	**0.65**	**0.73**
Tuckey	<2.2	26.17	123.00	0.55	0.39
	2.2-2.5	35.38	159.21	0.73	0.43
	2.5-2.8	43.56	100.19	0.98	0.78
	>2.8	51.58	196.00	0.78	0.62
	Mean	**39.17**	**144.60**	**0.76**	**0.55**
Weetulta	<2.2	26.85	69.81	0.69	0.57
	2.2-2.5	39.19	86.22	0.84	0.69
	2.5-2.8	52.58	210.32	0.80	0.50
	>2.8	59.78	119.56	1.15	1.00
	Mean	**44.60**	**121.48**	**0.87**	**0.69**
Yeelanna	<2.2	30.40	124.64	0.60	0.17
	2.2-2.5	38.27	137.77	0.66	0.20
	2.5-2.8	45.67	150.71	0.74	0.28
	>2.8	51.73	170.71	0.86	0.31
	Mean	**41.52**	**145.96**	**0.71**	**0.24**

4.3.3 Coleoptile length

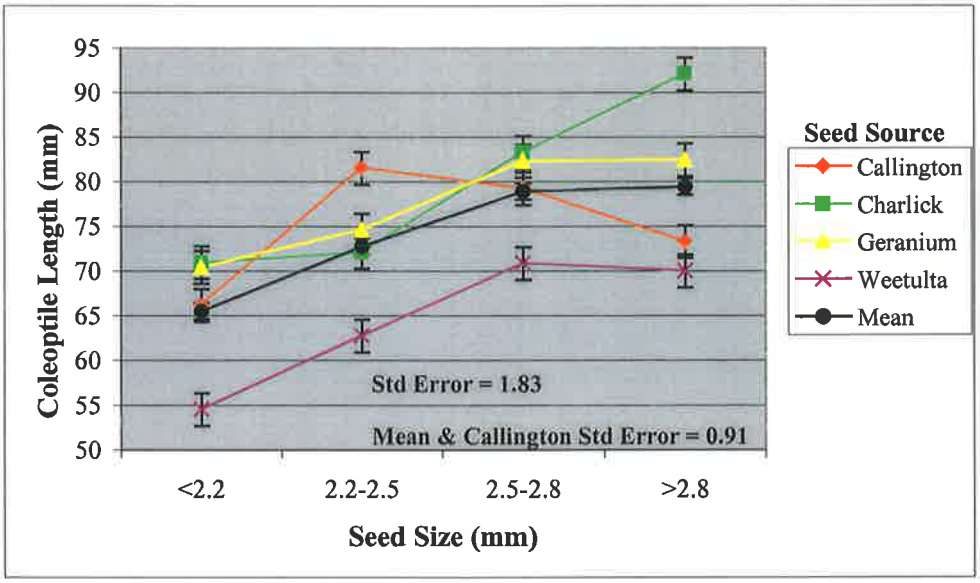
The effect of the interaction between seed source and seed size on coleoptile length was significant (P<0.001, Table 4.6). The coleoptile length of seed from many sources displayed a similar pattern to the mean (Geranium and Weetulta, Figure 4.1a; Callington, Clinton, Tuckey, Figure 4.1b). Coleoptile length increased with greater seed size up to 2.5-2.8mm (Figures 4.1a & b). Following this, coleoptile length remained essentially unaffected by seed size. The change in coleoptile length with seed size was particularly substantial at Callington and Tuckey in 2001. This finding is in general agreement with Cornish and Hindmarsh (1988) and Botwright *et al.* (2001), although they observed a linear relationship with seed weight and did not identify seed source as a significant contributor to coleoptile length (Botwright *et al.*, 2001). Two sites did display a tendency towards a linear increase in coleoptile length with greater seed size (Weetulta and Yeelanna in 2001, Figure 4.1b). The relationship at Charlick (Figure 4.1a) was linear from 2.2-2.5mm upwards. Coleoptile length peaked at 2.2-2.5mm for seed sourced from Callington (Figure 4.1a). The inferior length of coleoptile for the seed from Weetulta (1999) in the 2000 trials (Figure 4.1a) may reflect the fact that seed from this source was deficient in phosphorus.

Table 4.6: Mean squares from ANOVA of coleoptile length of seed from the 1999 (2000 Mundah seed size trials) and 2000 (2001 Mundah seed size trials) S2 breeders trials.

Source of Variation	d.f.	1999 (2000 trials)	d.f.	2000 (2001 trials)
Seed source	3	843.46***	4	166.54***
Seed size	3	537.75***	3	774.45***
Seed source x Seed size	8	104.65***	12	61.15***
Residual	41	12.69	56	7.28

***P<0.001

a)



b)

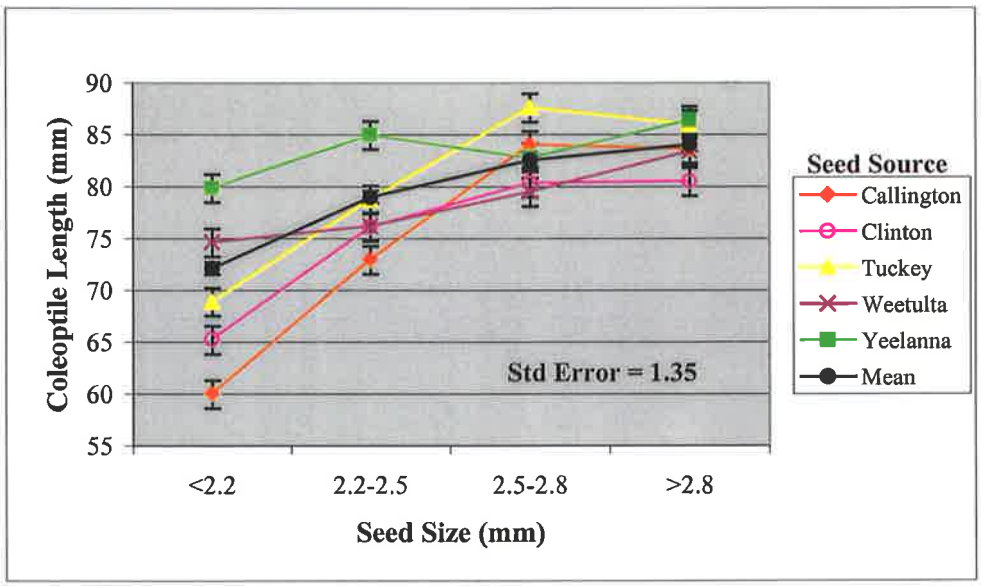


Figure 4.1: Influence of seed source and seed size on coleoptile length.

a) **2000.** Regression coefficient (R^2) for the mean effect was 0.90.

b) **2001.** Regression coefficient (R^2) for the mean effect was 0.91.

Coleoptile length for seed from Weetulta (2000) in the 2001 trials was superior to the equivalent seed in the 2000 trials (Figures 4.1a & b), presumably because of a higher content of phosphorus in the seed. However, the coleoptile length of Weetulta (2000) seed at each seed size fraction above 2.2-2.5 mm was similar to Clinton (2000) seed (Figure 4.1b), even

though the phosphorus content of Clinton seed was generally higher (Table 4.5). The weight of seed from Weetulta was greater than the seed from Clinton within each size fraction (Table 4.5). It may be that seed size, which is closely associated with embryo size (Lopez-Castaneda *et al.*, 1996), and the reserves of starch in the endosperm, is also an important contributor to improved coleoptile length.

4.3.4 Establishment

Establishment increased with seed size (Tables 4.7 and 4.8), and with the exception of Darke Peak and Charlick, the effect on plant establishment was significant ($\underline{P}<0.01$, $\underline{P}<0.001$, Table 4.9). The substantial improvement in establishment with larger seed is clearly visible from the photograph of plots at Geranium in 2001 (Plate 4.1). The significant effect of seed source ($\underline{P}<0.05$), and the interaction between seed source and seed size ($\underline{P}<0.01$), on establishment at Geranium and Tuckey, in 2000, respectively, may be related to the poor quality of seed sourced from the S2 trial at Weetulta in 1999 (Table 4.10). Seed from this site was characterized by low phosphorus content, and may explain the inferior establishment counts achieved using this seed. The shorter coleoptile of seed from Weetulta (1999) may also contribute to the poor establishment. A number of authors have produced evidence to show the relationship between establishment and coleoptile length (Whan, 1976; Bacaltchuk and Ulrich, 1990; Hughes and Mitchel, 1987; Radford, 1987; Radford and Wildermuth, 1987; Sharma, 1990).

Seed from the 2000 S2 trial at Weetulta may also account for the significant ($\underline{P}<0.001$) seed source effect at Brinkworth. Despite a low phosphorus content, this source of seed produced the highest establishment counts at Brinkworth. The superior fertility of the soil at Brinkworth may have overridden any effect that the poor nutrient status of the seed had on establishment (Table 4.3). In contrast the poor fertility of the sandy soils may have exacerbated the effect of nutrient deficient seed.

Table 4.7: Agronomic data for the Mundah seed size trials in 2000. Coleoptile length was analysed by ANOVA. Establishment means were determined from REML analysis. Early vigour, grain yield, 1000 grain weight and screenings data were analysed by MET analysis. LSDs were determined from ANOVA.

a) Geranium

Seed Size (mm)	Average Seed Weight (mg)	Coleoptile length (cm)	Establishment (plants m^{-2})	Early Vigour (g m^{-2})	Grain Yield (t ha^{-1})	1000 grain weight (g)	Screenings (%)
<2.2	24.63	65.36	103.2	43.8	2.76	49.15	19.62
2.2-2.5	35.80	72.75	135.7	52.8	3.15	49.44	19.01
2.5-2.8	47.20	78.92	153.0	68.4	3.33	49.66	15.85
>2.8	54.78	79.45	172.4	77.0	3.36	49.75	15.48
*LSD (P<0.05)	N/A	*1.43*	*19.11*	*15.35*	*0.85*	*1.54*	*4.29*

N/A=not applicable

b) Tuckey

Seed Size (mm)	Average Seed Weight (mg)	Coleoptile length (cm)	Establishment (plants m^{-2})	Grain Yield (t ha^{-1})
<2.2	24.63	65.36	79.0	1.64
2.2-2.5	35.80	72.75	78.5	1.32
2.5-2.8	47.20	78.92	87.4	1.56
>2.8	54.78	79.45	103.8	1.58
*LSD (P<0.05	N/A	*1.43*	*15.97*	*0.22*

N/A=not applicable

Table 4.8: Agronomic data for the Mundah seed size trials in 2001. Coleoptile length was analysed by ANOVA. Establishment means were determined from REML analysis. Early vigour, grain yield, 1000 grain weight and screenings data were analysed by MET analysis. LSDs were determined from ANOVA.

a) Geranium

Seed Size (mm)	Average Seed Weight (mg)	Coleoptile length (cm)	Establishment (plants m^{-2})	Early Vigour (g m^{-2})	Grain Yield (t ha^{-1})	1000 grain weight (g)	Screenings (%)
<2.2	26.61	69.84	111.1	15.55	2.29	49.12	14.43
2.2-2.5	37.26	77.85	153.3	22.76	2.51	48.72	14.38
2.5-2.8	47.17	82.84	149.5	27.00	2.56	48.58	14.35
>2.8	54.79	83.98	139.4	29.12	2.62	48.56	14.32
**LSD (P<0.05*	*N/A*	*0.86*	*15.25*	*3.28*	*0.28*	*0.75*	*1.34*

N/A=not applicable

b) Lowbank

Seed Size (mm)	Average Seed Weight (mg)	Coleoptile length (cm)	Establishment (plants m^{-2})	Early Vigour (g m^{-2})	Grain Yield (t ha^{-1})	1000 grain weight (g)	Screenings (%)
<2.2	26.61	69.84	104.4	17.44	1.08	53.00	4.53
2.2-2.5	37.26	77.85	125.9	24.52	1.14	52.06	4.34
2.5-2.8	47.17	82.84	138.5	27.57	1.17	51.89	4.51
>2.8	54.79	83.98	144.6	30.14	1.18	51.73	4.62
**LSD (P<0.05*	*N/A*	*0.86*	*17.55*	*5.86*	*0.14*	*1.01*	*0.87*

N/A=not applicable

Table 4.8: Agronomic data for the Mundah seed size trials in 2001. Coleoptile length was analysed by ANOVA. Establishment means were determined from REML analysis. Early vigour, grain yield, 1000 grain weight and screenings data were analysed by MET analysis. LSDs were determined from ANOVA.

c) Darke Peak

Seed Size (mm)	Average Seed Weight (mg)	Coleoptile length (cm)	Establishment (plants m^{-2})	Early Vigour (g m^{-2})	Grain Yield (t ha^{-1})	1000 grain weight (g)	Screenings (%)
<2.2	26.61	69.84	98.9	4.03	1.93	52.83	6.65
2.2-2.5	37.26	77.85	120.4	5.54	2.05	52.09	6.64
2.5-2.8	47.17	82.84	124.9	6.49	2.09	51.93	6.79
>2.8	54.79	83.98	125.5	7.01	2.12	51.68	6.75
**LSD (P<0.05)*	*N/A*	*0.86*	*18.63*	*1.48*	*0.20*	*0.56*	*0.44*

N/A=not applicable

d) Brinkworth

Seed Size (mm)	Average Seed Weight (mg)	Coleoptile length (cm)	Establishment (plants m^{-2})	Early Vigour (g m^{-2})	Grain Yield (t ha^{-1})	1000 grain weight (g)	Screenings (%)
<2.2	26.61	69.84	92.3	17.70	2.90	52.91	5.38
2.2-2.5	37.26	77.85	113.9	26.25	2.94	52.78	5.30
2.5-2.8	47.17	82.84	132.4	31.57	2.93	52.78	5.23
>2.8	54.79	83.98	131.9	34.51	2.93	52.82	5.19
**LSD (P<0.05)*	*N/A*	*0.86*	*15.36*	*4.42*	*0.24*	*0.55*	*0.94*

N/A=not applicable

e) Charlick

Seed Size (mm)	Average Seed Weight (mg)	Coleoptile length (cm)	Establishment (plants m^{-2})	Early Vigour (g m^{-2})	Grain Yield (t ha^{-1})	1000 grain weight (g)	Screenings (%)
<2.2	26.61	69.84	107.4	18.09	3.92	58.96	2.78
2.2-2.5	37.26	77.85	114.4	25.41	3.99	58.39	2.79
2.5-2.8	47.17	82.84	122.4	29.97	4.00	58.29	2.77
>2.8	54.79	83.98	121.3	32.48	4.03	58.23	2.77
**LSD (P<0.05)*	*N/A*	*0.86*	*12.83*	*3.92*	*0.25*	*2.66*	*0.55*

N/A=not applicable

Table 4.9: Wald statistics from the REML analysis of various traits for the Mundah seed size trials, 2000-2001.

Site	Source of Variation	d.f.	Establishment	Early Vigour ($g\ m^{-2}$)	Grain yield	1000 grain weight	Screenings
Geranium 2000	Seed source	3	9.57*	4.62	1.94	1.38	6.33
	Seed size	3	30.73***	19.97***	17.05***	12.25**	32.50***
	Seed source x Seed size	6	4.50	13.86	21.40**	6.26	11.52
Tuckey 2000	Seed source	3	5.69	No	4.76	No	No
	Seed size	3	31.70***	Data	14.57**	Data	Data
	Seed source x Seed size	8	21.29**		23.81**		
Geranium 2001	Seed source	8	11.94	31.88***	15.49*	191.13***	18.04*
	Seed size	3	25.18***	114.05***	17.22***	0.49	0.25
	Seed source x Seed size	12	16.84	14.09	8.40	29.77**	38.23***
Darke Peak 2001	Seed source	4	2.13	7.53	12.20*	9.97*	7.66
	Seed size	3	4.58	13.25**	10.77*	24.19***	2.61
	Seed source x Seed size	11	7.75	7.09	14.15	31.66***	13.13
Lowbank 2001	Seed source	4	6.22	5.72	34.55***	7.22	57.49***
	Seed size	3	25.63***	50.28***	4.75	14.48	1.99
	Seed source x Seed size	12	18.05	12.39	16.25	14.11	8.67
Charlick 2001	Seed source	8	0.54	16.98*	12.42	3.16	3.76
	Seed size	3	5.09	49.57***	23.95***	2.37	1.18
	Seed source x Seed size	12	14.00	23.78*	36.68***	4.99	5.29
Brinkworth 2001	Seed source	8	29.88***	58.79***	14.99	103.61***	41.06***
	Seed size	3	28.45***	140.98***	3.48	1.02	2.56
	Seed source x Seed size	12	16.48	29.79***	22.18*	49.24***	22.96*

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

Plate 4.1: Field plots at Geranium in 2001 planted with Mundah seed from the 2000 Weetulta S2 trial. Left: planted with seed less than 2.2mm. Right: planted with seed greater than 2.8mm.

Table 4.10: Average plant establishment for seed sourced from four sites in 1999 when grown at Geranium, 2000. Data was analysed by REML analysis. LSDs were determined from ANOVA.

Seed source (1999)	Average establishment (plants m^{-2})
Charlick	153.8
Geranium	140.6
Callington	130.7
Weetulta	114.5
LSD (P<0.05)	*19.11*

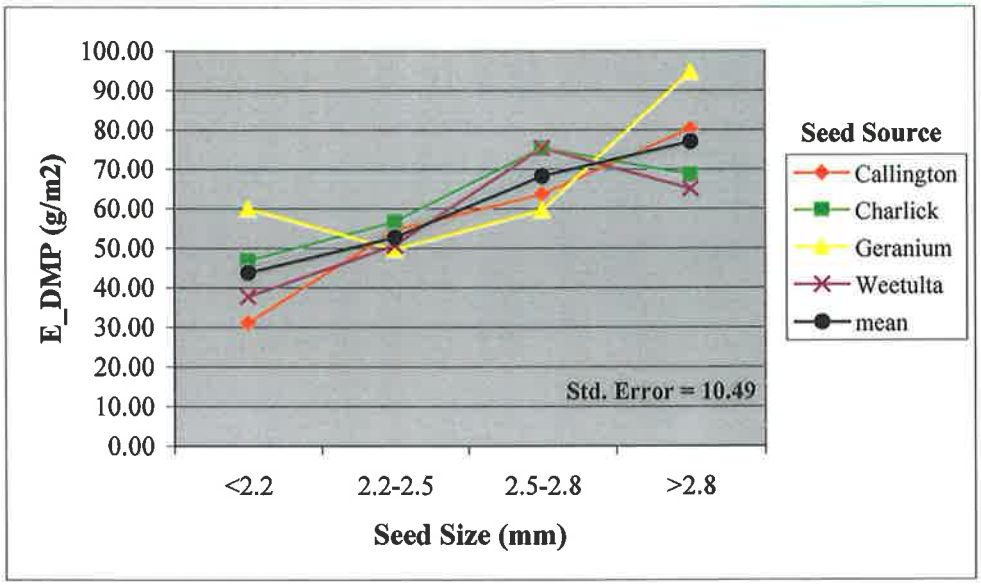
4.3.5 Early vigour

Examination of early vigour (as dry matter production at early tillering) clearly illustrated that larger seed size substantially improved biomass production (Tables 4.7-4.8; Figure 4.2) and accounted for between 99-100% of the variation in early vigour when averaged over seed source (Figure 4.2a-f). Seed size was shown to be important for superior early growth on sand, but it was also evident that seed size impacted on growth on loamier soil types, of higher inherent fertility.

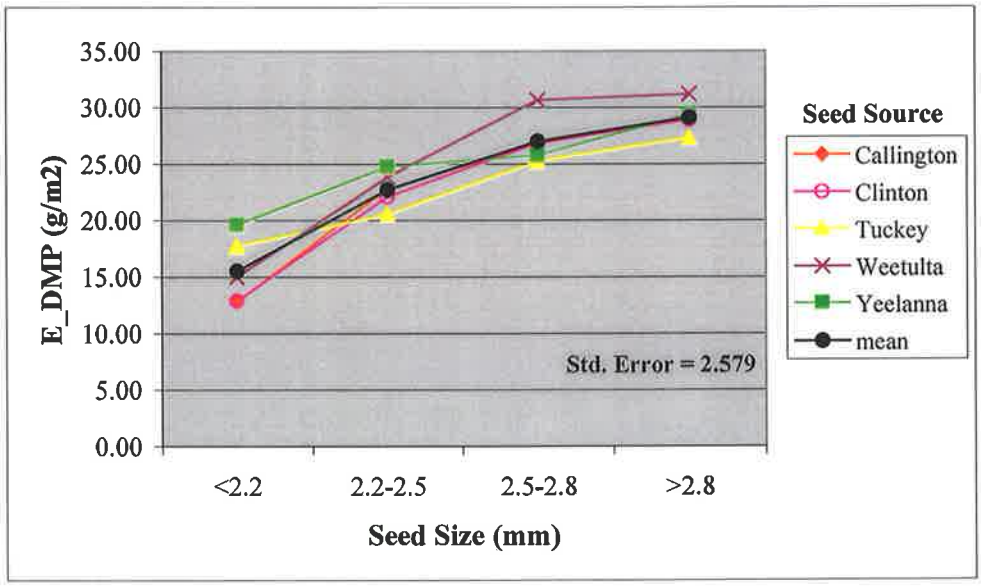
At all sites, seed size was a statistically significant determinant of early vigour ($P<0.01$, $P<0.001$, Table 4.9). Sowing seed greater than 2.8mm increased early vigour by 73% at Lowbank, 74% at Darke Peak, 76% at Geranium (2000), and 87% at Geranium (2001) relative to the biomass production from seed screened to below 2.2mm. This difference in early vigour is clearly illustrated in the photograph of two plots at Geranium (2001) in Plate 4.1.

At Charlick ($P<0.05$) and Brinkworth ($P<0.001$), early vigour was determined by a significant interaction between seed source and seed size (Figures 4.2e & f). On these heavier soils the relatrionship between early vigour and seed size was at least as strong as that observed on the sandy soils (*e.g.* 80% at Charlick and 95% at Brinkworth).

In addition to seed size, seed source was a significant ($P<0.001$) source of variation for early vigour at Geranium (2001). Weetulta and Yeelanna seed produced the greatest average biomass (25.14g and 24.81g respectively) at early tillering, while the biomass produced from seed from Callington, Tuckey and Clinton was 8-10% lower relative to the former two sites.



a)



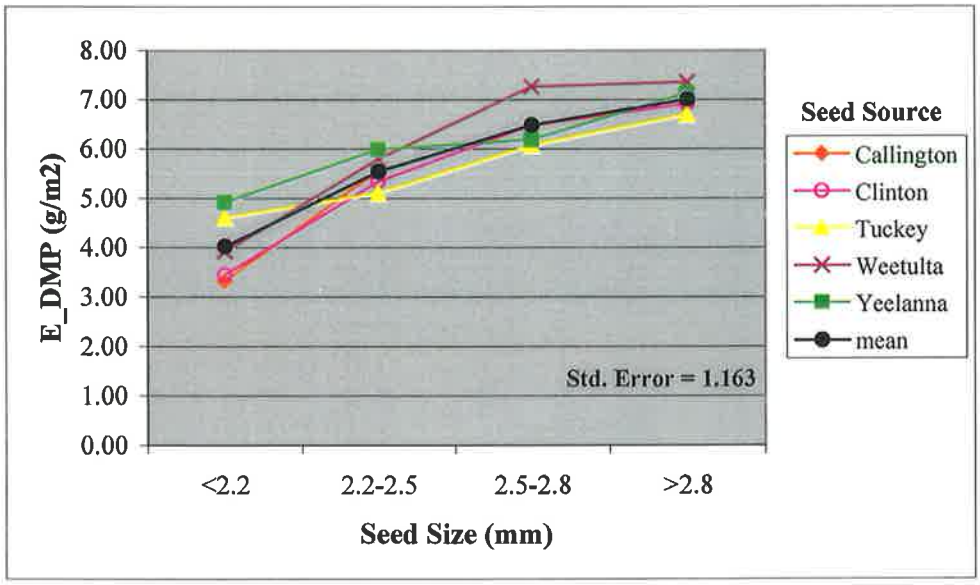
b)

Figure 4.2: The influence of seed source and seed size on E_DMP. Mundah seed size experiments 2000-2001. Data was analysed by MET analysis.

a) **Geranium 2000.** Regression coefficient (R^2) for the mean effect was 0.98.

b) **Geranium 2001.** Regression coefficient (R^2) for the mean effect was 0.997.

c)



d)

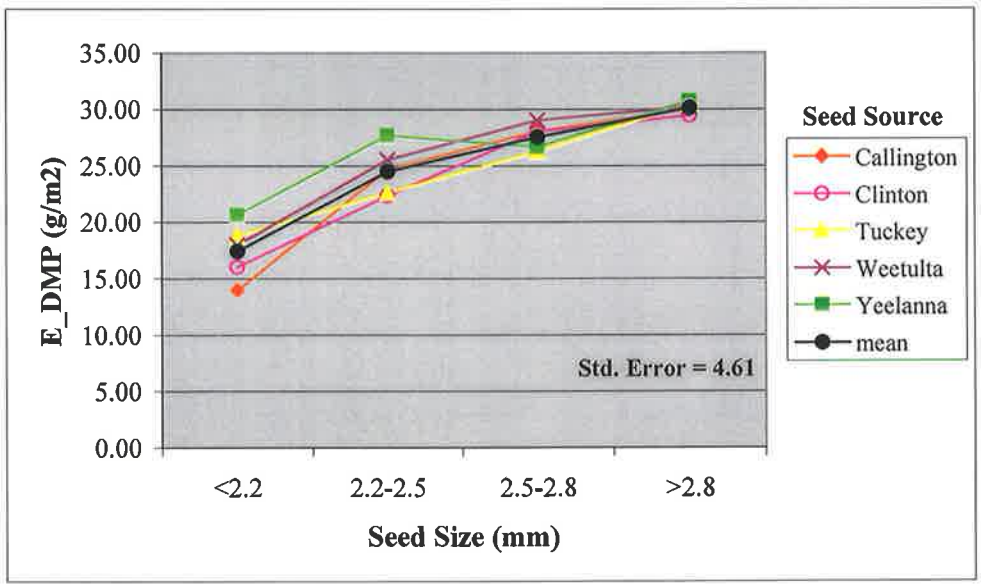
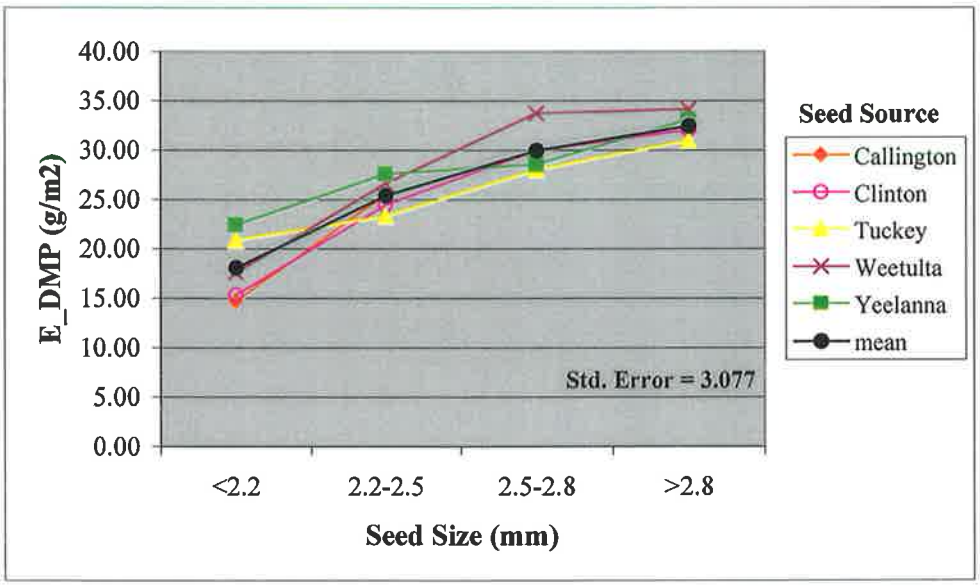


Figure 4.2: The influence of seed source and seed size on E_DMP. Mundah seed size experiments 2000-2001. Data was analysed by MET analysis.

c) **Darke Peak.** Regression coefficient (R^2) for the mean effect was 0.95.

d) **Lowbank.** Regression coefficient (R^2) for the mean effect was 0.996.

e)



f)

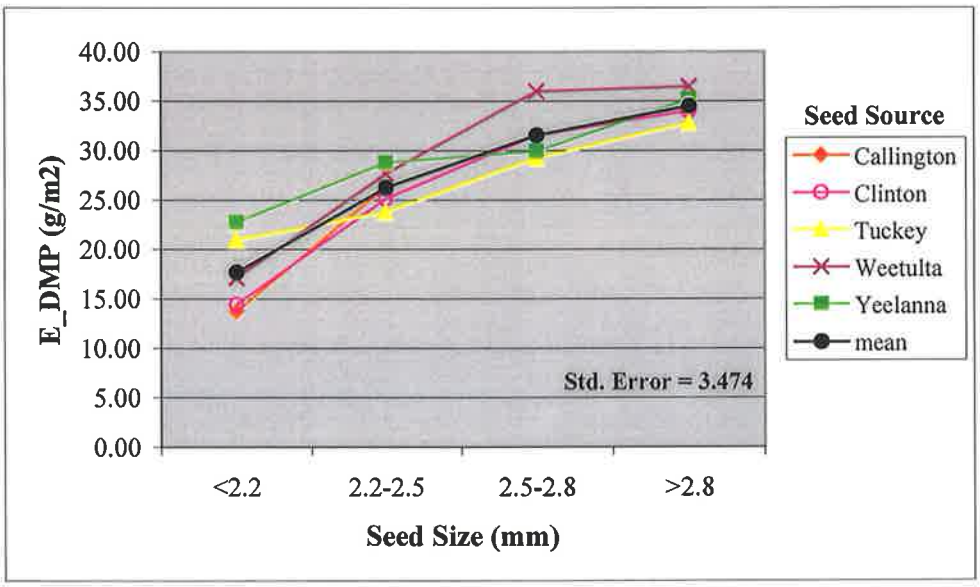


Figure 4.2: The influence of seed source and seed size on E_DMP. Mundah seed size experiments 2000-2001. Data was analysed by MET analysis.

e) **Charlick.** Regression coefficient (R^2) for the mean effect was 0.95.

f) **Brinkworth.** Regression coefficient (R^2) for the mean effect was 0.95.

4.3.6 Grain yield

The low observed site means for grain yield highlighted the low yield potential of sandy soils compared with heavier soil types in higher yielding environments (P<0.001, Table 4.11). The exception was Geranium (2000) with a mean grain yield between Brinkworth and Charlick. However, the accompanying coefficient of variation (CV) illustrates the substantial variation in grain yield across treatments; a typical response on sandy sites.

Table 4.11: Site mean for grain yield, soil type, and variation in grain yield for the Mundah seed size trials, 2000-2001. LSD calculated from ANOVA.

Trial site	Site Mean (t ha^{-1})*	Soil type	Variance for grain yield (within site)	CV (%)
Geranium (2000)	3.34	Sandy loam	0.297	17.832
Geranium (2001)	2.55	Sandy loam	0.023	5.988
Tuckey	1.49	Sandy loam	0.151	24.595
Lowbank	1.17	Sandy loam	0.005	5.940
Darke Peak	2.07	Sand	0.008	4.227
Brinkworth	2.93	Loam	0.000	0.721
Charlick	4.00	Loam	0.003	1.274
LSD (P<0.05)	*0.13*			

* Site means determined from MET analysis

Statistical analysis of grain yield showed a significantly higher grain yield with each incremental rise in seed size at Geranium 2000 (P<0.001), Tuckey (P<0.01), Geranium 2001 (P<0.001), Darke Peak (P<0.05) and Charlick (P<0.001) (Tables 4.7-4.8; Figure 4.3a-f; Table 4.9). The increase in grain yield at Charlick (Figure 4.3f), although significant (P<0.001), was small. The effect was not so clear however at Tuckey, where there was effectively no improvement in grain yield with larger seed size (Figure 4.3b). No significant differences for grain yield between seed size intervals was determined for Lowbank, although grain yield

tended to improve with each seed size increment (Figure 4.3e). There was no significant effect of seed size on grain yield at Brinkworth (Figure 4.3g). At Tuckey, the significant interaction ($\underline{P}<0.01$) was erratic, and was likely to be caused by experimental error (CV=24.6%, Table4.11).

The underlying feature at most sites was that grain yield was greater with each seed size increment, with seed size acounting for 2 (Tuckey)-97% (Geranium 2000) of the variation in grain yield averaged over seed source (Figure 4.3).

a)

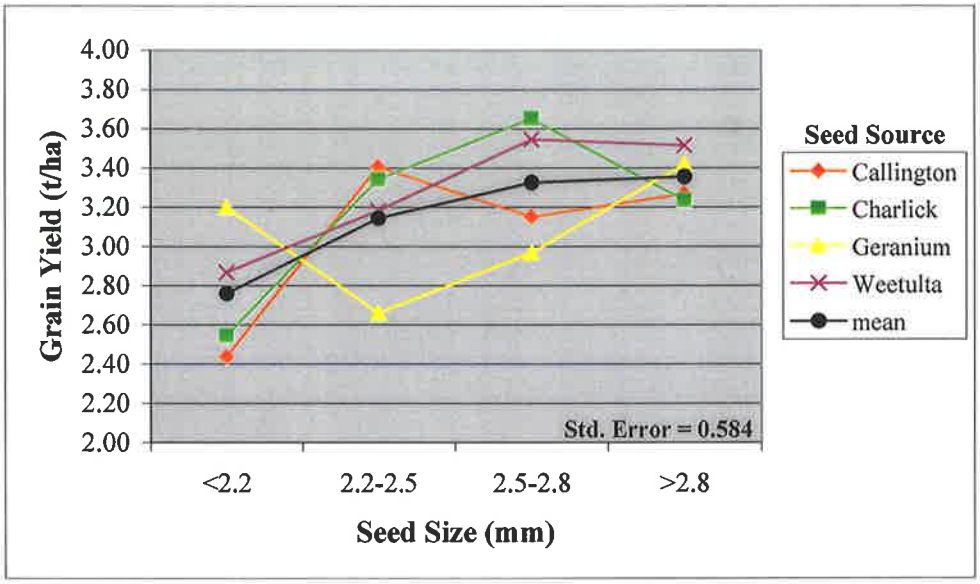
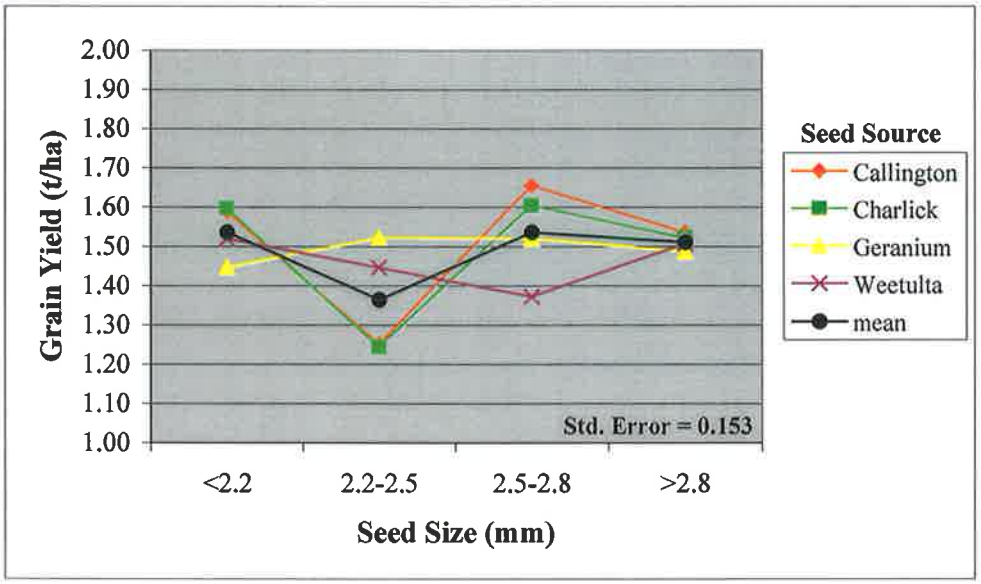


Figure 4.3: The influence of seed source and seed size on grain yield. Mundah seed size experiments 2000-2001. Data was analysed by MET analysis.

a) **Geranium 2000.** Regression coefficient (R^2) for the mean effect was 0.85.

b)



c)

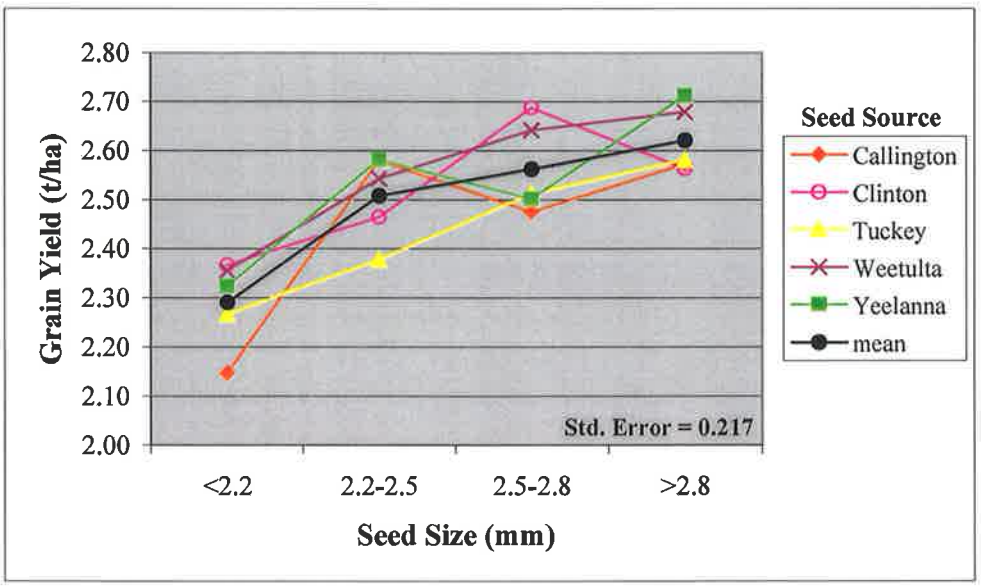
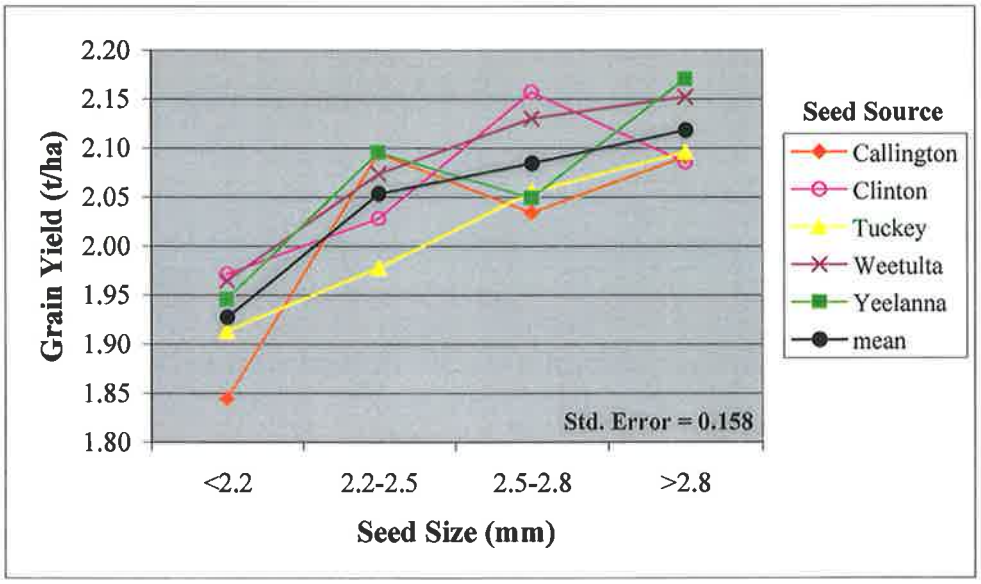


Figure 4.3: The influence of seed source and seed size on grain yield. Mundah seed size experiments 2000-2001. Data was analysed by MET analysis.

b) **Tuckey.** Regression coefficient (R^2) for the mean effect was 0.02.

c) **Geranium 2001.** Regression coefficient (R^2) for the mean effect was 0.97.

d)



e)

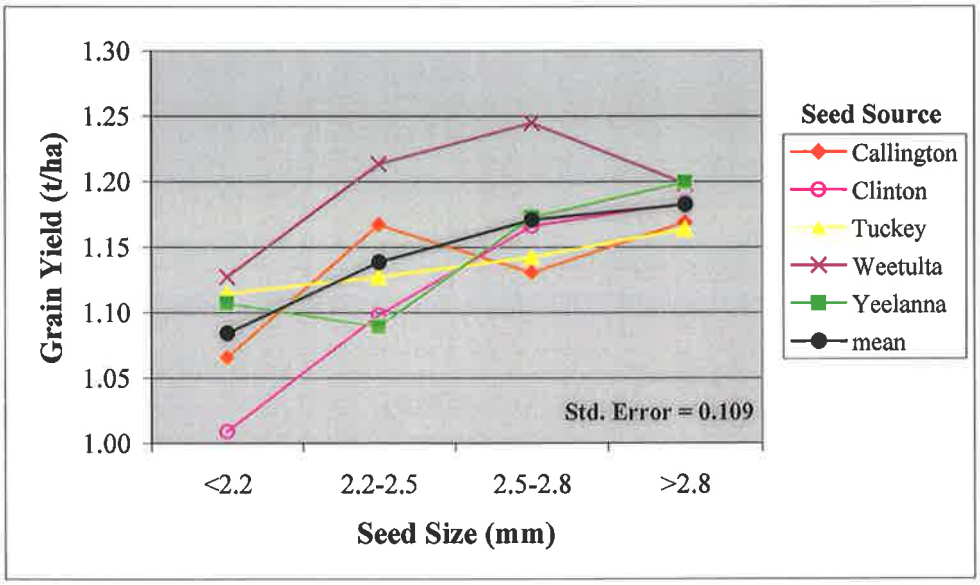
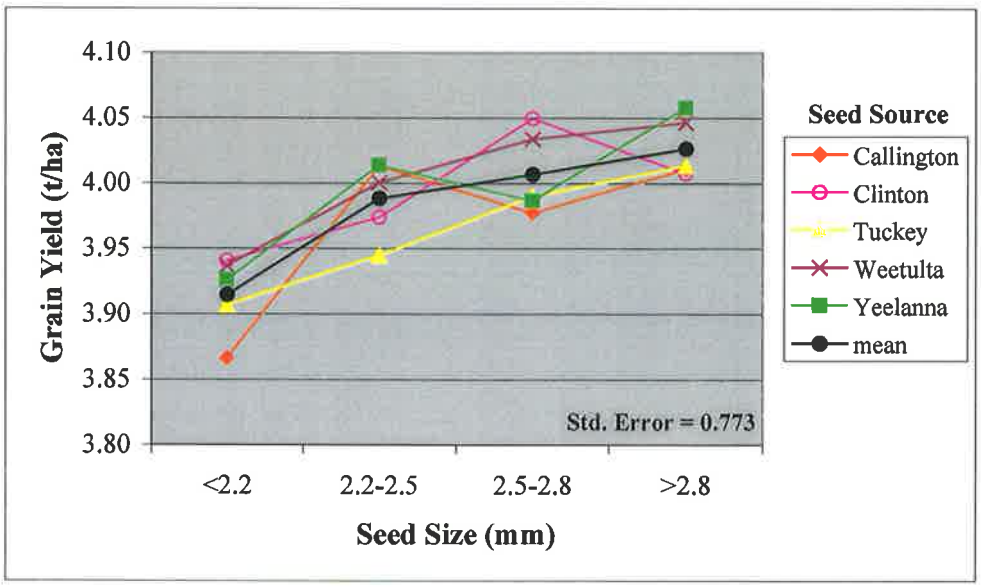


Figure 4.3: The influence of seed source and seed size on grain yield. Mundah seed size experiments 2000-2001. Data was analysed by MET analysis.

d) **Darke Peak.** Regression coefficient (R^2) for the mean effect was 0.88.

e) **Lowbank.** Regression coefficient (R^2) for the mean effect was 0.92.

f)



g)

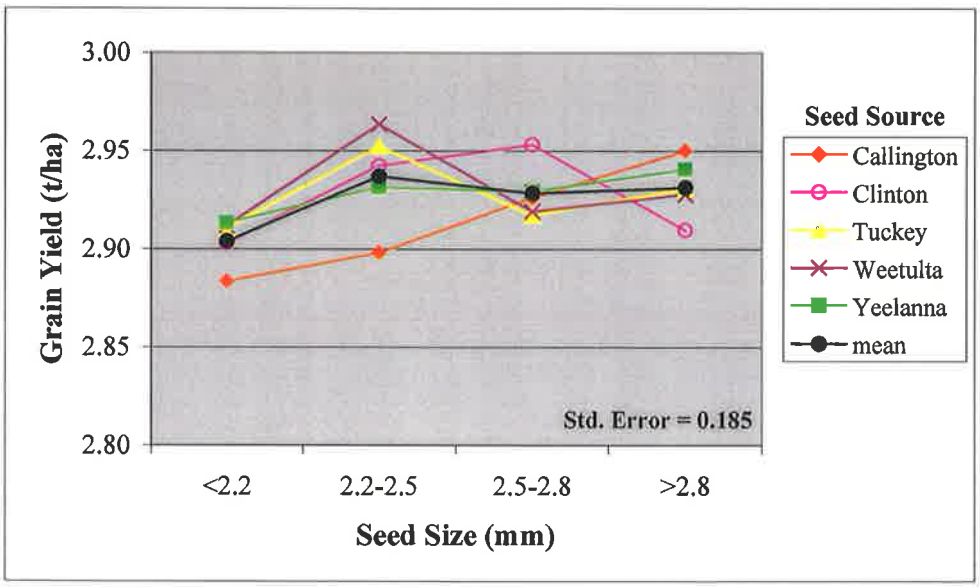


Figure 4.3: The influence of seed source and seed size on grain yield. Mundah seed size experiments 2000-2001. Data was analysed by MET analysis.

f) **Charlick.** Regression coefficient (R^2) for the mean effect was 0.88.

g) **Brinkworth.** Regression coefficient (R^2) for the mean effect was 0.42.

4.3.7 1000 grain weight and screenings percentage

Grain weight and screenings percentage were determined by a significant effect of seed size at Geranium 2000 ($P<0.01$), a significant interaction between seed source and size at Geranium 2001 ($P<0.01$) and Brinkworth ($P<0.001$) (Table 4.9). The interaction between seed source and size was significant for grain weight at Darke Peak ($P<0.001$).

Analysis of grain weight and screenings at Lowbank and Charlick did not indicate significant differences due either to seed size or the interaction between source and size. Despite this, there was a trend for declining grain weight and increasing screenings at Lowbank. Similarly, grain weight declined at Charlick, although screenings percentage was unaffected. Seed source had a significant ($P<0.001$) effect on screenings at Lowbank, with higher values for seed from Tuckey and Callington. This may reflect the generally smaller seed from these sites (Table 4.5), even though there was no significant effect of seed size on screenings percentage at Lowbank.

The inferior grain weight evident at Geranium (2000-49.50g; 2001-48.75g) is almost certainly related to the higher site mean for screenings (2000-17.49%; 2001-14.37%), and contrasts with the values of these yield parameters at the other sites. Charlick had the highest grain weight and lowest screenings for all sites used in these experiments.

While there was a variable response to seed size and/or seed source, and the significance of the responses, at each site, essentially the effects on grain weight and screenings percentage were small (Table 4.7-4.8).

4.4 Discussion

4.4.1 Effect of seed size on coleoptile length

The coleoptile length study as part of the field evaluation of barley and other cereals on sand, described in chapter 2, indicated that while seed size may have contributed to the differences in coleoptile development between cereals, the overriding source of variation in coleoptile length between barley varieties was genotype. The findings in this component of the study, however, lend weight to those of Cornish and Hindmarsh (1988). Seed of differing size categories, not confounded by genotype, does impact on coleoptile length. The mean coleoptile length improved appreciably up to the 2.5-2.8 mm seed size fraction.

Seed source was also a significant factor, although the basis of this effect could not be easily defined. The lower coleoptile lengths for seed from Weetulta in the 2000 trials for each seed size fraction, however, may relate to low phosphorus content of the seed. Seed weight may have also contributed to the observed differences in coleoptile length between Weetulta seed from 1999 and 2000.

4.4.2 Effect of seed size on establishment

Low establishment was significantly ($\underline{P}<0.001$) associated with small seed (<2.2 mm). This effect of seed size may relate to the significant ($\underline{P}<0.001$) differences in coleoptile length, particularly since ample evidence has been produced demonstrating that a long coleoptile improves establishment (Whan, 1976; Bacaltchuk and Ulrich, 1990; Hughes and Mitchel, 1987; Radford, 1987; Radford and Wildermuth, 1987; Sharma, 1990). This association between establishment and coleoptile length could not be confirmed in these experiments since covariate analysis using REML could not assign degrees of freedom or calculate errors associated with coleoptile length. This occured because the coleoptile length for each treatment replicate was the mean value determined from the filter paper test. The regression

coefficients between establishment and coleoptile length (Table 4.12) were low to very low, suggesting other factor(s) were at play in determining variation in establishment. Small seed will invariably have reduced endosperm reserves, and it may be reasonable to suggest that the reduced capacity of the smaller seed did not adequately sustain development between germination and emergence. This may be exacerbated further on sandy soils if seed is sown too deep.

Table 4.12: Regression coefficient and level of significance for the relationship between establishment and coleoptile length, and early vigour (E_DMP) and coleoptile length.

Site	Trait 1	Trait 2	Regression coefficient (R^2)	Level of Significance
Brinkworth	Establishment	Coleoptile length	11.5	P=0.003
	E_DMP	Coleoptile length	22.1	P<0.001
Charlick	Establishment	Coleoptile length	1.8	P=0.137
	E_DMP	Coleoptile length	34.3	P<0.001
Darke Peak	Establishment	Coleoptile length	2.6	P=0.102
	E_DMP	Coleoptile length	12.9	P<0.01
Geranium 2000	Establishment	Coleoptile length	21.3	P<0.01
	E_DMP	Coleoptile length	0.8	P=0.264
Geranium 2001	Establishment	Coleoptile length	1.9	P=0.130
	E_DMP	Coleoptile length	34.6	P<0.001
Lowbank	Establishment	Coleoptile length	5.2	P<0.05
	E_DMP	Coleoptile length	14.1	P<0.001

4.4.3 Effect of seed size on early vigour

Early vigour was substantially greater with sowing seed from the largest fraction (>2.8 mm), with increases in dry matter production between 73% and 95% relative to that observed with sowing seed screened below 2.2 mm. This equates to an average increase in dry matter of 28.4% per 10 mg increase in seed weight. The improvement in early vigour over the same range on the sandy soils (27.1%) was only marginally lower than for the heavier soil types (31.1%). These results compare to the observations of Richards and Lukacs (2002) for wheat where plant dry weight increased by 21% per 10 mg increase in seed weight, albeit over a broader range of seed weights (15-65 mg). The association between early vigour and coleoptile length (Gul and Allan, 1976; Gorny and Patyna, 1981; Redona and Mackill, 1996; Rebetzke and Richards, 1996; Rebetzke *et al.*, 1999) could not be confirmed by covariate analysis for this set of data for the reasons stated for the relationship between coleoptile length and establishment. However, the regression coefficient between E_DMP and coleoptile length (Table 4.12) suggests that coleoptile length is a small, but significant, factor in determining early vigour. Establishment, in conjunction with seed source and/or seed size, was also a significant ($\underline{P}<0.001$) contributor to early vigour (Table 4.13). This is reasonable because more plants per unit area will contribute to a greater biomass per unit area. However, at Geranium 2000 ($\underline{P}=0.358$) and Lowbank ($\underline{P}=0.400$) establishment was not a contributing factor to early vigour, despite a significant effect of seed size ($\underline{P}<0.001$) on establishment at both sites. In other words, improvements in early vigour were directly related to the sowing of larger seed.

4.4.4 Effect of seed size on grain yield

The yield potential set up at early tillering through greater early vigour did not seem to follow through into grain yield at Brinkworth and Charlick. Grain yield of the largest seed fraction was only 1% and 3% higher relative to that observed for seed smaller than 2.2mm at Brinkworth and Charlick respectively (Table 4.8d & e). Analysis of early vigour as a covariate to seed source and seed size verified that grain yield was not significantly ($\underline{P}$=0.726 and $\underline{P}$=0.103 respectively) related to early vigour at either of these sites. It is likely that the better properties of these soils and the more favourable environments in which they exist better sustained grain development, and thereby negated the yield potential established by superior early vigour arising from the sowing of large seed.

This contrasts with the sandier soils where grain yield was significantly (Table 4.9) higher with seed from the >2.8mm fraction. Grain yield was 22%, 14%, 10% and 9% higher than seed screened to below 2.2mm at Geranium (2000), Geranium (2001), Darke Peak and Lowbank respectively. While it would be intuitive to attribute this improvement in grain yield associated with sowing larger seed, to the superior early vigour at early tillering, covariate analysis (Table 4.13) showed that early vigour did not contribute to the observed improvement in grain yield on the sandy soils. Rather seed source and seed size, and in some instances the interaction between seed source and seed size, were the most important factors contributing to grain yield (Table 4.9). Only at Lowbank, where seed size did not have a significant effect on grain yield, was early vigour crucial to grain yield potential ($P<0.001$, Table 4.13). The significant relationship between grain yield and early vigour at all sandy sites (Figure 4.4) seemingly contradicts the covariate analysis, but the amount of variation in grain yield explained by early vigour, even at Lowbank, was small. This may relate to a confounding effect of seed source on grain yield and/or early vigour.

Table 4.13: Wald statistics from the covariate analysis (REML) of early vigour (E_DMP) and grain yield for the Mundah seed size trials, 2000-2001.

Site	Trait	Covariate	d.f.	Wald Statistic	Significance
Geranium 2000	Early vigour	Establishment	1	0.85	P=0.358
	Grain yield	Early vigour	1	0.91	P=0.341
Geranium 2001	Early vigour	Establishment	1	73.80	P<0.001
	Grain yield	Early vigour	1	0.00	P=0.958
Darke Peake	Early vigour	Establishment	1	14.61	P<0.001
	Grain yield	Early vigour	1	0.06	P=0.806
Lowbank	Early vigour	Establishment	1	0.71	P=0.400
	Grain yield	Early vigour	1	13.21	P<0.001
Charlick	Early vigour	Establishment	1	43.95	P<0.001
	Grain yield	Early vigour	1	0.72	P=0.397
Brinkworth	Early vigour	Establishment	1	28.82	P<0.001
	Grain yield	Early vigour	1	0.67	P=0.412

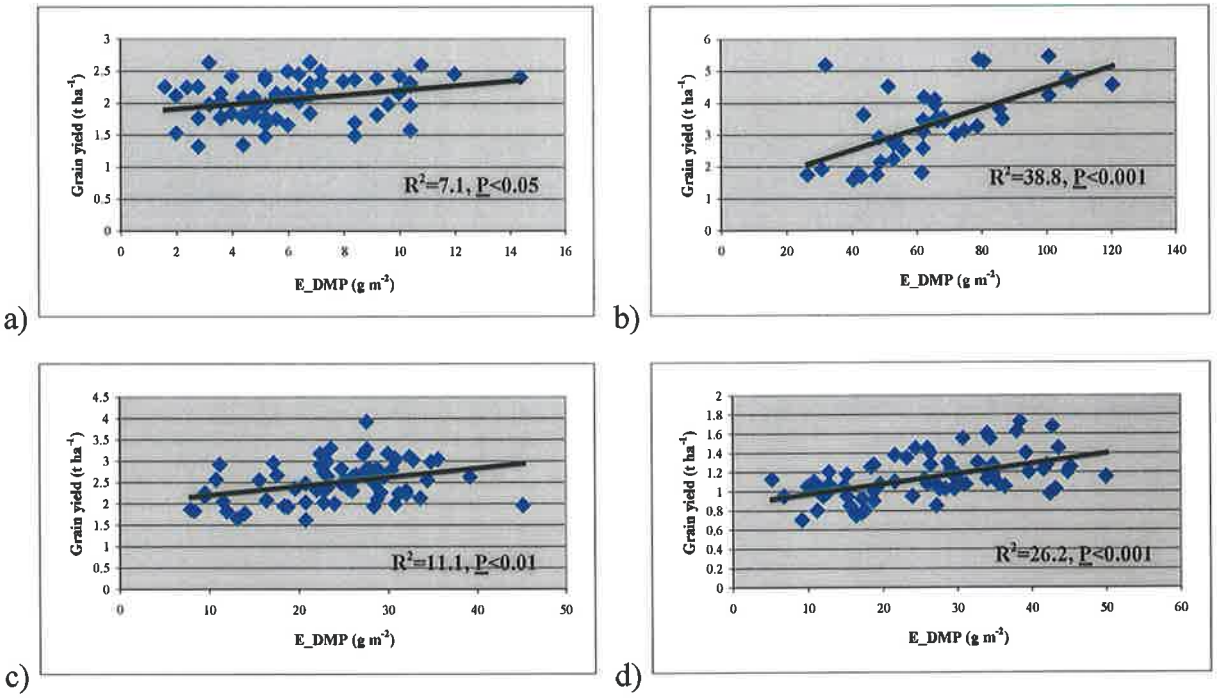


Figure 4.4: Association between grain yield and early vigour (E_DMP) for the raw data at a) Darke Peak, b) Geranium 2000, c) Geranium 2001 and d) Lowbank.

Nevertheless, it has been clearly demonstrated that early vigour, both in terms of early biomass production (E_DMP) and leaf area development, is a critical component of the superior adaptation of Mundah on sandy soils because it can set yield potential (see chapter 2). In addition, other authors have shown the importance of early vigour to grain yield potential (Brown *et al.*, 1987; Ceccarelli, 1987; Acevedo *et al.*, 1991). Importantly, this data also shows, and this has been a common theme throughout this study, that a combination of key traits is critical to sand adaptation. Early vigour is fundamental to sand adaptation because of the relationship with yield potential, as is seed size in improving early vigour and yield potential. A faster developing canopy also has implications for water use efficiency, and the capture of light for crop photosynthesis. The benefits of strong early vigour also extend to an improved competitiveness with weeds, a reduction in erosion, and the possibility of a reduction in the effects of sand "blasting".

A number of authors (Wood *et al.*, 1977; Roebuck and Trenerry, 1978; Spilde, 1989; Giles, 1990) have demonstrated that harvested grain weight is not affected by seed size at sowing. Despite the statistical significance of some of the current results (Table 4.9), the relative effects on the yield components (*i.e.* 1000 grain weight and screenings percentage) related to seed size can be reasonably considered as negligible in agronomic or absolute terms. Accordingly, the results presented here are in broad agreement with those of the authors listed above. The factor responsible for the differences in grain yield, and given the negligible changes in the grain yield components, cannot be clearly defined. However the variation in the number of grains per square metre (Table 4.14) provides circumstantial evidence to suggest that the degree of fertile tillering may differ between seed size categories. At the majority of sites, grain number was significantly ($\underline{P}<0.01$; $\underline{P}<0.001$) higher in plots sown with the large seed relative to those plots sown with the smallest seed fraction (Table 4.14). Even at Lowbank where grain number was not significantly associated with seed size ($\underline{P}=0.138$), a trend towards higher harvested grain number with larger seed size at sowing was evident. Seed size did not have a significant effect on grain number ($\underline{P}=0.419$) at Brinkworth. A

significant seed source x seed size interaction was determined from REML analysis at Charlick ($P<0.05$) and Geranium 2000 ($P<0.01$) (Figure 4.5). The general relationship at Geranium 2000, however, was one of increasing grain number with larger seed size (Figure 4.5). Giles (1990) and Richards and Rebetzke (2002) have provided evidence to show that plants derived from small seed have a lower capacity to develop tillers. Accordingly, it is possible that the higher or equivalent grain weight associated with plants from the sowing of small seed in these experiments relates to the distribution of carbohydrate and other resources amongst fewer grains, a consequence of fewer tillers per plant. The values for grain number per square metre is most likely an under estimate because they were calculated from grain yield and 1000 grain weight and would therefore be dependent on harvest efficiency (*i.e.* a function of the amount of grain lost through the harvester). However the principle that fewer grains per square metre resulted from the sowing of small seed remains valid.

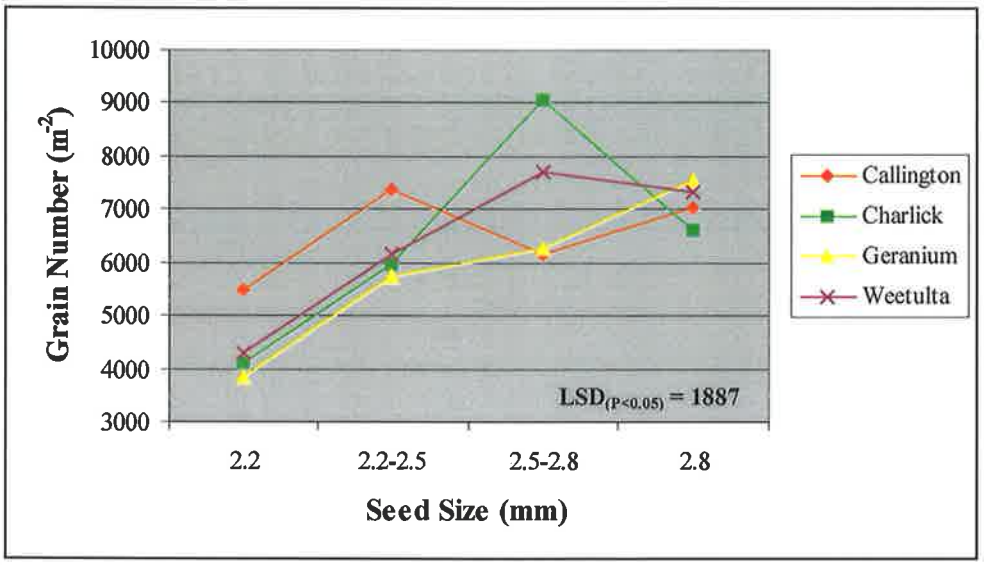
The higher grain yield at Geranium 2000 and 2001, despite a lower grain weight and higher screenings percentage, compared to the other sand sites, may also relate to the higher number of grains per square metre, presumably a result of increased tiller development. It is possible that late season environmental constraints limited the development of grain on the later tillers and these contributed to the higher screenings and reduced the overall grain weight.

Table 4.14: Wald statistics from the REML analysis and means for each seed size fraction of grain number per square metre for the Mundah seed size trials, 2000-2001.

Site	Source of Variation	d.f.	Grain number (m^{-2})	<2.2	2.2-2.5	2.5-2.8	>2.8	LSD (P<0.05)
Geranium	Seed source	3	1.78					
2000	Seed size	3	13.74**	4463	6363	7018	7162	*1426*
	Seed source x Seed size	6	19.64**					
Geranium	Seed source	6	30.12***					
2001	Seed size	3	19.03***	4802	5470	5701	6010	*544*
	Seed source x Seed size	12	8.22					
Darke Peak	Seed source	6	12.67*					
2001	Seed size	3	12.48**	3686	4147	4340	4372	*371*
	Seed source x Seed size	11	12.01					
Lowbank	Seed source	6	64.83***					
2001	Seed size	3	5.51	2275	2466	2506	2590	*242*
	Seed source x Seed size	12	10.76					
Charlick	Seed source	6	15.00*					
2001	Seed size	3	17.58***	6653	7343	7111	7399	*454*
	Seed source x Seed size	12	24.53*					
Brinkworth	Seed source	6	7.78					
2001	Seed size	3	2.28	5386	5916	5680	5618	*611*
	Seed source x Seed size	12	14.18					

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

a)



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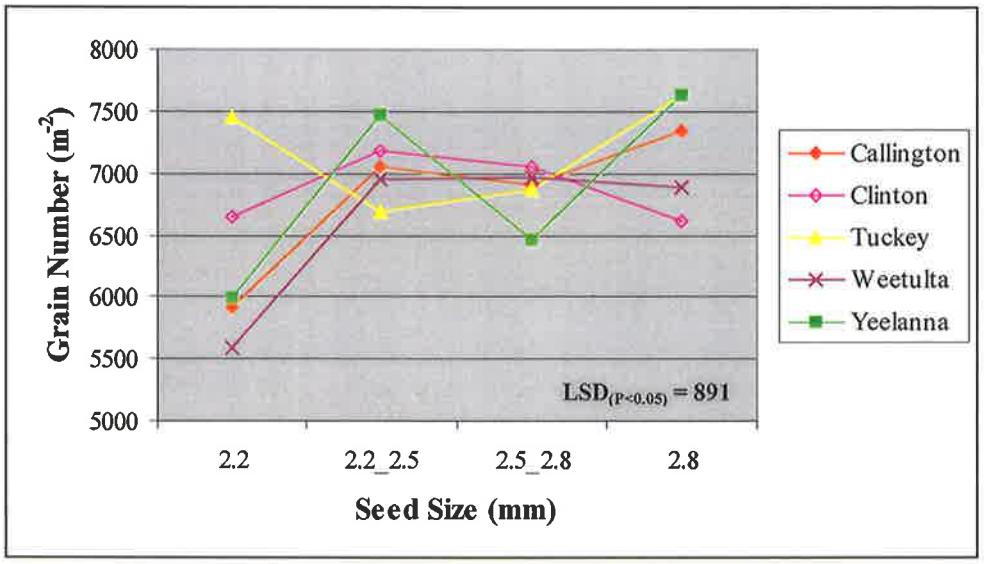


Figure 4.5: Influence of seed source and seed size on grain number (number m^{-2}) at a) Geranium 2000 and b) Charlick. Data analysed by REML analysis.

4.5 Conclusions

In this study, natural variation in seed size was exploited to clearly demonstrate that sowing large seed improves the growth and yield of barley on sandy soils. It has also been demonstrated that seed size can influence the establishment, early growth and grain yield on sand of a variety that displays inherent adaptation to this environment. Large seed produced longer coleoptiles, which in conjunction with better seed resources (nutrients and carbohydrate) led to enhanced establishment. Large seed combined with better establishment also improved early vigour. This superior early vigour can set the potential for improved grain yield, provided that environmental conditions remain favourable during grain development.

In practical terms this study has shown that growers can probably improve the growth and yield of barley on sandy soils through simple, yet effective, management strategies, including:

1. Selecting seed from the plumpest seed crop,
2. Selecting seed from favourable environments and soil types that produce seed of higher average size, weight and nutrition or,
3. Screen seed heavily to improve the average size and weight.

However, if it is common practice for growers to sow seed on a weight per hectare (kg ha^{-1}) basis, the benefits of sowing seed of larger average size will be lost because plant density and early biomass production on an area basis (early vigour) will be lower than that for seed of smaller average size. Accordingly, it is advisable to calculate seeding rate based on a recommended plant density, and adjusted for average seed weight.

Further, seed for all field evaluation, whether variety testing, agronomic or mapping populations, should be sourced from the same site and cleaned over a 2.5 mm slotted screen to remove seed source effects and allow a 'true' estimation of genetic effects.

Chapter 5. Development of a linkage map of the Mundah/Keel mapping population and the mapping of chromosome regions associated with improved growth and grain yield on sandy soils of low fertility.

5.1 Introduction

Progress towards the development and selection of superior barley varieties with adaptation to sand has been challenged by the very nature of sandy soils. Such soils can be characterised as low yielding environments in which yield and agronomic improvement is undermined by low genetic variance and high experimental error variance. For this reason plant and agronomic traits typically have a low heritability (h^2). The low genetic variance for sand adaptation in Australia has, in part, been due to the objectives of the breeding programs such as the South Australian Barley Improvement Program, which, historically, has focused on selection for improved malting quality in more favourable areas. Consequently, a large proportion of germplasm introduced into the breeding program as parents has typically been of superior malting quality and sourced from higher yield potential environments such as Europe, Canada and Japan, and as such are generally not agronomically suited to the soil types and growing conditions of southern Australia. The high experimental error variance of barley yield trials on sand soils is related to variability across the site due to factors such as uneven seeding depth, depth of sand, disease, the small size of breeding plots, and the limited replication number and availability of seed. In addition, traditional statistical approaches for analysing yield differences fail to adequately adjust for site variabilility and tended to favour varieties and environments of high grain yield potential. Consequently the application of conventional

breeding methodologies can result in material with potential for low yielding environments being culled because their unique adaptation has not been identified. Genetic progress for traits important to sand adaptation may also be stalled due to the measurement of traits being time consuming, difficult, laden with error, and expensive.

Molecular marker technology provides a unique opportunity to improve the efficiency of breeding for low h^2 traits, such as for sand adaptation. Early generation material can be screened with markers of known linkage to a gene/quantitative trait loci (QTL) controlling a trait of interest for the target environment without the need for destructive sampling and the analysis of grain. Breeding efficiency is also improved because the vagaries associated with the effect of environment on trait expression are overcome. In this chapter the development, and construction, of a mapping population and linkage map to characterise, and map, chromosome regions conferring improved growth and grain yield on sandy soils is discussed.

5.2 Methods and Materials

5.2.1 Mundah/Keel RIL Population

The Mundah/Keel RIL population was developed from a cross between two agronomically adapted varieties for southern Australia. Mundah has the superior adaptation to sandy soils in this cross, although the parents do contrast for a number of other traits (Table 5.2).

5.2.1.1 Mundah

Mundah is a two-row feed grade barley, bred and selected in Western Australia from a cross between the feed varieties Yagan (an introduction from the International Maize and Wheat Improvement Center (CIMMYT) of unknown parentage; Portmann, 1989) and O'Connor (Proctor/CI3576(WI2231)/3/ (XBVT212)Atlas57//(A14)Prior/Ymer). Mundah was selected

for its improved yield and agronomic features in the medium to low rainfall zones (Collins, 1998). Since its introduction into South Australian Research and Development Institute (SARDI) evaluation trials (1992), Mundah has been a consistently high yielding line that also produces very large grain. Mundah has a particularly high yield potential on sandy soils (Table 5.1) and this has been related to its early vigour, early flowering, and tolerance of low fertility situations and higher levels of boron (Table 5.2, Rob Wheeler, *pers. comm.*). Mundah has a short basic vegetative phase (BVP) and is daylength insensitive (Moody, *pers. comm.*). That is, the timing of phenological events (*e.g.* floral initiation and anthesis) seems to be unrelated to daylength. The ability of Mundah to perform well on sand has led to its recommendation as the variety of choice for sandy soils in SA. It is, however, limited by a lack of resistance to significant foliar diseases in S.A. (Table 5.2).

Table 5.1. Long term grain yield (MET analysis) of Mundah and Keel on sandy and non-sandy soils in South Australia, 1988-2000. (SAFCEP Annual Reports).

	Sandy Soils (t/ha)	No. Observations	Non-sandy Soils (t/ha)	No. Observations
Mundah	1.70	13	2.97	139
Keel	1.55	13	3.02	102

Table 5.2: Comparison of Mundah and Keel for key traits when grown under South Australian conditions.

Trait type	Phenotype	Mundah	Keel
Disease	Cereal Cyst Nematode *(Heterodera avenae)*	S	R
	Leaf Rust *(Puccinia hordei)*	MS	VS
	Leaf Scald *(Rhynchosporium secalis)*	S	MR/MS
	Powdery Mildew *(Erysiphe graminis* f. sp. *Hordei)*	MS/S	MR/MS
	Spot form net blotch (SFNB) *(Pyrenophora teres* f. *maculata)*	S	MR
	Net form net blotch (NFNB) *(Pyrenophora teres* f. *teres)*	MR	R
Plant type	Flowering	Early	Early
	Stature	Medium	Short-Medium
	Spikelet	2 row	2 row
	Basic vegetative phase	Short (photoperiod insensitive)	Short (photoperiod sensitive)
	Growth habit	Erect	Intermediate
	Early vigour	Very high	Moderate
	Tillering	Moderate	High
Grain size		Large	Moderate

Key: VS = very susceptible, S = susceptible, MS = moderately susceptible, MR = moderately resistant, R = resistant

5.2.1.2 Keel

Keel is a two-row feed quality barley, developed and released by the S.A. Barley Improvement Program from a cross between Clipper (Prior A/Proctor), CPI-18197 and WI-2645 (Mari/California Mariout 67). Keel has high yielding potential across most areas of SA, which is predominately realised on soils of higher fertility (Table 5.1). On sandy soils, characteristically low in fertility, the performance of Keel has been quite poor compared to

Mundah (Rob Wheeler, *pers. comm.*). Keel has some similar attributes to Mundah in that it is early flowering and has plump grain, however, it is photoperiod sensitive (Barr, *pers. comm.*, Table 5.2). In addition, Keel has good resistance to some leaf diseases and cereal cyst nematode (CCN), a distinct advantage over Mundah. Keel also has a higher fertile spikelet to primordia ratio (Coventry, *pers. comm.*), which seems to be related to its improved yield potential under water stress conditions.

5.2.2 Population Development

The Mundah/Keel population was developed as F_3 derived recombinant inbred lines (RILs) through single seed descent from an F_2 bulk sown at Charlick, S.A. in 1997. Originally 50 random lines were multiplied and phenotyped for traits 'desirable' for adaptation on sandy soils in 1999 and 2000. An additional 60 random lines from the F_2 bulk were multiplied over summer 2000/01 and added to the original 50 lines. In the 2001 sand screening trials 95 of the possible 110 lines were phenotyped.

5.2.3 Linkage Map Construction

5.2.3.1 DNA extraction

The extraction of DNA from leaf material followed the midi-prep procedure devised by Karakousis and Langridge (submitted). Two grams of frozen leaf material was weighed into a 50ml polypropylene screw cap tube (V shaped). Two large and four medium size stainless steel ball bearings were placed in the tubes with the leaf tissue, and then re-frozen in liquid nitrogen. Maintaining the tissue at low temperatures inhibits nucleases from degrading the DNA. The tubes were shaken using a flask shaker for one minute until the tissue was ground to a fine powder, then placed back in liquid nitrogen. The ball bearings were removed and the tissue allowed to thaw. DNA extraction buffer (4.5ml) was mixed with the ground tissue on a vortex. Phenol/chloroform/iso-amyalcohol (25:24:1, 4.5ml) was added, mixed in thoroughly

and mixed for a further 10 minutes at low speed on a multiple tube vortex. The solution was poured off into a 10ml collection tube and spun in a swing-out bench centrifuge at 4000 rpm for 10 minutes. The supernatant was carefully poured into a silica matrix tube and re-extracted with phenol/chloroform/iso-amyalcohol (25:24:1, 4ml) for 5 minutes on an orbital mixer. The samples were re-spun in the centrifuge. Supernatant was poured into a fresh 14ml tube, to which was added 400µl Sodium Acetate (3M, pH4.8) and 4ml of isopropanol. The mixture was gently rotated on the orbital mixer for 5 minutes to precipitate the DNA. The DNA was carefully spooned out using a pasteur pipette and transferred to a 2ml eppendorf tube and washed with 1ml of 70% ethanol. The ethanol was poured off and after spinning at 14000 rpm for 5 minutes the excess ethanol was removed. The DNA was resuspended in *R40* (350µl) and frozen until required for AFLP and SSR analysis. An explanation of the function of reagent solutions in the extraction of DNA from plant tissue is listed in Table 5.3.

Table 5.3: Explanation of the function of specific reagent solutions used in the DNA extraction method.

DNA extraction buffer	
1% Sarkosyl	Dissolves cell membranes, strips protein off the DNA and inactivates enzymes that denature DNA.
10mM EDTA	Collating agent that complexes Mg^{2+} (nuclease cofactor) to inactivate enzymes that denature DNA.
2% PVPP (Polyvinyl-polypyrrolidone)	Blocks activity of polyphenol oxidase.
100mM Sodium Chloride	Solubilizes the DNA.
phenol/chloroform/iso-amyalcohol (25:24:1)	Denatures proteins aiding in the purification of the DNA.
Sodium Acetate (3M, pH4.8)	Reduces the solubility of DNA.
R40	An Rnase that denatures RNA molecules.

5.2.3.2 Mapping

The complete population (110 lines) was screened with fluorescently labelled amplified fragment length polymorphism (AFLP) primer combinations, microsatelite (single sequence repeats, SSR) primers and RFLPs to construct a partial linkage map. A linkage map based on AFLPs can be constructed quickly with the aid of a set of anchored SSR and RFLP markers.

5.2.3.2.1 Amplified fragment length polymorphisms (AFLPs)

The AFLP analysis of the Mundah/Keel population was based on the protocol developed by Vos *et al.* (1995). Restriction and ligation of the genomic DNA (1μg) was performed in a final volume of 60μl and incubated at 37°C for 3 hours. The reaction mix contained 5 units of each of Mse1 (0.25μl) and Pst1 (0.5μl), 6μl of 10x restriction-ligation (R-L) buffer, 1ul Pst1 (5μM) and 1μl Mse1 (50μM) adaptors, 1μl of T4 DNA ligase (1μg/μl), and 47.05 μl of nanopure water. The ligation of Pst1 and Mse1 adaptors to their corresponding restriction sites created a specific sequence that facilitated the binding of PCR primers to the DNA fragments. The Restricted-Ligated DNA fragments (4μl) were pre-amplified in a reaction mix (25μl) containing the Pst1+1 primer (75ng/μl), the Mse1+1 primer (75ng/μl), 1.25mM dNTP, 10x buffer, 50mM $MgCl_2$, 5U/μl Taq DNA polymerase and nanopure water. See Table 5.4 for sequence details on the Pst1 and Mse1 primers. The PCR reaction for pre-amplification was 20 cycles of the following steps; 94°C for 30 seconds, 56°C for 1 minute and 72°C for 1 minute. 200μl of nanopure water was added to the mix to complete the template DNA. In total, nine primer combinations, divided into 3 sets of 3 primer combinations, were used to selectively amplify the template DNA. To visualise the AFLP products the PstI primers were labelled with 6-carboxy-fluorescein (6-FAM), tetrachloro-fluorescein (TET) or hexachloro-fluorescein (HEX) fluorescent dyes, while the MseI primer was unlabelled (Table 5). For each set of primer combinations one microliter of each of the three types of fluorescently labelled PCR products were pooled. The combined sample was

dried then mixed with 0.25 μL of a N,N,N',N'-tetramethyl-6-carboxyrho-damin (TAMRA)-labelled internal length standard GeneScan-500 TAMRA (PE/Applied Biosystems) and 1.75 μL of formamide, denatured for 3 min at 90°C and quickly chilled on ice. Table 5.5 details the primer combinations used in the selective amplification of the templated DNA and their corresponding fluorescent dyes. The buffer mix also contained 10x PCR buffer, 1.25mM dNTP, 50mM $MgCl_2$, 5U/μl Taq DNA polymerase and nanopure water. Selective PCR of the fluorescently labelled R-L DNA fragments (5μl) was as follows. One cycle of 94°C for 30 seconds, 65°C for 30 seconds and 72°C for 1 minute. This cycle was followed by 9 cycles of the above steps, except that the annealing temperature was decreased by 1°C per cycle until an annealing temperature of 56°C was reached. Following this there was 25 cycles of 94°C for 30 seconds, 56°C for 30 seconds and 72°C for 1 minute. Once the selective PCR was completed the 3 primer combinations that made up one set were combined for each sample (*i.e.* DNA from each line). Prior to the analysis of each sample, 2μl of loading buffer was added and the sample denatured for 3 minutes at 90°C before storing on ice. AFLP products were separated on a 6% polyacrylamide sequencing gel run at 40W for 6 hours in a 1x TBE running buffer. After separation of the products the data was displayed as chromatographs and analysed for polymorphisms with the assistance of Genescan software.

Table 5.4: The primer sequences used in the pre-amplification and selective amplification of DNA fragments.

Primer	Core sequence (selective nucleotides shown as N)	Pre-amplification (plus selective Nucleotide)	AFLP nomenclature	Selective amplification (plus selective Nucleotides)	AFLP nomenclature
Pst1	5'-GACTGCGTACATGCAGNNN-3'	Pst1 + A	P01	Pst1 + AA	P11
				Pst1 + AC	P12
				Pst1 + AG	P13
				Pst1 + AT	P14
Mse1	5'-GATGAGTCCTGAGTAANNN-3'	Mse1 + C	M02	Mse1 + CAA	M47
				Mse1 + CAC	M48
				Mse1 + CAG	M49
				Mse1 + CAT	M50
				Mse1 + CTG	M61

Table 5.5: The primer combinations used and their corresponding fluorescent dye

Primer combination	Fluorescent dye
P13/M47	FAM
P14/M47	TET
P12/M50	HEX
P13/M49	FAM
P14/M61	TET
P13/M50	HEX
P11/M48	FAM
P14/M47	TET
P12/M50	HEX

5.2.3.2.2 Microsatelites (Single Sequence Repeats, SSR)

To complete a partial map of the population, suitable for QTL analysis, an even spread of anchored SSR markers across all 7 chromosomes was essential. To achieve this Mundah and Keel were screened with 145 SSR primers of known location on the barley consensus map (Karakousis *et al.*, 2003). PCR reactions to amplify and analyse SSR products for polymorphisms between the two parents was performed in a final volume of 12μl (12.5μl for genotyping of population) using a standard PCR protocol with an annealing temperature of 50°C (Table 5.6). The reaction mix contained 0.5U *Taq* DNA polymerase, PCR buffer, 50mM MgCl2, 5mM dNTP mix, 0.15μg/μl primer mix and approximately100ng/μl of template DNA.

Table 5.6:PCR protocol for parental screening

	PCR Steps	
1.	Denaturation	94°C for 30 seconds
2.	Annealing	59°C for 30 seconds
3.	Extension	72°C for 30 seconds
4.	Repeat steps 1-3 reducing the annealing reached.	temperature by 0.5 °C until 50°C is
5.	Denaturation	94°C for 30 seconds
6.	Annealing	50°C for 30 seconds
7.	Extension	72°C for 30 seconds
8.	Repeat steps 5-7 for another 28 cycles.	
9.	Extension	72°C for 5 minutes
10.	Cool to 25oC	5 minutes
11.	End	

For both the parental screening and the mapping of the population, PCR products were separated on an 8% polyacrylamide denaturing gel for between 3 to 5 hours at 300 volts. The products were stained with ethidium bromide for 30 minutes, de-stained and visualised for

under a fluorescent light. The gel was photographed and saved as a picture file (tag image file format) and scored for polymorphisms.

5.2.3.2.3 Restriction fragments length polymorphisms (RFLPs)

Forty-six RFLP markers were selected based on their position on the consensus map, collated by Karakousis *et al.* (2003), to fill regions of the genome not covered by SSRs. This was to ensure an even spread of anchored markers along each chromosome to integrate the AFLP markers and provide adequate marker density for the QTL analysis to be valid. Mundah and Keel were digested with 6 restriction enzymes (Bam HI, Dra I, Eco RI, Eco RV, HindIII and Xba I) and screened with the 46 RFLP markers for each digest to select the marker-restriction enzyme combination that detected polymorphic bands between the two parents. Those combinations which scored positive for polymorphism detection, and which were suitably positioned to cover the gaps on the consensus map, were used to screen the entire population (110 lines).

The general procedure for both the screening of parents for polymorphic markers and the screening of the mapping population was as follows:

Genomic DNA (5ul) was digested in a buffer mix containing 0.5μl of restriction enzyme (40U/ul) at 37°C for 5 hours. 6x Ficoll dye was added and the samples separated on a 1% agarose gel, in 1x TAE buffer, run overnight at 33V. Included in each run was a Lambda DNA size marker (Lambda HindIII). Digested DNA was transferred onto a Hybond N^+ nylon membrane using the Southern transfer method (Southern, 1975), rinsed in 2x SSC, sealed in a plastic bag and stored in the fridge until used for hydridisation of the radioactive-labelled DNA probes. DNA fragments (RFLP markers) were radio-labelled with [a-^{32}P]dCTP , hybridised to the membranes, using standard protocols, washed in 2x SSC (0.1% SDS) and analysed by autoradiography (Rafalski and Tingey, 1993). The membrane was exposed to film for 5 days at –70°C and developed using an automatic film developer. In the case of the

parental screens, enzyme-marker combinations were scored for their ability to detect polymorphisms. For the population, the autorads were analysed to score each line for the origin of the DNA segments (*i.e.* which parent contributed the DNA to that specific line) detected by each individual probe (marker).

5.2.3.3 Construction of the Mundah/Keel linkage map

For each DNA marker the population was scored based on whether the line carried the Mundah or Keel allele, or whether it was heterozygous at that locus. These data were imported into Mapmanager QTxb13 software (Manly and Cudmore 1997) and analysed using the Kosambi mapping function (Kosambi 1944; Lander *et al.* 1987), at a threshold value of P=0.001, to construct initial linkage groups. Linkage groups were then assigned a chromosome number according to the known chromosomal position of the majority of DNA markers within each linkage group. Linkage groups assigned with the same chromosome number, and single markers (SSRs or RFLPs) with no significant linkage with previously allocated markers, were cross-referenced to the consensus map of Karakousis *et al.* (submitted) to allow the assignment of 'best fit' chromosome positions. Any subsequent markers were integrated into the linkage map using the 'find best location' function.

5.2.4. Field Trials

5.2.4.1 Sites

The Mundah/Keel RIL population, including parents, was sown at nine site x year trials in South Australia from 1999 to 2001; at Minnipa (MAC99, MAC00, MAC01), Tuckey (TUC99, TUC00), Sandalwood (SAN99), Lowbank (LOW00, LOW01) and Cooke Plains (CPL01). The location of these trials is illustrated on the map of South Australia in Appendix 1.

5.2.4.2 Establishment of Trials

Trials in 1999 and 2000 consisted of the original 50 RILs and the two parents. These were established as randomised complete block designs (RCBD), with each entry replicated twice. Plot area at sowing was 10.5m^2 at Minnipa (1999) and Sandalwood (1999), 18m^2 at Minnipa and Lowbank (2000), and 4.1m^2 at Tuckey (1999, 2000). Depth of seeding was set for 2.5-3cm. Plot area at harvest was 7.5m^2 at Minnipa and Sandalwood (1999), 15m^2 at Minnipa and Lowbank (2000); and 3.92m^2 at Tuckey. In 2001, 95 RILs were phenotyped in field trials at three locations (Minnipa, Lowbank and Cooke Plains). All experiments were established as a RCBD, with entries spatially randomised using SpaDes® (Coombes, 1999) and replicated twice. Plot area was 10.5m^2 at sowing before being reduced to 7.5m^2 at harvest. Details pertaining to the sowing and harvest dates, fertilizer regime, soil type and rainfall for each site is summarised in Tables 5.7-5.9.

Table 5.7: Site Details-Sowing date, fertilizer rates, harvest date, soil type and April-October rainfall

	1999		
	Minnipa	**Tuckey**	**Sandalwood**
Sowing Date	3rd June	8th June	3rd June
Fertilizer Rate	75 kg/ha of 17:19 (5% Zn)	45 kg/ha of 27:11	116 kg/ha of 9:17 (5% Zn)
Harvest Date	19th November	4th December	30th November
Soil Type	Sandy loam (0-10cm) over loam	10 cm of sandy loam over loamy clay over clay	Sandy loam (0-80cm)
April-October Rainfall (mm)	200	241	178

Table 5.8: Site Details-Sowing date, fertilizer rates, harvest date, soil type and April-October rainfall

	2000		
	Minnipa	**Tuckey**	**Lowbank**
Sowing Date	2nd June	25th May	2nd June
Fertilizer Rate	75 kg/ha of 17:19 (5% Zn)	130 kg/ha of 13:15	143 kg/ha of 9:17 (5% Zn)
Harvest Date	24th November	17th December	15th November
Soil Type	Sand over sand over clay	Sandy loam(10cm) over loamy clay over clay	Sand (pH9.0)
April-October Rainfall (mm)	293.3	302	175.5

Table 5.9: Site Details-Sowing date, fertilizer rates, harvest date, soil type and April-October rainfall

	2001		
	Minnipa	**Lowbank**	**Cooke Plains**
Sowing Date	31st May	18th June	1st June
Fertilizer Rate	75kg/ha of 17:19 (5% Zn)	98.6 kg/ha of 17:19 (5% Zn)	98.6 kg/ha of 17:19 (5% Zn)
Harvest Date	22nd November	8th January 2002	3rd Januatry 2002
Soil Type	Sandy loam	Sandy loam	Sandy loam
April-October Rainfall (mm)	240	215	267

5.2.4.3 Measurements

5.2.4.3.1 Early Vigour Measurements

At approximately 8 weeks post-sowing and at a plant development stage of Z14/Z21-22 (early tillering) establishment was scored by counting the number of plants in a 0.25 m^2 quadrat at two locations in each plot. The plants in each of these quadrats were harvested, counted to determine establishment, and dried at 80°C for 48 hours to determine dry matter production. Dry matter production (E_DMP, g m^{-2}) was used as a measure of early vigour. In addition

early vigour (1 =low, 7 = high) and plant growth habit (1 = prostrate, 2 = intermediate 3 = erect) were scored using a ranking system based on visual assessment of the plots.

5.2.4.3.2 Disease scores

The incidence of spot form of net blotch (SFNB) in the Mundah/Keel population was scored at Tuckey (2000), and Pt. Wakefield (2000, PTW00), a low rainfall site with a heavy soil type, using a ranking system based on the visual assessment of plots (1 = resistant, 9 = very susceptible).

5.2.4.3.3 Agronomic Measurements

Scores for maturity were made in late September to early October using the decimal growth stage method (Zadoks *et al.*, 1974). From the harvested material grain yield (t ha^{-1}), screenings (proportion of grain below 2.5mm, %), and 1000 grain weight (g) were measured.

5.2.4.4 Statistical Analysis

All field trial data was analysed by a linear mixed model analysis using residual maximum likelihood (REML) (Patterson and Thompson, 1971). Analysis was performed using Genstat statistical software (Genstat® for Windows™, 5th edition, Lawes Agricultural Trust). REML produces Wald statistics to test the significance of fixed (treatment) effects. Early dry matter production (early vigour), growth habit, maturity, 1000 grain weight, screenings and grain yield were also analysed using a MET (multi-environment trial) statistical analysis (Cullis *et al.*, 1998, Smith *et al.*, 2001). The analyses employ spatial techniques to adjust the means of data to accommodate variability across a field trial site (soil depth, fertility) and also variability due to cultural practices (*e.g.* harvesting in two directions). In addition, the MET analysis adjusts across site means to accommodate variability between sites, and provide information on the environmental stability of varieties and the h^2 of the traits analysed. Since

quantitative traits display a continuous variation in a population, h^2 describes the proportion of the total phenotypic variance that is attributable to the additive genetic variance (*i.e.* genetic variance due to alleles that act additively) (Snustad *et al.*, 1997). The MET analysis was conducted using ASREML (Gilmour *et al.*, 1999).

The application of least significant differences (LSDs) to compare varieties against control varieties is unsuitable when data is analysed using MET data (Smith *et al.*, 2001). Instead, the predicted mean of traits for each variety at the 'average' site are interpreted on the basis of the probability that the variety is truly superior to the control (Mundah in these experiments) for that trait (Smith *et al.*, 2001). The calculation of the 'average' site is dependent on the sites used in the analysis. The MET analysis generates loadings for each site that describes the weight of each sites contribution in calculating the performance ranking of varieties for any trait (*e.g.* grain yield) at the 'average' site. Site loadings also illustrate the correlation of each site with the 'average' site and the correlation between sites used in the analysis, and accommodates the environmental similarities and differences between sites (Smith *et al.*, 2001). Accordingly, site loadings can be utilized to graphically represent site environments, and the environmental effects (*i.e.* genotype x environment interaction) on traits at each site. Figure 5.3 presents the grain yield response of Mundah and Keel on this basis.

5.2.5 QTL Analysis

The method used to characterise associations between markers and Quantitative Trait Loci (QTL) was a single marker regression of the trait values on the markers incorporating environmental effects and genetic correlations between sites in a single stage analysis (Eckermann *et al.*, 2001; Verbyla *et al.*, 2003). In other words the QTL analysis was combined with spatial analysis (MET analysis) of the phenotypic data. Marker effects were fitted separately for each site, with significant markers being added to the model as cofactors when searching for further markers.

The general model fitted was of the form:

QTL = marker effects + residual genetic variation + environmental variation + error.

In the case of SFNB the Q-gene analytical package (Nelson 1997) was used to characterise associations between markers and Quantitative Trait Loci (QTL) using the single point analysis function.

Maximum likelihood statistics (LOD values) were used to confirm marker-trait associations for both approaches of analysing the quantitative traits. LOD scores between 2 and 3 indicated possible QTLs, and scores above 3 were regarded as significant marker-trait associations. A LOD score of 2 relates to a p-value (level of significance) of 0.0024, and was chosen because it is possible to miss significant QTL's with a LOD of 3 (Patrick Lim, *pers. comm.*). A LOD score of 3 was still used as the critical value of QTL significance when using Q-gene software, since the inherent variation in the data is not accounted for in this type of analysis (Patrick Lim, *pers. comm.*).

5.3 Results and Discussion

5.3.1 Mundah/Keel linkage map

With the aim of identifying chromosome regions associated with traits involved in sand adaptation (*i.e.* improved grain yield, 1000 grain weight, reduced screenings, superior early vigour, erect growth habit, early maturity), a skeletal map of strategically placed markers, based on their chromosomal locations on the barley consensus map (Karakouisis *et al.*, 2003), was produced for the Mundah/Keel population. A similar strategy was adopted by Pallotta *et al.* (2003) in constructing a linkage map for the Amagi Nijo/WI2585 doubled haploid mapping population. The availability of the barley consensus map provided a more efficient and time effective approach to mapping, in that molecular markers could be selected at regular intervals along each chromosome to ensure suitable coverage of the genome. A linkage map with an even spread of markers across the genome may well provide an equally or more effective strategy for QTL analysis than one developed from screening a vast number of markers without reference to chromosomal location. From the outset, a lack of polymorphism between the parents was evident, and consequently, even the development of a reasonable skeletal map proved to be problematic. Of the 146 SSRs screened, only 21% were found to be polymorphic between parents (Table 5.10). This compares with the 34-46% achieved in other mapping populations developed and characterised by the National Barley Molecular Marker Program (NBMMP) (Karakousis *et al.*, 2003). In addition, the number of polymorphic loci detected by AFLP analysis with 9 primer combinations (32) was considerably less than in the other NBMMP mapping populations; such as Alexis/Sloop (71), Chebec/Harrington (49), Galleon/Haruna nijo (101), Arapiles/Franklin (75) and ND11231/VB9524 (84).

The small number of polymorphic SSRs detected and mapped proved insufficient, not only for a suitable skeletal map, but also in providing anchored markers to aid in the assignment of AFLP markers to linkage groups. Therefore selected RFLP markers, located within

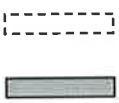
chromosome regions not covered by SSRs, were used to improve the skeletal map, and allow AFLP markers to be integrated. However, the RFLP markers also displayed low levels of polymorphism between the parents (26%, Table 5.10). Even with the addition of extra 12 markers, only 14 of the 32 AFLP markers could be placed on to the linkage map. Consequently the subsequent linkage map for the Mundah/Keel population (Figure 5.1 and 5.2) was particularly skeletal in nature. The limited number of polymorphic markers available for developing the linkage map of the Mundah/Keel population also required that 'best-fit' chromosomal locations of the 'linkage blocks', in addition to the location of single unlinked markers, be estimated from the consensus map of Karakousis *et al.* (2003).

Table 5.10: Markers used in the construction of the Mundah/Keel linkage map.

Marker type	Number Screened on parents to detect polmorphism	Number screened across population	Number of markers mapped
AFLP		32	14
RFLP	46	12	12
SSR (Adelaide Uni)	146	31	28
TOTAL	191	75	54

Each chromosome on the map was characterised by 'linkage blocks' of two or more linked markers, and/or single, unlinked markers. In addition, each chromosome had large regions without marker coverage (*e.g.* 6H). In some cases these regions covered large genetic distances. Chromosomes 1H, 3H and 4H were poorly represented with markers, covering only 10% (plus one single unlinked locus), 23% (plus one single unlinked locus) and 22% (plus two single unlinked loci) of their respective chromosomes. Chromosome 6H was of particular concern, with only three loci on a chromosome estimated to be 130cM (Karakousis *et al.*, 2003). Markers covered 62% and 63% of chromosomes 2H and 7H respectively, although the genetic distances between markers within 'linkage blocks' on 7H were

Figure 5.1: Linkage map of the Mundah/Keel RIL mapping population.



Size of chromosome based on the barley consensus map (Karakousis *et al.*, 2003).

Chromosome regions covered by markers in the Mundah/Keel RIL population.

Key: * Loci for growth habit (GH) and maturity (MAT) are predictive and should be treated with caution because the data does not follow a normal distribution

The locus for spot form of net blotch (SFNB) resistance is predictive since the QTL is not significant

C = location of centromere based on the barley consensus map (Karakousis *et al.*, 2003)

= candidate genes controlling flowering time (*Ppd-H1*, Laurie *et al.*, 1994 and *eps2s*, Laurie *et al.*, 1995)

GY = grain yield (t ha-1)

GWT = 1000 grain weight (grams)

SCR = screenings percentage (% below 2.5mm)

E_DMP = early dry matter production (g m-2)

EV = early vigour score (1-7)

GH = growth habit score (1-3)

MAT = maturity (decimal growth stage)

SFNB = spot form of net blotch score (1-9)

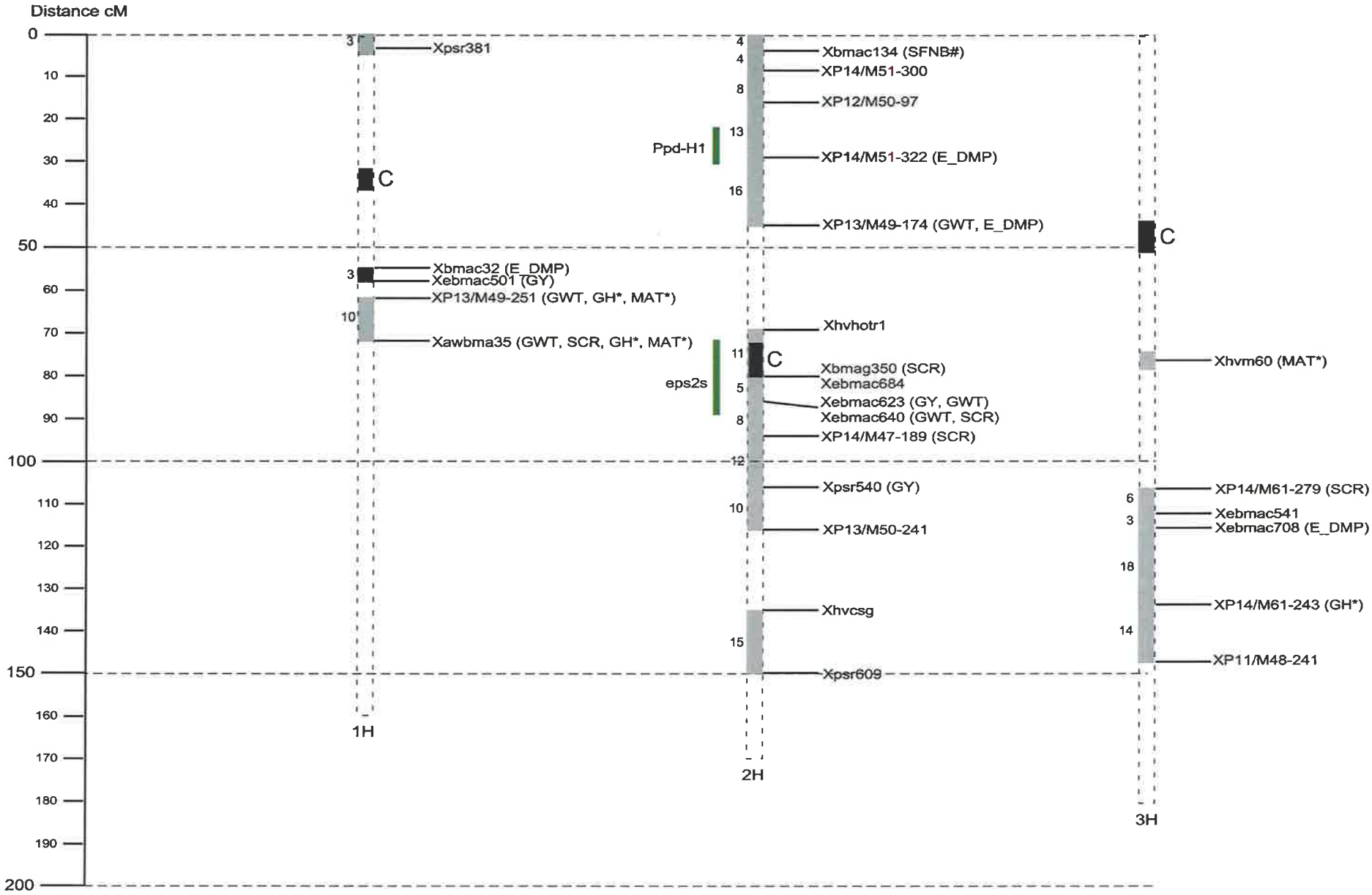

Distance cM
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
3
Xpsr381
C
3
Xbmac32 (E_DMP)
Xebmac501 (GY)
10
XP13/M49-251 (GWT, GH*, MAT*)
Xawbma35 (GWT, SCR, GH*, MAT*)
1H
4
4
Xbmac134 (SFNB#)
XP14/M51-300
8
XP12/M50-97
Ppd-H1
13
XP14/M51-322 (E_DMP)
16
XP13/M49-174 (GWT, E_DMP)
Xhvhotr1
11
C
Xbmag350 (SCR)
Xebmac684
eps2s
5
Xebmac623 (GY, GWT)
Xebmac640 (GWT, SCR)
8
XP14/M47-189 (SCR)
12
Xpsr540 (GY)
10
XP13/M50-241
Xhvcsg
15
Xpsr609
2H
C
Xhvm60 (MAT*)
6
XP14/M61-279 (SCR)
Xebmac541
3
Xebmac708 (E_DMP)
18
XP14/M61-243 (GH*)
14
XP11/M48-241
3H

Figure 5.2: Linkage map of the Mundah/Keel RIL mapping population.

Size of chromosome based on the barley consensus map (Karakousis *et al.*, 2003).

Chromosome regions covered by markers in the Mundah/Keel RIL population.

Key: * Loci for growth habit (GH) and maturity (MAT) are predictive and should be treated with caution because the data does not follow a normal distribution

C = location of centromere based on the barley consensus map (Karakousis *et al.*, 2003)

= candidate genes controlling flowering time (Laurie *et al.*, 1995)

GY = grain yield (t ha-1)

GWT = 1000 grain weight (grams)

SCR = screenings percentage (% below 2.5mm)

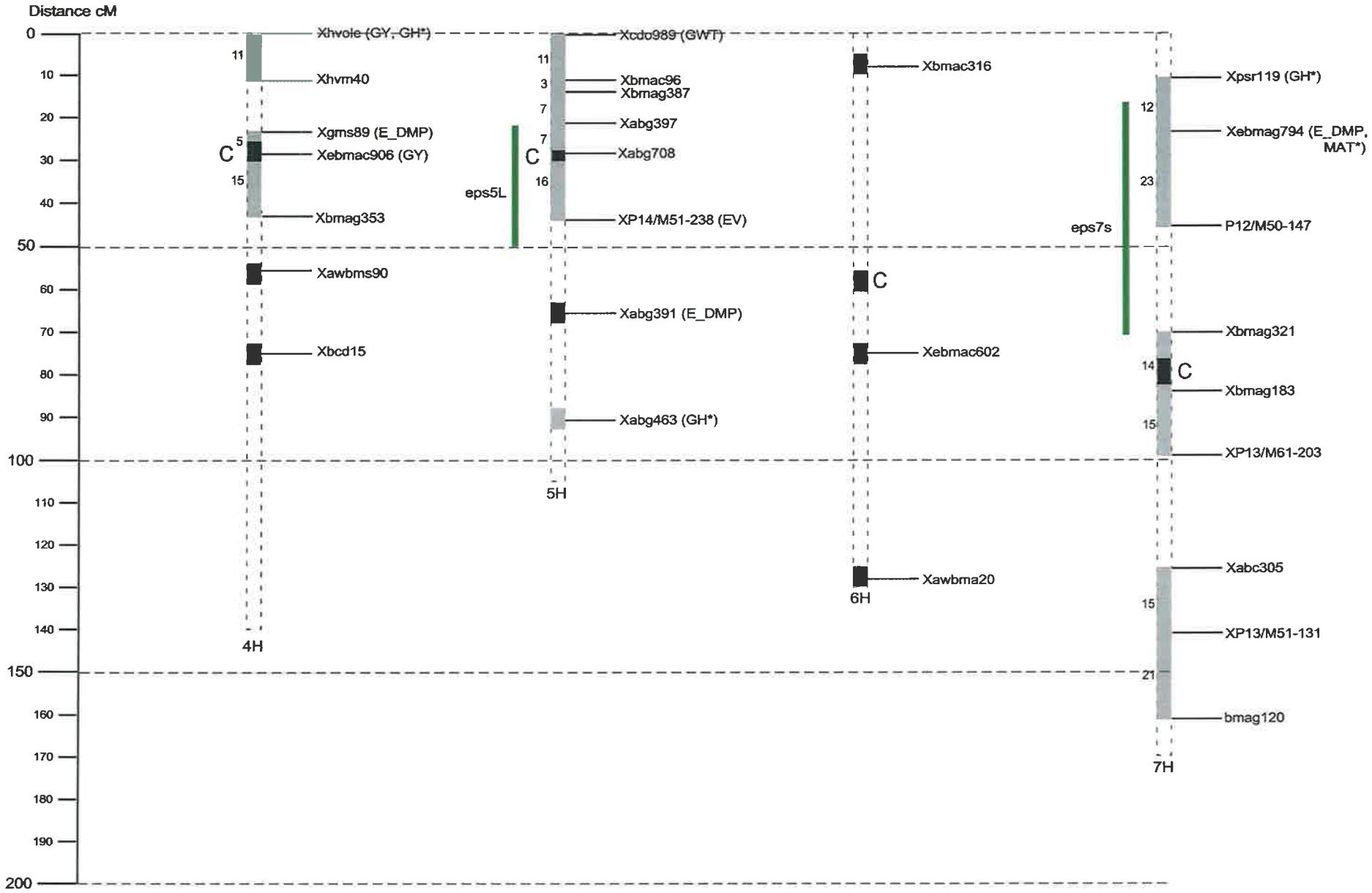
E_DMP = early dry matter production (g m-2)

EV = early vigour score (1-7)

GH = growth habit score (1-3)

MAT = maturity (decimal growth stage)

SFNB = spot form of net blotch score (1-9)


Distance cM
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
Xhvole (GY, GH*)
11
Xhvm40
Xgms89 (E_DMP)
C
5
Xebmac906 (GY)
15
Xbmag353
Xawbms90
Xbcd15
4H
eps5L
Xcdo989 (GWT)
11
Xbmac96
3
Xbmag387
7
Xabg397
7
C
Xabg708
16
XP14/M51-238 (EV)
Xabg391 (E_DMP)
Xabg463 (GH*)
5H
Xbmac316
C
Xebmac602
Xawbma20
6H
eps7s
Xpsr119 (GH*)
12
Xebmag794 (E_DMP, MAT*)
23
P12/M50-147
Xbmag321
14
C
Xbmag183
15
XP13/M61-203
Xabc305
15
XP13/M51-131
21
bmag120
7H

reasonably large. The genetic distances between loci within linkage blocks, between linkage blocks, between unlinked single loci, and between linkage blocks and unlinked single loci were extremely large, such that the capability of characterising and mapping all possible QTLs associated with any one trait were significantly diminished. In other words, some, or the majority of, possible QTLs associated with a specific trait would not be detected; a consequence of having a linkage map sparsely populated with markers.

The pedigree histories of Mundah and Keel identified some commonalities in their genetic backgrounds that may provide some explanation for the low level of polymorphism encountered. O'Connor (parent of Mundah) and Clipper (parent in the cross from which Keel was selected) both have significant contributions from Proctor, and Prior A or Prior (selections from Priors Chevalier) cultivars in their pedigrees (see sections 5.2.1.1 and 5.2.1.2, and Appendix 2). In addition, Yagan (unknown parentage) and WI2645 (2EBYT-23) are CIMMYT derived lines. The possibility exists, although quite speculative, that WI2645 and Yagan may have common genetic backgrounds. In retrospect, Mundah and Keel were probably too genetically similar to produce a high quality linkage map with the number of markers used. However, the Mundah/Keel RIL population is still likely to be a very useful resource, with further development of the linkage map, because it is a cross between two agronomically adapted varieties, whereas the mapping populations of the National Barley Molecular Marker Program (NBMMP) are predominately of the type "adapted x unadapted". Within these populations almost all grain yield QTL detected were associated with chromosome regions related to maturity, height and disease resistance, and not yield *per se*. An "adapted x adapted' population may uncover regions associated with yield *per se* in addition to the regions where yield is regulated by genes controlling phenological and disease resistance traits.

5.3.2 Phenotypic data

Averaged across all nine site x year field experiments, Keel was higher yielding and produced more dry matter at early tillering (E_DMP) than Mundah (Table 5.11). In addition, MET analysis showed Keel had a highly significant probability (Table 5.11) of exceeding Mundah for both these traits. Further evidence of this for E_DMP can be seen by differences in mean dry matter production between Mundah and Keel at each experimental site (Table 5.12), and especially reflects the differences observed at TUC99, LOW01 and MAC01. Largely unexpected, especially at the sites characterised by a 'sand' response (see chapter 2), this response was preserved even when the data was adjusted for variation in plant establishment.

Table 5.11: Predicted means, and the probability of Keel exceeding Mundah, for four agronomic traits in Mundah and Keel calculated from the MET analysis of nine site x year field trials.

	Mundah	Keel	Probability
Grain yield (t ha^{-1})	1.582	1.651	0.88
1000 grain weight (g)	47.56	42.92	0.00
Screenings (%)	12.73	16.23	0.99
Early dry matter production (g m^{-2})	50.78	57.08	0.99

Table 5.12: Means, and heritability (h^2) for four agronomic traits in Mundah (M), Keel (K) and their 95 RIL progeny. Data was analysed by MET analysis.

Site	Grain yield (t ha^{-1})						1000 grain weight (g)					
	M	K	RILs (mean)	RILs (min.)	RILs (max.)	h^2	M	K	RILs (mean)	RILs (min.)	RILs (max.)	h^2
CPL01	1.619	1.673	1.619	1.389	1.770	0.159	45.89	47.22	46.53	45.60	48.06	0.000
LOW00	1.440	1.480	1.431	1.327	1.545	0.392	49.18	41.70	44.53	38.88	50.20	0.886
LOW01	1.545	1.501	1.553	1.355	1.731	0.377	53.18	46.35	50.06	43.61	54.43	0.563
MAC99	1.695	1.802	1.702	1.224	1.346	0.568	48.52	41.41	44.25	37.43	50.35	0.800
MAC00	1.350	1.333	1.296	2.048	2.693	0.227	49.75	42.46	45.43	38.63	51.62	0.915
MAC01	2.376	2.277	2.394	1.338	1.964	0.498	48.99	43.29	46.45	37.11	49.90	0.889
SAN99	1.448	1.427	1.459	1.315	1.639	0.307	50.22	44.40	46.70	38.63	53.02	0.826
TUC99	0.937	1.106	1.014	1.719	1.936	0.396	41.69	34.76	37.88	40.46	45.55	0.701
TUC00	1.814	1.790	1.808	0.849	1.181	0.078	41.05	43.81	42.70	31.60	42.02	0.003

Site	Screenings (<2.5mm, %)						E_DMP (g m^{-2})					
	M	K	RILs (mean)	RILs (min.)	RILs (max.)	h^2	M	K	RILs (mean)	RILs (min.)	RILs (max.)	h^2
CPL01	13.12	12.70	12.92	11.24	13.56	0.000	24.76	26.41	25.38	24.20	27.14	0.001
LOW00	10.90	14.76	14.22	5.68	33.76	0.887	53.04	62.49	56.43	49.87	66.64	0.258
LOW01	3.76	7.34	4.84	2.77	9.81	0.783	19.88	20.78	19.73	18.34	21.84	0.189
MAC99	8.78	12.38	12.06	5.15	26.33	0.781	96.05	101.23	108.36	28.32	40.91	0.460
MAC00	8.39	17.55	11.62	4.29	30.92	0.863	29.54	33.51	33.91	19.81	19.89	0.207
MAC01	9.70	13.37	11.81	2.79	36.65	0.821	19.83	19.87	19.84	72.79	138.06	0.002
SAN99	11.41	12.49	12.64	3.51	54.13	0.941	54.25	91.04	65.77	37.52	99.08	0.681
TUC99	34.72	32.44	34.07	12.91	21.19	0.906	41.08	53.84	46.56	90.15	113.93	0.355
TUC00	19.59	18.10	18.64	15.34	67.65	0.000	100.82	102.00	101.78	38.76	62.15	0.234

Graphic representation of the deviation in grain yield from the site mean against site loadings determined from the MET analysis (see section 5.2.4.4) indicated a genotype x environment interaction for grain yield (Figure 5.3 and Table 5.12) despite the higher predicted mean of Keel (Table 5.11). Site loadings indicated that the varietal ranking for grain yield at SAN99 (site 5) most closely resembled the ranking at the 'average' site (Table 5.13). Mundah achieved a higher grain yield than Keel at SAN99 (site 5), LOW01 (site 6), MAC00 (site 7), MAC01 (site 8), and TUC00 (site 9), while the opposite was apparent at MAC99 (site 1), CPL01 (site 2), TUC99 (site 3) and LOW00 (site 4) (Figure 5.3). The deviations from the site means also revealed the stable adaptation response of Mundah across environments. Conversely, the adaptation response of Keel was noticeably influenced by prevailing environmental conditions.

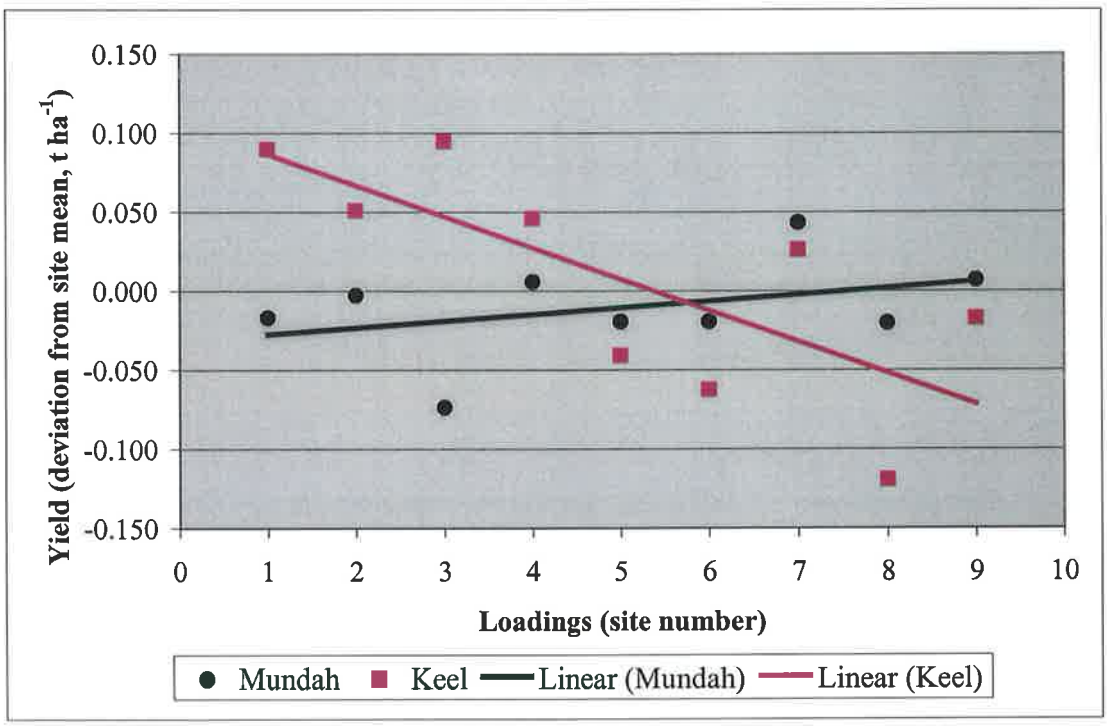


Figure 5.3: Deviation of grain yield from the site means for Mundah and Keel in the Mundah/Keel RIL population field trials, 1999-2001. Legend: 1-MAC99, 2-CPL01, 3- TUC99, 4- LOW00, 5- SAN99, 6- LOW01, 7- MAC00, 8- MAC01, 9-TUC00. Data analysed by MET analysis.

Table 5.13: Site loadings generated from the MET analysis of the Mundah/Keel RIL population at all sites

Trial Site	Site Number	Site Loading
Minnipa 1999	1	-0.976
Cooke Plains 2001	2	-0.958
Tuckey 1999	3	-0.500
Lowbank 2000	4	-0.269
Sandalwood 1999	5	-0.231
Lowbank 2001	6	-0.022
Minnipa 2000	7	0.098
Minnipa 2001	8	0.189
Tuckey 2000	9	0.908
Average		*-0.196*

Sites 5 to 9 were more typical of a sand response because the grain yield ranking of Mundah and Keel followed expectation for this environment, based on long term grain yield analysis of SARDI sand trials (Table 5.1). Seasonal rainfall at these sites was average to above average, and other environmental constraints (*e.g.* frost) were not evident.

In contrast, the grain yield at MAC99 (site 1) and TUC99 (site 3) was a response to moisture stress. Although rainfall during early grain filling was above average, seasonal rainfall at MAC99 (site 1) was below average, with the period between June and August particularly deficient. No seasonal averages were available for TUC99 (site 3), but the significant awn curling evident was likely to be a result of quite severe moisture stress. At both sites the distinct grain yield advantage of Keel over Mundah was likely to be related to developmental growth stage (Figure 5.4), and suggests that drought avoidance played a primary role. However, the grain yield response of the RILs points to some other factor(s) influencing response at these sites (Figure 5.4). The response of Keel in these experiments was not

unexpected since a similar result was observed in the variety comparison experiment at MAC99 (Chapter 2). Keel may also display superior drought stress properties via a higher fertile spikelet to primordia ratio (Coventry, *pers. comm.*) or other drought tolerance mechanisms.

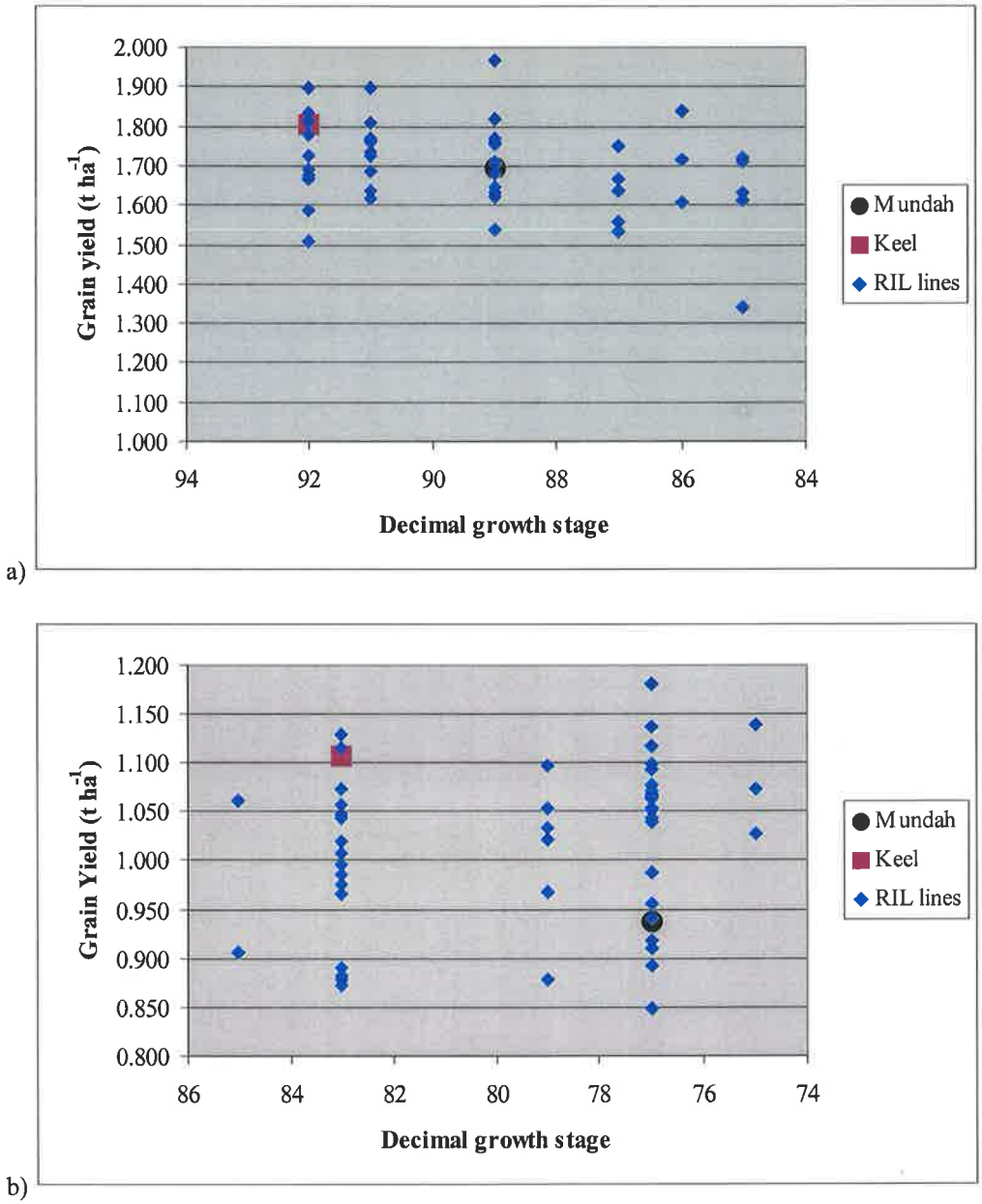


Figure 5.4: The association of grain yield with maturity at sites exhibiting moisture stress. a) MAC99 and b) TUC99.

Keel also out performed Mundah at CPL01 (site 2) where a significant frost event occurred on the 29th October 2001. An earlier flowering phenology was seemingly not a component of adaptation in this case. Rather Keel (3% damage) was marginally less affected by frost than Mundah (5% damage) (Reinheimer, *pers com.*). Across the entire population, the maximum physical damage scored was 10%. Rainfall was about average for this site, and with the minimal damage due to frost, suggests that some other unrecorded factor, possibly disease resistance, was responsible for the better yield potential of Keel. The conclusion that can be drawn, primarily from the observed variation in adaptation of Keel, is that the nature of sandy soils is such that a sand 'response' can easily be confounded by the prevailing environmental conditions.

Mundah had a higher 1000 grain weight and lower screenings percentage than Keel (Table 5.11). The probability that Keel would be superior to Mundah for 1000 grain weight was zero, but would consistently have higher screenings percentage on sandy soils (Table 5.11). Only at CPL01 did Keel display better grain weight and lower screenings than Mundah.

The histograms in Figure 5.5 for four agronomic traits indicate that the pattern of variation across the Mundah/Keel population was essentially normal and continuous. Figure 5.5 and the maximum and minimum values for the RIL progeny (Table 5.12) also highlight the existence of positive and negative transgressive segregants within the population. Transgressive segregants occur where the recombination of genes from a cross between different cultivars produces a number of progeny that receives either a more complete repertoire of 'favourable' genes than either parent (positive), or a combination of genes that confer inferior adaptation response than either parent (negative) (Paterson *et al.*, 1991). Accordingly, it is plausible that some progeny, or a single line, within the population may have received alleles favourable for sand adaptation from Mundah and favourable alleles,

including those conferring improved disease resistance, early maturity or drought tolerance, from Keel. The detection of the genes/QTL underlying this superior performance, however, relies on a high quality map with a dense coverage of markers. The nature of the linkage map devised from the Mundah/Keel RIL population makes the discovery of the genes/QTL for superior sand adaptation almost impossible. The inability to identify all possible QTL is discussed later.

The moderately low h^2 for grain yield and E_DMP observed (Table 5.12) implies that the variation in these traits in the population was predominantly due to environmental and/or extraneous factors (Snustad *et al.*, 1997). Although grain yield at MAC99 and MAC01, and E_DMP at MAC99, were equally influenced by genetic and environmental variances. Early dry matter production at CPL01 and MAC01 was entirely determined by environmental factors. Conversely, the variation in the 1000 grain weight and screenings data was predominantly attributable to genetic influences. Strong genetic effects were observed for grain weight and screenings at MAC00 and LOW00, for grain weight at MAC01, and for screenings at SAN99 and TUC00. However, at CPL01 and TUC00 the h^2 for all traits was zero. While at TUC00, the equivalent or better adaptation response of Keel for these two traits appeared to be a direct response to moisture stress and/or disease severity.

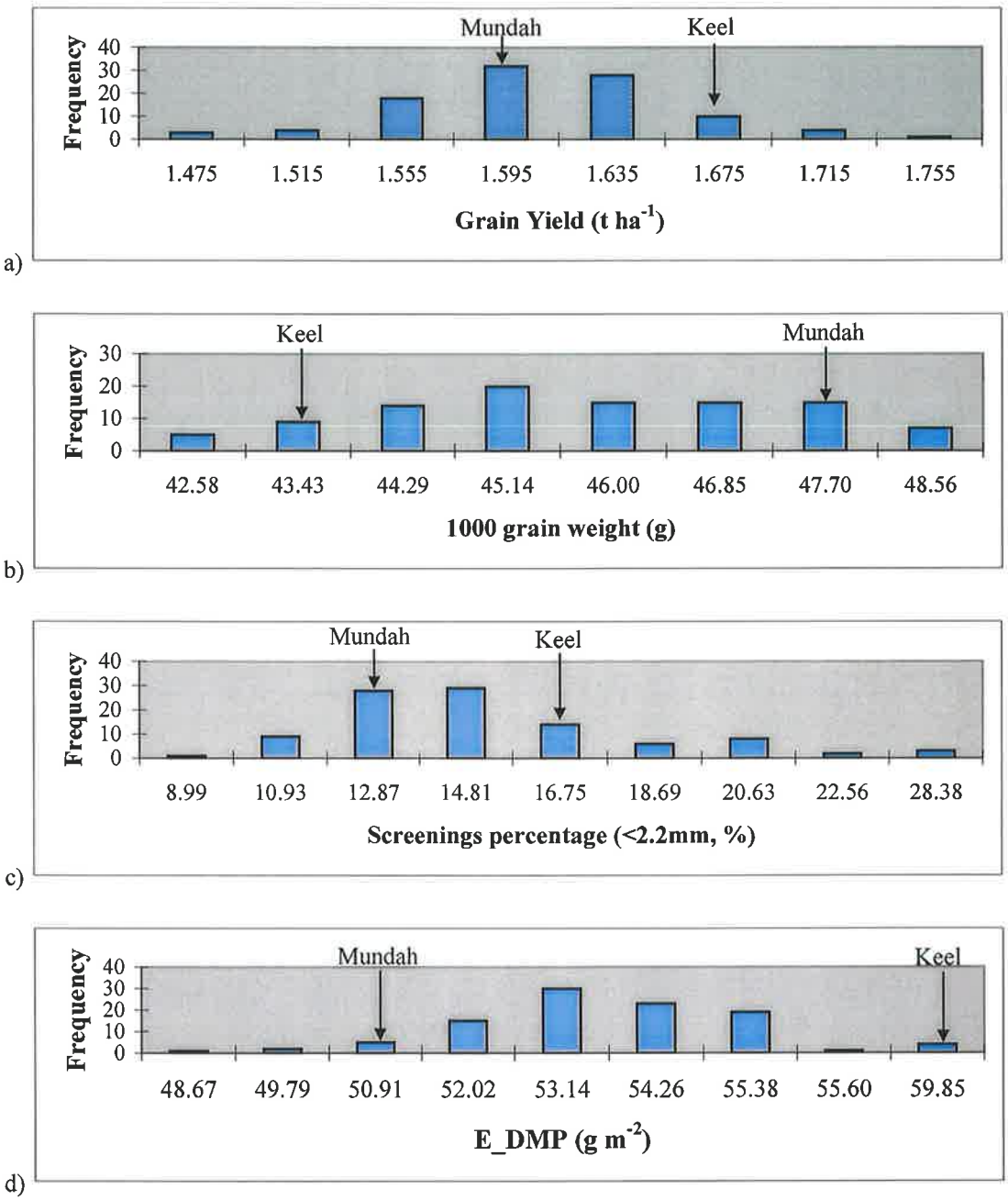


Figure 5.5: Histograms of spatially adjusted means for a) grain yield, b) 1000 grain weight, c) screenings percentage, and d) early dry matter production across all nine site x year trials for the Mundah/Keel population. Values for the parents (Mundah and Keel) are indicated with arrows.

5.3.3 QTL analysis: Identification of loci associated with traits conferring superior sand adaptation

5.3.3.1 Grain yield, 1000 grain weight and screenings percentage

The overall picture that emerged from the QTL analysis of the Mundah/Keel population is that the underlying genetic control of sand adaptation, in terms of grain yield, grain weight and screenings percentage, appears to predominately reside on chromosomes 1H and 2H (Figure 5.1).

Positive QTL for grain yield (Table 5.14) were identified on the long arm of chromosome 2H at *Xpsr540* (Mundah allele) and *Xebmac623* (Keel allele); on chromosome 1HL at *Xebmac501* (Keel allele); and on chromosome 4HS at *Xhvole* and *Xebmac906* (Keel alleles). None were expressed at more than one site, although two Keel alleles, at *Xebmac501* (1HL) and *Xebmac623* (2HL), were both significantly associated with grain yield at the same site (MAC01). The predicted size of the effect of these loci on grain yield (0.209 t ha^{-1} and 0.214 t ha^{-1} respectively) was approximately twice the contribution of the single positive Mundah allele on 2HL (0.114 t ha^{-1}). In addition these QTL were expressed at sites exhibiting a 'sand response' effect. Contrary to the generally moderately poor performance of Keel on sandy soils (see chapter 2), the QTL analysis of grain yield, and for grain weight and low screenings (discussed below), in addition to the superior yield potential of Keel under moisture stress on sand (see chapter 2 and section 5.3.2), seem to point to drought tolerance as a component of adaptation response on sand.

Two putative loci on chromosome 4HS were found to be associated with grain yield on sand (Table 5.13, Figure 5.2). Both positive yield alleles were contributed by Keel alleles, and were expressed at sites with an underlying 'drought response' effect (MAC99 and TUC99, see section 5.3.2).

Table 5.14: Chromosome location, marker(s), favourable allele, and the size of the effect of that allele associated with the expression of grain yield, 1000 grain weight and screenings (% < 2.5mm). 'Size of effect' describes the improvement in trait value *per se* associated with the marker.

Trait	Chr.	Marker	Favourable allele	Site	LOD	Size of effect
Grain yield	1H	*XEBmac501*	Keel	MAC01	3.57	0.209
	2H	*Xpsr540*	Mundah	LOW00	2.34	0.114
		XEBmac623	Keel	MAC01	3.32	0.214
	4H	*Xhvole*	Keel	MAC99	2.37	0.188
		XEBmac906	Keel	TUC99	2.49	0.148
1000 grain weight	1H	*XP13/M49-251*	Mundah	LOW01	2.23	1.52
			Mundah	MAC99	7.93	3.78
			Mundah	MAC00	5.44	2.52
			Mundah	MAC01	4.58	2.52
			Mundah	TUC99	4.28	2.93
			Keel	TUC00	2.49	2.54
			Mundah	SAN99	10.64	3.68
		Xawbma35	Mundah	LOW00	3.87	2.26
	2H	*Xebmac623*	Keel	LOW01	3.28	1.94
			Keel	MAC01	5.81	3.02
		Xebmac640	Keel	MAC00	3.98	2.17
			Keel	SAN99	7.82	3.14
		XP13/M49-174	Keel	SAN99	2.27	1.82
	5H	*Xcdo989*	Mundah	LOW00	2.88	2.46
			Mundah	LOW01	4.22	3.02
			Mundah	MAC99	2.11	2.45
			Mundah	MAC00	2.56	2.19
Reduced Screenings Percentage	1H	*Xawbma35*	Mundah	MAC99	2.08	7.10
			Mundah	MAC01	3.12	4.14
			Mundah	TUC99	4.69	14.59
	2H	*XP14/M47-189*	Keel	MAC99	2.80	8.42
			Keel	MAC01	8.09	6.31
		Xbmag350	Keel	LOW00	7.20	9.59
			Keel	LOW01	5.61	1.90
		Xebmac640	Keel	MAC00	3.23	5.29
			Keel	TUC99	3.07	10.53
	3H	*XP14/M61-279*	Mundah	TUC00	3.21	7.47

Significant and putative QTL for high 1000 grain weight (Table 5.14) were identified on chromosome 1HL (*XP13/M49-251* and *Xawbma35*), 2HL (*Xebmac623* and *Xebmac640*), 2HS (*XP13/M49-174*) and 5HS (*Xcdo989*). The favourable alleles at loci on chromosomes 1HL and 5HS were predominantly contributed by Mundah, and were associated with between 1.52 and 3.78 grams per 1000 grains higher grain weight than lines carrying Keel alleles at those loci. Keel contributed favourable alleles at three loci on 2H which were associated with between 1.82 and 3.14 grams superior grain weight per 1000 grains. The QTL analysis showed Keel alleles to be positive factors in grain weight at sites typical of a 'sand response', and one Mundah allele for improved grain weight (*XP13/M49-251* on 1HS) was expressed at sites with a 'sand response', and sites where moisture stress was an issue (MAC and TUC99)(Table 5.14).

A QTL x environment interaction was apparent for grain weight at *XP13/M49-251* on chromosome 1HL. The alternate parent (Keel allele) conferred improved grain weight at TUC00, while Mundah contributed the positive allele at TUC99, and at the other sites where this marker loci had a significant association with grain weight.

Two significant QTL for low screenings were identified at loci also significantly associated with grain weight (*e.g. Xawbma35* on chromosome 1HL and *Xebmac640* on chromosome 2HL). Only *Xebmac640* at MAC00 was associated with large grain weight and low screenings percentage at the same site. Additional markers found to be associated with low screenings included *Xbmag350* (2HL), *XP12/M47-189* (2HL) and *XP14/M61-279* (3HL). All loci contributed quite sizable effects on screenings percentage (Table 5.14).

QTL for grain yield, 1000 grain weight and low screenings detected on the long arm of chromosome 2H (Figure 5.1) in the Mundah/Keel population was also found to be associated with these traits in the NBMMP populations. Namely, grain yield and grain weight in the Alexis/Sloop mapping population, grain weight and screenings percentage in the Chebec/Harrington mapping population and grain weight in the Amagi Nijo/WI2585 mapping

population. The low screenings QTL at *XP13/M61-279* on chromosome 3HL also corresponded to the same region in the Alexis/Sloop population for grain weight, and the Arapiles/Franklin population for grain yield.

As discussed previously, the Mundah/Keel RIL population is of the type "adapted x adapted", unlike the majority of the NBMMP populations. Accordingly, this may favour the detection of QTL for yield *per se*, rather than revealing regions confounded by genes affecting phenology. However, many of the QTL for yield were coincident with candidate genes for photoperiod sensitivity and earliness *per se*. The QTL for grain yield, grain weight and low screenings, conferred by Keel alleles, mapped on the long arm of chromosome 2H (Figure 5.1) were in a region characterised by the earliness *per se* gene, *eps2s* (Laurie *et al.*, 1994). In addition, the QTL for grain weight on chromosome 2HS at *XP13/M49-174*, also conferred by the Keel allele, was in a region possibly influenced by the photoperiod sensitive gene, *Ppd-H1*, regulating flowering under long day length (Laurie *et al.*, 1995). This would seem reasonable because Keel is photoperiod sensitive (Barr, *pers comm.*), although to date, an association between Ppd-H1 and grain weight has not been shown in NBMMP mapping populations (Coventry *et al.*, 2003).

While it would seem that some yield QTL were coincident with regions regulating phenology, suggesting pleiotropic associations, the QTL on chromosomes 3HL (low screenings), 4HS (grain yield) and 5HS (1000 grain weight) did not appear to be linked with genes associated to phenology. The QTL for grain weight on Chromosome 1HL, controlled by Mundah alleles, was at loci also possibly associated with developmental growth stage (see section 5.3.3.2 regarding the validity of maturity loci), but was not coincident with putative genes for earliness.

5.3.3.2 Early vigour, maturity, growth habit and SFNB reaction

As discussed in section 5.3.2, Keel unexpectedly produced equivalent or greater biomass at early tillering to that of Mundah across all experiments. Hence, QTL analysis showed the favourable alleles for biomass at early tillering were conferred by Keel (Table 5.15). The QTL for early dry matter production were expressed on chromosomes 1HL, 2HS, 5HL and 7HS; the most significant was on 5HL at *Xabg391* (Figures 5.1 and 5.2).

In all field experiments of the Mundah/Keel population an equivalent weight of seed for each RIL was sown per plot. Consequently inherent (genetic) differences in seed size between the RILs will result in variation in plant density in yield plots. Therefore, QTL for E_DMP may reflect differences in plant density rather than real genetic differences in E_DMP. To account for this, establishment counts were carried out in conjunction with dry matter cuts at early tillering, and the data was adjusted for plant density. QTL analysis of E_DMP data adjusted for seed size was also performed because of the influence of seed size on establishment and early vigour (Chapter 4).

The putative QTL for unadjusted E_DMP data located at *Xbmac32* on chromosome 1HL was the only QTL not to be significantly associated with E_DMP after adjustment for plant density. The fact that adjusting for establishment had only a minimal impact on the QTL-marker associations suggests no effect of plant density on E_DMP; rather there was a real contribution of Keel alleles to E_DMP. Analysis of E_DMP adjusted for seed size presented new QTL for E_DMP on 2HS (*XP13/M49-174*), 3HL (*Xebmac708*) and 4HS (*Xgms89*). This approach to analysing the data resulted in the detection of a QTL for E_DMP, at *XP13/M49-174*, that was conferred by a Mundah allele. However the size of the effect on E_DMP at this locus was small compared to the other loci, all of which were characterised by Keel alleles (Table 5.15).

Table 5.15: Chromosome location, marker(s), favourable allele, and the size of the effect of that allele associated with the expression of early dry matter production, early vigour, growth habit, maturity and SFNB. 'Size of effect' describes the improvement in trait value *per se* associated with the marker.

Trait	Chr.	Marker	Favourable allele	Site	LOD	Size of effect
E_DMP	1H	*Xbmac32*	Keel	CPL01	2.09	4.90
	2H	*XP12/M50-322*	Keel	SAN99	4.17	21.04
	5H	*Xabg391*	Keel	SAN99	2.16	16.52
	7H	*Xebmag794*	Keel	MAC00	2.04	10.56
E_DMP (adjusted for establishment)	2H	*XP12/M50-322*	Keel	SAN99	2.45	15.86
	5H	*Xabg391*	Keel	SAN99	3.42	19.69
	7H	*Xebmag794*	Keel	MAC00	2.81	11.04
E_DMP (adjusted for seed size)	2H	*XP13/M49-174*	Mundah	MAC01	2.24	5.16
		XP12/M50-322	Keel	SAN99	2.79	15.94
	3H	*Xebmac708*	Keel	MAC00	2.48	12.46
	4H	*Xgms89*	Keel	SAN99	2.54	14.46
	7H	*Xebmag794*	Keel	SAN99	3.23	16.78
Early vigour	5H	*XP14/M51-238*	Keel	MAC01	2.03	0.552
Growth habit	1H	*XP13/M49-251*	Mundah	LOW00	2.20	0.406
			Mundah	TUC00	2.01	0.388
		Xawbma35	Mundah	MAC00	3.35	0.494
	3H	*XP13/M61-243*	Keel	CPL01	2.50	0.336
			Keel	MAC01	3.47	0.384
			Keel	TUC00	2.48	0.444
	4H	*Xhvole*	Keel	LOW00	3.54	0.587
	5H	*Xabg463*	Keel	LOW00	2.36	0.478
	7H	*Xpsr119*	Keel	MAC01	2.81	0.358
Early Maturity	1H	*XP13/M49-251*	Mundah	CPL01	5.11	5.58
		Xawbma35	Mundah	LOW00	2.70	3.54
			Mundah	MAC00	2.27	4.28
			Mundah	TUC00	2.10	5.41
	3H	*Xhvm60*	Keel	CPL01	2.30	3.97
	7H	**Xebmag794**	Keel	LOW00	4.56	5.07
Low SFNB symptoms	2H	*Xbmac134*	Keel	TUC00	1.14	
				PTW00	2.02	

The QTL for E_DMP (adjusted for seed size) detected on 3HL is of interest since Richards and Lukacs (2002) identified in wheat-barley addition lines, that barley chromosome addition line 3HL was associated with improved leaf area and dry weight, both measures of early vigour, over Chinese Spring wheat, although this chromosome was only determined to account for 25% of the difference between barley and wheat. They also found that greater SLA, another measure of early vigour, was associated with the short arm of barley chromosome 3H. The deficiency of markers mapped to chromosome 3HS in the Mundah/Keel population could account for the fact that no QTL for E_DMP were detected in this region. While it could be argued that seeding rate should have been modified according to a set plant density, and seed size, these results justify not adjusting seeding rate, for there was no real alteration to the QTL-marker associations when taking these two factors into account. Analysis of the early vigour scores also successfully detected a QTL on the long arm of chromosome 5H (*XP14/M51-238*) contributed by Keel, and unrelated to other significant QTL identified in this study.

The fact that only one QTL for E_DMP was detected with Mundah conferring the favourable allele highlights the poor quality of the linkage map and possibly the interaction between 'sand response' and 'drought response'. It is a very real prospect that further QTL conferred by Mundah alleles reside in regions not covered by molecular markers available here. While alleles conferred by Keel may contribute significantly and directly to improved E_DMP on sandy soils, it may be that the presence of Keel alleles associated with QTL for E_DMP relates to other components of these environments.

Some of the QTL for E_DMP, on chromosomes 2HS, 5HL and 7HS, and one QTL for early vigour on chromosome 5HL were associated with the regions carrying genes controlling earliness of flowering (*Ppd-H1* on chromosome 2HS, *eps5L* on chromosome 5HL and *eps7s* on chromosome 7HS). As with the QTL for yield, earliness genes may also be involved with regulating early growth in this population.

Putative QTL for maturity were identified on chromosomes 1HL (Mundah alleles at *XP13/M49-251* and *Xawbma35*), 3HL (Keel allele at *Xhvm60*), and 7HS (Keel allele at *Xebmag794*). Single marker regression also revealed possible loci associated with growth habit (Table 5.15). A note of caution with the QTL for maturity and growth habit is warranted, since the phenotypic scoring of these traits did not follow a normal distribution, an assumption of QTL analysis. In terms of maturity, the reason for this resides in the fact that maturity was scored based on the decimal growth stage method (Zadoks *et al.*, 1974). The vagaries of this method are such that scores for plant developmental stage are not continuous. In addition, logistics prevented the trials from being scored at a similar point in time, such that maturity at each site did not relate to the same developmental period. A better strategy may have been to score anthesis at all sites and relate maturity to the number of days after sowing. This method was discounted as a viable option, however, because the travel involved, and the locality of the trials, prevented the daily monitoring of each trial necessary to score anthesis for each line. For growth habit only three scores were possible (1 = prostrate, 2 = intermediate & 3 = erect), which is not enough to, with assurance, show that variation followed a normal distribution. For this reason it wasn't possible to fit any spatial effects or random genetic effects to the model. Accordingly, the QTL effects may be confounded with environmental effects (Eckermann, *pers. comm.*).

Single point analysis (Q-gene analytical package, Nelson 1997) of the SFNB scores at TUC00 and PTW00 intimated that a possible, although insignificant (LOD=1.14), QTL for low symptoms, conferred by the Keel allele, existed at *Xbmac134* on chromosome 2HS. In section 5.3.2 it was postulated that moisture stress and/or disease may have impacted to reduce 1000 grain weight and increase screenings losses of Mundah at TUC00, such that Keel performed better for these two agronomic traits. However, no yield QTL was detected at *Xbmac134*, and therefore it cannot be concluded that this locus was associated with the yield response in this environment.

5.4 Conclusions

The paucity and irregular density of mapped markers, and the large number of regions without any marker coverage, produced a linkage map of the Mundah/Keel RIL population that was not of a quality to sufficiently perform a detailed QTL analysis using the interval mapping method. Given the quality of the linkage map, the application of single regression analysis as per Eckermann *et al.* (2001), and the equivalent option in the Q-gene analytical package (Nelson, 1997), proved to be a valuable alternative in characterising loci significantly associated with traits conferring sand adaptation.

Chromosome regions (QTL) were found to be associated with traits related to sand adaptation. Major loci associated with grain size and growth habit regulated by Mundah alleles were detected at *XP13/M49-251* and *Xawbma35* on the long arm of chromosome 1H. Other contributing loci were identified on chromosomes 2HL (higher grain yield), 3HL (lower screenings) and 5HS (higher grain weight). Loci characterised by Keel contributing the favourable allele were expressed at sites affected by moisture stress (MAC99 and TUC99), but also at sites characterised by a sand response. It has also been demonstrated that Keel carries QTL associated with agronomic traits that can contribute to sand adaptation. In addition, Mundah alleles for low screenings and higher grain weight emerged at drought affected sites, implicating some degree of moisture stress tolerance can be attributed to Mundah. This QTL study has therefore identified drought tolerance is an intrinsic component of sand adaptation, but it was very site specific. At some site/season trials, some drought tolerance QTL contributed more to yield, and in general, both parents contributed valuable traits to adaptation. Further evidence for this is the observation of positive "transgressive segregants" for grain yield at many site/season experiments (Table 5.12, Figure 5.5).

Despite the advantage of having a population constructed from two agronomically adapted parents, many of the QTL for grain yield, grain weight and lower screenings, were coincident with major phenological genes. Although, possible QTL for yield *per se* were associated with

loci on chromosome 3HL (*XP14/M61-279*, Mundah allele), chromosome 4H (*Xebmac906* and *Xhvole*, Keel alleles), and chromosome 5HS (*Xcdo989*, Mundah allele) that were unrelated to loci controlling phenology.

While the significance of most of the marker-trait associations is not in dispute, the nature of the linkage map, arising from the genetic similarity of the parents, raises several issues. Firstly, other chromosomal regions may have an association with the agronomic traits assessed that are significantly greater than any of the marker-trait associations discussed in sections 5.3.3.1 and 5.3.3.2. However, the low level of polymorphism for the markers screened against the parents and their RIL progeny resulted in a linkage map of poor quality, with insufficient markers, to be able to detect alternate loci with significantly better association with traits for sand adaptation. The other important issue of concern is the position of the markers on the linkage map. The minimal number of polymorphic markers available to construct the linkage map necessitated the use of the barley consensus map (Karakousis *et al.*, 2003) as a template to position the 'linkage blocks' and single markers on the Mundah/Keel map, and it cannot be assumed that the agreed positions of markers were accurate. Only the construction of a linkage map solely determined from recombination data of a sufficient number of markers will provide the true genetic position of markers. However they can be, with confidence, associated with the chromosomes detailed, for the reason that the SSRs and RFLPs used have been anchored to specific chromosomes. In addition, the QTLs mapped in the Mundah/Keel population also map to similar regions in other mapping populations. Both issues highlight the importance of screening large numbers of molecular markers for map construction purposes, and validating marker-trait associations detected from a genetic map such as this, to confirm linkage, before the markers can be implemented in marker assisted selection.

The presence of Keel alleles associated with QTL for E_DMP in this study are likely to be an artifact of the phenotyping environment, rather than QTL for sand adaptation, and highlights

the limited scope of the genotype by environment study of the Mundah/Keel population, due to the time constraints of a Ph.D. study. In order to obtain a more complete data set to identify QTL for sand adaptation, the population needs to be evaluated over more sites and years to reduce the risk of incorrectly ascribing QTL due to specific QTL by year and QTL by location interactions.

The effect that 'genetically similar' parents have on constructing an appropriate linkage map has also been highlighted. Either an extremely large number of molecular markers need to be screened, to obtain a satisfactory number for mapping, or parental selection should be more carefully considered, such that they are still divergent for the trait(s) of interest, but be sufficiently different genetically to avoid the problem of low polymorphism. Ultimately, for the Mundah/Keel linkage map to be of broader application, more markers need to be screened and integrated to improve the current linkage map to the point where alternative and possibly more significant marker-trait associations are detected, and the map location of QTL can be better defined.

Chapter 6. Identification and evaluation of current breeding material and alternate germplasm for adaptation on sandy soils

6.1 Introduction

The domestication, and ongoing selection and breeding of cultivated barley (*Hordeum vulgare* L.) has, over time, reduced the genetic variance in the cultivated barley germplasm pool. Genetic variation within Australian barley breeding germplasm for traits associated with adaptation to sandy soils is limited, because the objectives of breeding programs have historically focused on selection for improved malting quality. Accordingly, germplasm introduction into Australia has generally been limited to material of superior malting quality principally from Europe, Canada and Japan. However this germplasm is not agronomically suited to some of the soil types and growing conditions of southern Australia.

In recent times the barley breeders from the South Australian Barley Improvement Program (SABIP) have been seeking to improve genotypic variation for agronomically important traits, including adaptation to biotic and abiotic stresses, by introducing and utilising landrace varieties from centers of origin and diversity such as the fertile crescent, and the wild progenitor of barley (*Hordeum spontaneum* sp. Koch). Various authors have provided some evidence of the potential and realized value of both landraces and *Hordeum spontaneum* to the genetic improvement of cultivated varieties. Examples include genetic variation for root morphology (Brown *et al.*, 1987; Cooper *et al.*, 1987; Grando and Ceccarelli, 1995), early vigour (Ceccarelli, 1987; Cooper *et al.*, 1987; Acevedo *et al.*, 1991; Wacker *et al.*, 2002) and disease resistance (van Leur *et al.*, 1989; Jana and Nevo, 1991; Abbott et al., 1992; Ivandic *et al.*, 1998). Grain yield potential in limiting environments, phenotypic variation for drought tolerance *per se* and mechanisms of drought tolerance within wild barley and landrace types have been extensively investigated (Ceccarelli and Grando, 1991; Hadjichristodoulou, 1993;

Ivandic *et al.*, 2000; Eglinton *et al.*, 2000). Further, progress towards the identification of novel alleles from wild and landrace barley has been enhanced by the development of mapping populations, between landrace barley and ICARDA breeding lines, to detect molecular markers associated with drought tolerance traits (Eglinton *et al.*, 2001).

Access to this genetic resource provides the potential to improve Australian breeding germplasm for traits important in reducing the impact of agronomic problems such as soils with inherently poor properties (*e.g.* sandy soils). Yagan, an introduction from the International Maize and Wheat Improvement Center (CIMMYT) of unknown parentage (Blakely Paynter *pers. comm.*), is a prime example, in this case, of the direct benefit of novel germplasm to agronomic improvement for abiotic stress tolerance in Australia. Yagan was directly introduced into Western Australia, and selected for its superior grain yield potential on sandy soils. In turn, Yagan has been utilised to incorporate 'sand adaptation' into new and improved cultivars, the most significant to date being Mundah.

Breeding material from the barley breeding program at the International Centre for Agricultural Research in Dry Areas (ICARDA) in Syria was introduced to Australia to identify alternate germplasm with potentially novel genes for early vigour and grain yield in low rainfall areas. These lines have been specifically bred with adaptation to low yielding environments from diverse germplasm (*e.g.* landraces and wild barley) sourced from a range of habitats throughout of the centres of origin of barley (*e.g.* The Fertile Crescent and North Africa), and material from breeding programs in other countries (*e.g.* Australia) that target dryland agricultural environments.

Lines from this pool of material may provide an alternative resource to improve genetic variation and adaptation on sandy soils within current breeding germplasm.

6.2 Methods and Materials

6.2.1 Populations and Breeder Lines

In the first year (2000) 221 lines selected in low rainfall environments by the Barley Breeding Program at ICARDA, previously imported by SABIP for the identification of alternate germplasm for drought tolerance, were evaluated for growth and grain yield on sandy soils. Following the trial in 2000, the 14 highest yielding lines, that were also 2-row types and had an appropriate developmental pattern (spring types with early maturity), were selected for evaluation in 2001, along with 7 advanced breeders lines from SABIP. A list of the lines evaluated in trials in 2001, and their pedigree information, is provided in table 6.1.

6.2.2 Field Experiments

6.2.2.1 2000

In 2000, the full complement of 221 lines was sown at Tuckey on the Eyre Peninsula of SA as an unreplicated field trial with augmented grids (Appendix 1). Check lines were established Australian varieties of varying adaptation on sandy soils such as Mundah, Forrest, Keel, Schooner, Clipper and Franklin. Plot area at sowing was $4.1m^2$, before being reduced to $3.9m^2$ for harvest. Further site information such as sowing date, fertilizer rates, harvest date, soil type and April-October rainfall are listed in table 6.2.

6.2.2.2 2001

Fourteen ICARDA entries selected from the trial at Tuckey in 2000 were included in the 2001 trials along with seven SABIP advanced breeding lines (Table 6.1). The 2001 entries were sown as a randomized complete block design (RCBD) of 2 replicates, randomised using a spatial design in the SpaDes® package (Coombes, 1999), at Minnipa, Darke Peak and Cooke Plains (Appendix 1). Lines were sown into plots of area $10.5m^2$ before being reduced to 7.5

m^2 for harvest. Further site information such as sowing date, fertilizer rates, harvest date, soil type and April-October rainfall are listed in table 6.2.

Table 6.1: List of ICARDA and SABIP breeding lines evaluated in 2001.

Line Designation	Source	Pedigree
ICARDA#31	ICARDA	ER/Apm//Lignee 131/3/Lignee 131/Arabi Abiad
ICARDA#53	ICARDA	WI2269/Line 251-11-2/5/11012 2/Impala//Birence/3/ Arabi Abiad/4/5604/1025
ICARDA#54	ICARDA	Unknown
INTER SPRING BARLEY YIELD-LRA (M)-7	ICARDA	Hml-02/Arabi Abiad//ER/Apm
INTER SPRING BARLEY YIELD-LRA (M)-9	ICARDA	WI2269//WI2197/Cam
ISBON--LRA-M-1	ICARDA	Harmal
ISBON--LRA-M-107	ICARDA	7028/2759/3/69-82//Ds/Apro/5/WI2291/4/11012-2/70-22425/3/Apm/IB65//A16
ISBON--LRA-M-109	ICARDA	WI2197/Cam/3/OP/Zy//Alger/Union 385-2-2
ISBON--LRA-M-65	ICARDA	BF891M-597/3/ER/Apm//Lignee 131
ISBON--LRA-M-84	ICARDA	Lth/3/Nopal//Pro/11012-2/4/Antares//12201/Attika/3/ RM1508/Por//WI2269
ISBYT-LRA(C) 96-97-15	ICARDA	Hml-02/Line 251-11-2
ISBYT-LRA(C) 96-97-16	ICARDA	Hml/Lignee 131
ISBYT-LRA(C) 96-97-6	ICARDA	Lignee 131
PARENT#5	ICARDA	WI2269/Line 251-11-2
WI3297	SABIP	WA-84SM:550 314.309
WI3386	SABIP	(WI2875-2/Mundah)/Barque
WI3453	SABIP	(WI2875-2/Mundah)/Barque
WI3630	SABIP	WI2978/Mundah-30
WI3653	SABIP	Forrest/Chariot/2/VB9624-48
WI3654	SABIP	Forrest/Chariot/2/Keel-15
WI3667	SABIP	Forrest/Chariot/2/VB9624-24

Table 6.2: Site Details-Sowing date, fertilizer rates, harvest date, soil type and April-October rainfall.

	2000	2001		
	Tuckey	**Minnipa**	**Darke Peake**	**Cooke Plains**
Sowing Date	25th May	31st May	7th June	1st June
Fertilizer Rate	130 kg/ha of 13:15	75kg/ha of 17:19 (5% Zn)	100 kg/ha of 17:19 (5% Zn	98 kg/ha of 17:19 (5% Zn)
Harvest Date	17th December	22nd November	10th December	3rd January 2002
Soil Type	Sandy loam(10cm) over loamy clay over clay	Sandy loam	Sand (57cm) over calcareous clayey sand	Sandy loam
April-October Rainfall (mm)	302	240	270	267

6.2.3 Measurements

Evaluation of the ICARDA and SABIP germplasm concentrated on a subset of important characteristics. These included establishment (no. of plants/m^2), growth habit (prostrate plant type verses erect plant type, scored prostrate, intermediate, erect), early vigour (low vigour verses high vigour, scored 1-9), relative developmental stage (Zadoks *et al.*, 1974), and grain yield (t ha^{-1}), 1000 grain weight (g) and screenings percentage (proportion of grain below 2.5mm, %).

6.2.4 Statistical Analysis

A moving mean analysis, using the row x column method (Genstat® for Windows, 5th edition), was employed to spatially analyse early vigour and grain yield in order to rank lines for selection into the 2001 field trials. Field trial data for 2001 was analysed by a linear mixed model analysis using residual maximum likelihood (REML) (Patterson and Thompson, 1971). Analysis was performed using Genstat Statistical software (Genstat® for Windows™, 5th edition, Lawes Agricultural Trust). REML produces Wald statistics to test the significance of fixed (treatment) effects. Early dry matter production (early vigour), 1000 grain weight, screenings and grain yield were also analysed using a MET (multi-environment trial)

statistical analysis (Cullis *et al.*, 1998, Smith *et al.*, 2001). The analyses employ spatial techniques to adjust the means of data to accommodate variability across a field trial site (soil depth, fertility) and also variability due to cultural practices (*e.g.* harvesting in two directions). MET analysis adjusts across site means to accommodate differences in genetic and environmental variance between sites, and provide information on the environmental stability of varieties and the heritability (h^2) of the traits analysed. The MET analysis was conducted using ASREML (Gilmour *et al.*, 1999).

The application of least significant differences (LSDs) to compare varieties against control varieties is unsuitable when data is analysed using MET data (Smith *et al.*, 2001). Instead, the predicted mean of traits for each variety at the 'average' site are interpreted on the basis of the probability that the variety is truly superior to the control (Mundah in these experiments) for that trait (Smith *et al.*, 2001). The calculation of the 'average' site is dependent on the sites used in the analysis. The MET analysis generates loadings for each site that describes the weight of each sites contribution in calculating the performance ranking of varieties for any trait (*e.g.* grain yield) at the 'average' site. Site loadings also illustrate the correlation of each site with the 'average' site and the correlation between sites used in the analysis, and accommodates the environmental similarities and differences between sites (Smith *et al.*, 2001). Accordingly, site loadings can be utilized to graphically represent site environments, and the environmental effects (*i.e.* genotype x environment interaction) on traits at each site. Figures 6.1 and 6.2 present the grain yield response of SABIP and ICARDA germplasm relative to Mundah on this basis.

6.3 Results

6.3.1 2000

Table 6.3 provides details of the top 14 ICARDA lines advanced into the 2001 trials. All were two-row types, and with the exception of ISBYT-LRA(C) 96-97-6, lines displayed mid to early maturity. The grain yield of Mundah was relatively poor in this season, and was exceeded by Keel. All the ICARDA lines yielded above Mundah, and four were superior to Keel. The majority of lines were of an erect growth habit, with varying early vigour scores, which appeared to be unrelated to potential yielding ability. Most of the 14 ICARDA lines selected were tall to very tall which is a disadvantage, because the risk of lodging is increased. Reduced grain yield and disease can become an issue, but this was not a problem at Tuckey in 2000.

6.3.2 2001

The 14 ICARDA lines and seven SABIP lines were assessed for growth habit, early vigour, maturity, grain yield, 1000 grain weight and screenings percentage. Genetic correlations of early vigour, grain yield, 1000 grain weight and screenings percentage, determined from the MET analysis, indicated that the ranking of varieties for every trait was highly correlated between all sites (Table 6.4). This was particularly evident for grain yield at Minnipa and Darke Peak, which produced identical rankings. The high genetic correlations may also reflect a minimal impact of genotype x environment effects on all traits in 2001 and the small number of lines assessed (14).

Table 6.3: Growth habit, height, early vigour, maturity and grain yield of the ICARDA lines advanced into 2001 experiments, at Tuckey in 2000. Data analysed by moving mean analysis.

Line	Growth Habit	Height	Early Vigour	Maturity	Grain Yield (t/ha)
ICARDA#31	prostrate	medium tall	4.9	early-mid	1.74
ICARDA#53	erect	very tall	3.5	early	1.91
ICARDA#54	erect	very tall	6.2	very early	1.99
INTER SPRING BARLEY YIELD-LRA (M)-7	erect	tall	8.3	early	1.62
INTER SPRING BARLEY YIELD-LRA (M)-9	erect	tall	4.8	early-mid	1.62
ISBON--LRA-M-1	erect	tall	4.2	early	1.65
ISBON--LRA-M-107	prostrate	tall	7.6	early-mid	2.36
ISBON--LRA-M-109	erect	tall	4.6	mid	1.84
ISBON--LRA-M-65	erect	tall	6.0	early-mid	1.76
ISBON--LRA-M-84	erect	tall	4.0	early-mid	1.89
ISBYT-LRA(C) 96-97-15	prostrate	very tall	6.7	early	1.98
ISBYT-LRA(C) 96-97-16	erect	tall	6.0	mid	1.73
ISBYT-LRA(C) 96-97-6	prostrate	medium	4.0	mid-late	1.50
PARENT#5	erect	tall	6.1	?	1.80
Mundah	erect	medium	6.0	early	1.48
Keel	intermediate	Short-medium	5.6	early	1.85
Site Mean			*5.5*		*1.14*

Table 6.4: Genetic correlations between sites in 2001 for early vigour, grain yield, 1000 grain weight and screenings percentage. Data analysed by MET analysis.

Site	Early Vigour			Grain Yield (t/ha)			1000 grain weight			Screenings percentage		
	Minnipa	Darke Peak	Cooke Plains	Minnipa	Darke Peak	Cooke Plains	Minnipa	Darke Peak	Cooke Plains	Minnipa	Darke Peak	Cooke Plains
Minnipa	1.000			1.000			1.000			1.000		
Darke Peak	0.908	1.000		1.000	1.000		0.950	1.000		0.722	1.000	
Cooke Plains	0.931	0.975	1.000	0.797	0.797	1.000	0.898	0.908	1.000	0.722	0.751	1.000

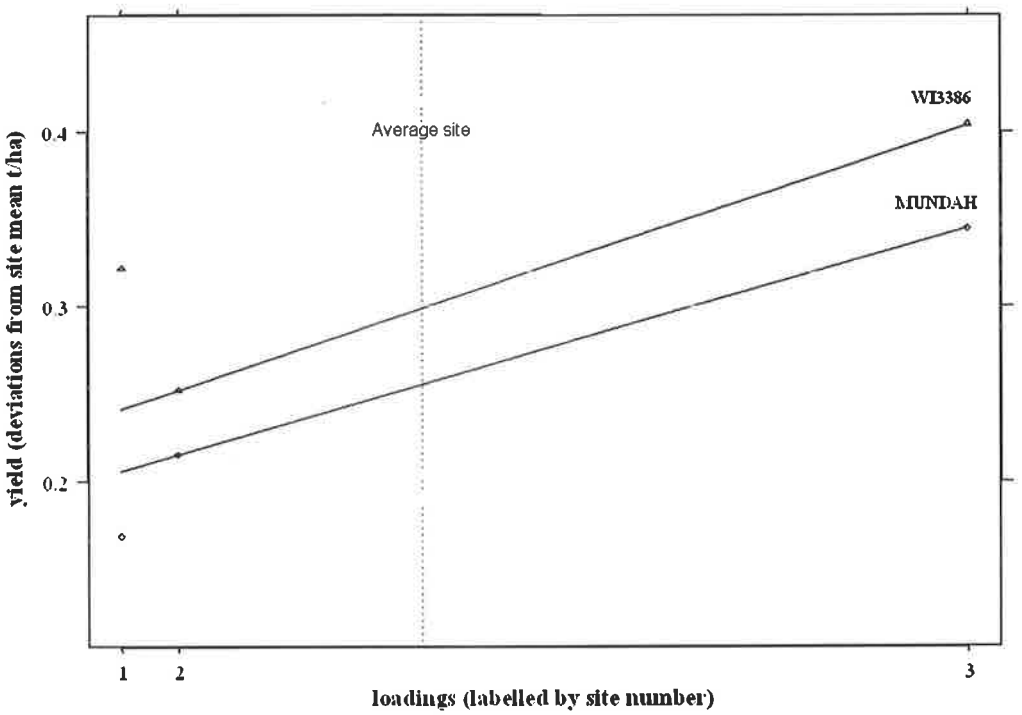
6.3.2.1 SABIP breeders' lines

The noticeable feature of the SABIP lines was not one cultivar was superior to Mundah for all traits on sandy soils, even those containing Mundah as a parent in the cross. The two feed types, WI3386 (WI2875-2/Mundah/2/Barque) and WI3453 (WI2875-2/Mundah/2/Barque) were, overall, the most prominent lines, in terms of grain yield (Table 6.5). Both averaged 2% better than Mundah and ranked first and second, respectively, at each site. The grain yield advantage of WI3386 and WI3453 was maintained at Minnipa despite greater head loss (24 heads/m^2 and 48 heads/m^2 respectively) compared to Mundah (12 heads/ m^2). While there was no genotype by environment (GxE) interaction for grain yield (Figures 6.1a and 6.1b), WI3386 performed best relative to Mundah at Cooke Plains. Both lines were inferior to Mundah for 1000 grain weight and had higher screenings percentage. WI3386 and WI3453 displayed early vigour scores comparable to Mundah in these trials, exhibited an erect growth habit, and were slightly later maturing than Mundah. MET analysis confirmed that WI3386 and WI3453 had a low probability of matching or exceeding Mundah in terms of grain weight and early vigour, but would consistently have higher screenings percentage (Table 6.6). The other SABIP lines evaluated were all inferior to Mundah, except for the screenings percentage of WI3667 (Forrest/Chariot/2/VB9624-24). WI3667 had 10% less screenings than Mundah and was estimated to have a very low probability of producing screenings losses above Mundah on sand (Table 6.6).

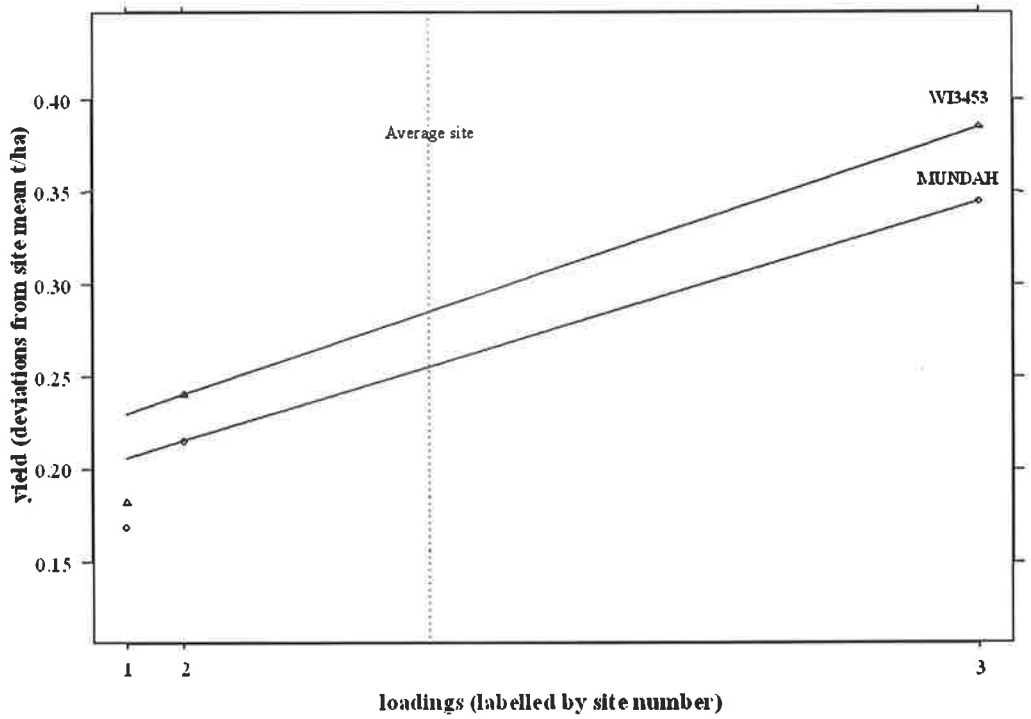
Table 6.5: Spatially adjusted means for early vigour, grain yield, 1000 grain weight and screenings in 2001. Data analysed by MET analysis.

Line	Growth Habit	Maturity	Early Vigour*	Grain Yield (t/ha)	1000 grain weight	Screenings percentage
ICARDA#31	intermediate	early-mid	4.0	1.59	48.91	5.25
ICARDA#53	erect	early-mid to early	4.4	1.31	45.81	6.70
ICARDA#54	erect	early	4.4	1.40	47.32	6.59
INTER SPRING BARLEY YIELD-LRA (M)-7	intermediate	early	4.4	1.21	52.00	6.08
INTER SPRING BARLEY YIELD-LRA (M)-9	erect	early-mid	4.7	1.49	47.88	6.47
ISBON--LRA-M-1	intermediate/ erect	early	4.1	1.20	46.13	10.92
ISBON--LRA-M-107	erect	early-mid	4.5	1.36	45.17	11.67
ISBON--LRA-M-109	intermediate	mid-late	3.8	1.56	45.95	10.46
ISBON--LRA-M-65	intermediate	early	4.2	1.30	44.58	10.18
ISBON--LRA-M-84	erect	early	3.8	1.25	44.46	9.71
ISBYT-LRA(C) 96-97-15	intermediate	early-mid to early	4.7	1.60	46.39	4.49
ISBYT-LRA(C) 96-97-16	intermediate	early-mid to early	4.4	1.56	42.93	20.08
ISBYT-LRA(C) 96-97-6	prostrate	very late	3.4	1.15	44.43	9.93
PARENT#5	intermediate/ erect	early-mid to early	4.5	1.25	40.53	19.74
WI3297	intermediate/ erect	mid	4.3	1.56	46.36	5.71
WI3386	erect	early-mid	4.8	1.81	44.34	9.63
WI3453	intermediate/ erect	mid	4.7	1.80	39.40	9.40
WI3630	erect	mid	4.3	1.68	44.48	17.84
WI3653	intermediate	mid	4.2	1.76	45.38	10.79
WI3654	intermediate	mid	4.5	1.68	40.21	19.11
WI3667	intermediate/ erect	mid-late	4.2	1.57	45.95	5.15
Mundah	erect	early	4.9	1.77	48.02	5.69
Keel	intermediate	early	4.4	1.55	49.41	4.83
Barque	intermediate	early-mid	4.0	1.73	48.45	10.64
Sloop	erect	mid	4.4	1.73	42.20	10.51
Galleon	prostrate	mid	3.5	1.44	40.77	5.25

*includes scores from Tuckey 2000. The WI lines, and Barque, Galleon and Sloop were not evaluated in 2000



a)



b)

Figure 6.1. Grain yield (deviation from site mean, t/ha) comparisons between a) WI3386 and Mundah, and b) WI3453 and Mundah across all sites in 2001. Data analysed by MET analysis. (Key: 1=Cooke Plains, 2=Darke Peak, and 3=Minnipa).

Table 6.6: Probabilities, determined from MET analysis, of ICARDA and SABIP lines exceeding Mundah for early vigour, grain yield, 1000 grain weight and screenings percentage.

Line	Early Vigour*	Grain Yield (t/ha)	1000 grain weight	Screenings percentage
ICARDA#31	0.00	0.07	0.87	0.42
ICARDA#53	0.08	0.00	0.00	0.68
ICARDA#54	0.04	0.00	0.21	0.66
INTER SPRING BARLEY YIELD-LRA (M)-7	0.04	0.00	1.00	0.57
INTER SPRING BARLEY YIELD-LRA (M)-9	0.17	0.01	0.43	0.65
ISBON--LRA-M-1	0.00	0.00	0.01	1.00
ISBON--LRA-M-107	0.10	0.00	0.00	1.00
ISBON--LRA-M-109	0.00	0.11	0.01	0.98
ISBON--LRA-M-65	0.01	0.00	0.00	0.99
ISBON--LRA-M-84	0.00	0.00	0.00	0.97
ISBYT-LRA(C) 96-97-15	0.19	0.07	0.02	0.28
ISBYT-LRA(C) 96-97-16	0.02	0.03	0.00	1.00
ISBYT-LRA(C) 96-97-6	0.00	0.00	0.00	0.96
PARENT#5	0.10	0.00	0.00	1.00
WI3297	0.03	0.07	0.02	0.50
WI3386	0.35	0.65	0.00	0.97
WI3453	0.30	0.58	0.00	0.96
WI3630	0.02	0.22	0.00	1.00
WI3653	0.02	0.48	0.00	0.99
WI3654	0.10	0.23	0.00	1.00
WI3667	0.01	0.04	0.00	0.40

*includes Tuckey 2000. The WI lines were not evaluated in 2000

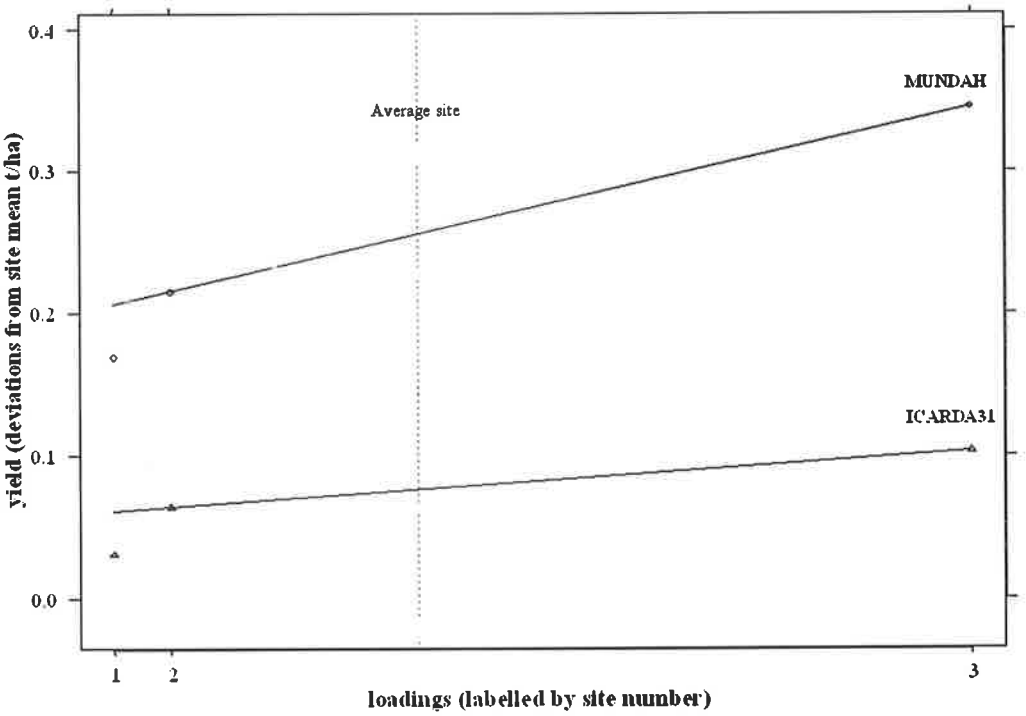
6.3.2.2 ICARDA germplasm

Nine lines of the ICARDA germplasm were inferior to all control varieties, including Keel and Galleon, which are considered moderately poor and poorly adapted to sandy soils

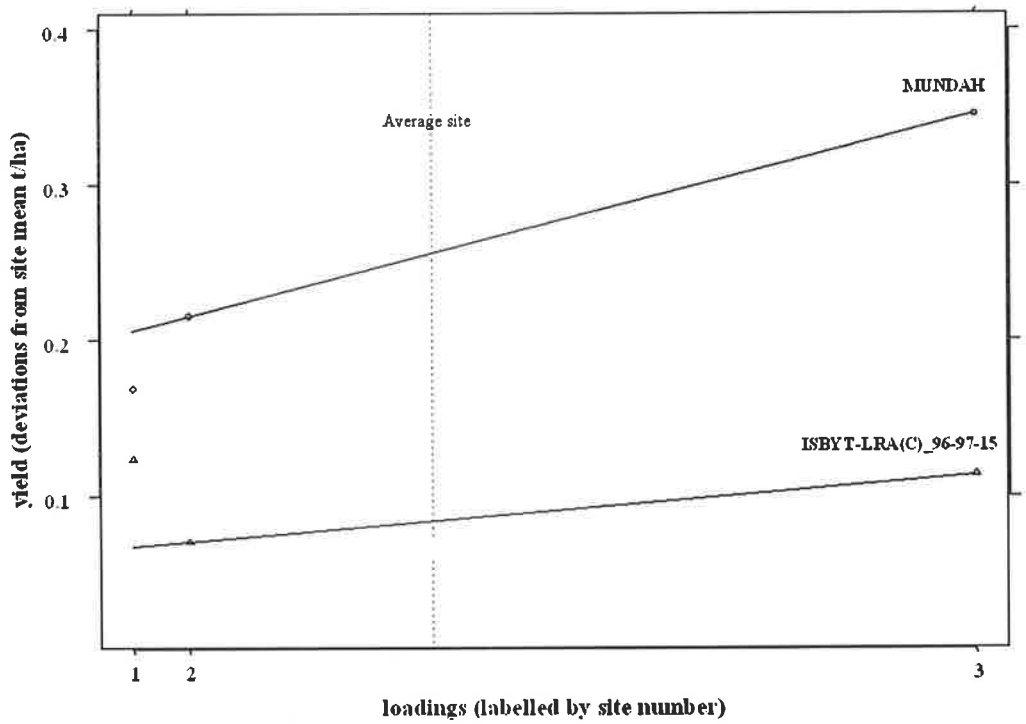
respectively (Table 6.5). The best performing ICARDA lines were ICARDA#31 and ISBYT-LRA(C) 96-97-15. Both lines were better than Keel for grain yield, but a lower 1000 grain weight may suggest an inferior capacity to adequately fill grain post-anthesis under the environmental and soil conditions specific to these field trials. Conversely, ICARDA#31 and ISBYT-LRA(C) 96-97-15 may simply have inherently lower grain weight.

Neither line was comparable to Mundah for grain yield on sand (Table 6.5). In addition, probability estimations from MET analysis of early vigour, grain yield, 1000 grain weight and screenings percentage suggested these lines would not exceed Mundah for adaptation in these environments (Table 6.6). A moderate GxE interaction was apparent relative to Mundah, evident from the diverging lines in Figures 6.2a-b (data analysed by MET analysis), although the varietal rankings did not vary between sites. ICARDA#31 maintained a relatively stable response across environments, compared to ISBYT-LRA(C) 96-97-15 (Figure 6.2). ISBYT-LRA(C) 96-97-15 performed best relative to Mundah at Cooke Plains, but both lines were particularly inferior to Mundah at MAC01. Head loss may have contributed to the poor grain yield of ISBYT-LRA(C) 96-97-15 (36 heads m^{-2} *cf.* 12 heads m^{-2} for Mundah) at Minnipa. However, these scores relate to the observation of one replicate only, and therefore the effect of head loss on grain yield can only be surmised and not statistically confirmed. ICARDA#31 produced larger grains and fewer screenings than Mundah (Table 6.5 and 6.6). Conversely, ISBYT-LRA(C) 96-97-15 had lower 1000 grain weight, but fewer screenings (Table 6.5 and 6.6).

ICARDA#31 flowered slightly later than Mundah, exhibited an intermediate growth habit, and was slightly inferior for early vigour (Table 6.5). ISBYT-LRA(C) 96-97-15 flowered at the same time as Mundah, had an equivalent early vigour and displayed an intermediate growth habit. Both ICARDA#31, ISBYT-LRA(C) 96-97-15 were taller than Mundah (data not shown).



a)



b)

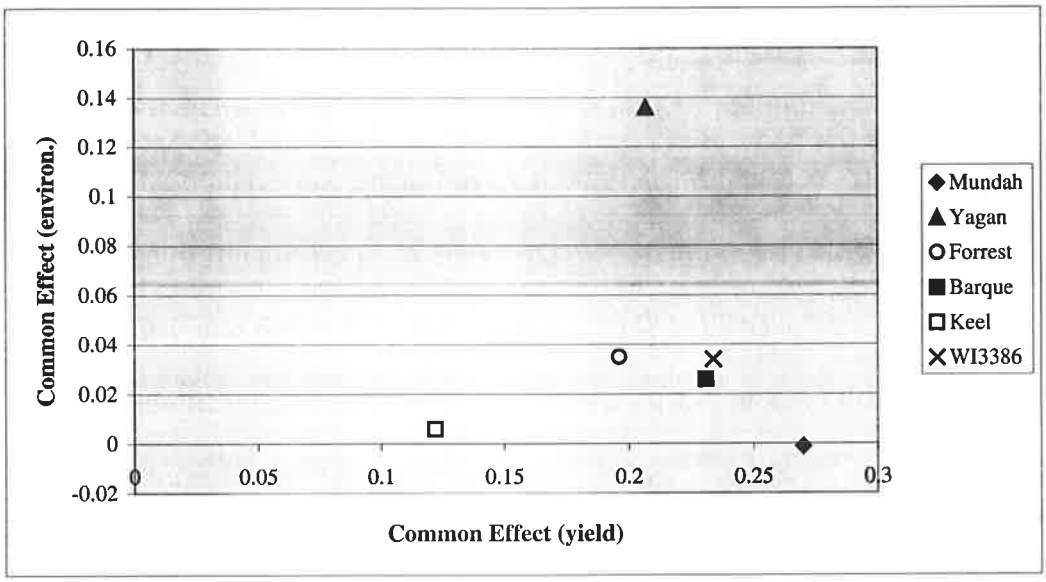
Figure 6.2. Grain yield (deviation from site mean, t/ha) comparisons between a) ICARDA#31 and Mundah, and b) ISBYT-LRA(C) 96-97-15 and Mundah across all sites in 2001. Data analysed by MET analysis. (Key: 1=Cooke Plains, 2=Darke Peak, and 3=Minnipa).

6.4 Discussion

6.4.1 SABIP Germplasm

The constraints of a Ph.D. study only allowed for a small-scale evaluation of SABIP germplasm and the available germplasm from ICARDA. However, two lines were identified that may have superior adaptation on sandy soils, namely WI3386 and WI3453. These lines were the highest yielding overall in these experiments with a high probability of consistently exceeding Mundah. Both lines are also known to display superior levels of resistance to economically important diseases to that of Mundah (Andrew Barr, *pers. comm.*). Keel also has superior disease resistance to that of Mundah, and is at least equivalent to WI3386 and WI3453. However, the poorer grain yield potential of Keel on sandy soils of low fertility suggests that the improved grain yield of WI3386 and WI3453 cannot be related to disease resistance alone. Rather soil type, and possibly other abiotic pressures, is the overriding factor(s) influencing the growth and grain yield potential of these advanced breeders' lines.

In South Australian Research and Development Institute (SARDI) variety evaluation trials, WI3386 has ranked highly for grain yield on sandy soils (Figure 6.5), although it has only been marginally better than Barque, and despite better disease resistance, has not displayed the improved grain yield potential over Mundah that has been highlighted in these trials. WI3386 may prove to be of breeding value to the program because of the combination of sand adaptation and superior disease resistance.



*Source: Rob Wheeler, SA Field Crop Evaluation Program (SARDI)

Figure 6.5: Long-term grain yield data for Mundah, Yagan, Forrest, Barque, Keel and WI3386 in SARDI sand evaluation trials (1988-2000)*. Data analysed by MET analysis.

Superior sand adaptation has also been identified in germplasm (WI3806, WI3804 and WI3802) developed from the cross Mundah/Keel//Barque. The overall performance of these breeding lines in SARDI and SABIP yield trials in 2002 was exceptional (Table 6.7). The breeding value of these lines was also confirmed by their improved yield potential on sandy soils relative to the cross parents (Table 6.7). From these preliminary results it would seem WI3804 and WI3806 have marginally lower 1000 grain weight than Mundah, while WI3802 is similar to Keel in this respect. WI3806 and WI3802 are early flowering, and WI3804 is slightly later (early-mid flowering). Provisionally these lines have very good resistance to the economically significant diseases such as spot form of net blotch (*Pyrenophora teres* f. sp. *maculata*), net form of net blotch (*Pyrenophora teres* f. sp. *teres*), cereal cyst nematode (cereal eelworm, CCN) (*Heterodera avenae*) and leaf scald (*Rhynchosporium secalis*). Given this wide spectrum of disease resistance, these lines are superior to Mundah and at least equivalent to Keel and Barque. The moderately susceptibile to susceptible status of WI3806

to leaf scald is a weakness. These lines continue to be evaluated in field trials to provide sufficient data to validate their adaptation on sand.

Table 6.7: Mean grain yield and 1000 grain weight of WI3806, WI3804 and WI3802 with the cross parents for trials conducted by SARDI* and SABIP in 2002. *Source: Rob Wheeler, SA Field Crop Evaluation Program (SARDI). Data analysed by REML and MET analysis.

Varieties/Lines	SARDI S3 Grain yield ($t\ ha^{-1}$) Non-sand	SARDI S3 1000 grain weight (g) Non-sand	SABIP Grain yield ($t\ ha^{-1}$) Non-sand	SARDI Grain yield ($t\ ha^{-1}$) Sandy soils	SABIP Grain yield ($t\ ha^{-1}$) Sandy soils
WI3802	3.52	42.92	1.47	1.11	1.38
WI3804	3.35	48.23	1.47	1.18	1.45
WI3806	3.47	48.80	1.54	0.93	1.45
Mundah	2.85	49.94	1.31	0.95	1.15
Keel	3.60	43.43	1.52	0.87	1.37
Barque	-	-	1.48	1.08	1.36

6.4.2 ICARDA Germplasm

Assessment of this current pool of ICARDA germplasm indicates no immediate or direct commercial value, although the breeders' lines ICARDA#31 and ISBYT-LRA(C) 96-97-15 may provide some potential as parents for targeted breeding for sand adaptation. The basis for this potential resides in the assumption that these lines will provide novel alleles for early vigour, growth habit and/or other attributes important in grain yield on sandy soils. Despite the lower overall potential of ICARDA#31 and ISBYT-LRA(C) 96-97-15 for early vigour and yield in these experiments, introgressing alternate alleles into germplasm of general (Barque and Keel) and specific (Mundah) agronomic adaptation may generate material with optimal combinations of genes that may lead to superior sand adaptation.

Most of the ICARDA germplasm assessed in these experiments originated from the Syrian program. The overall soil pattern for Syria is for the most part distinctly different to that found in South Australia (Figure 6.6 and 6.7, World Soil Resources Reports). Figure 6.6 and Figure 6.7 simplify the distribution of soils, and indicates the most common soils in the respective regions (World Soil Resources Reports). Many of the ICARDA selection environments are in Northern Syria where the soil pattern are broadly similar to that of South Australia (*i.e.* CL- Calcisols, Cambisols and Luvisols) (World Soil Resources Reports), however the northern Syrian soils are generally of a heavier texture (Andrew Barr, *pers. comm.*). In view of this, improving the growth and grain yield of barley on sandy soils would be better served by a more targeted approach to germplasm selection by focusing on accessing germplasm from regions where barley is not only an important agricultural commodity, and therefore cultivar development and selection is receiving serious attention, but also where sandy soils comprise a large proportion of the area of production. The CL soil pattern also occupies a significant proportion of North Africa (Morocco, Algeria and Tunisia) and possibly West Asia (Turkey) (Figure 6.6). The climates are Mediterranean to semi-arid (FAO/Unesco Soil map of the world, volume VI), and agriculture ranges from commercial production to subsistence farming. Barley production in North Africa is significant (Table 6.8) because it plays an important role in the diet of local inhabitants (Van Royen, 1954).

Table 6.8: Barley production and area for Morocco, Algeria, Tunisia and Libya for 2002 (FAO statistics, http://apps.fao.org/default.htm).

Country	Production (mt)	Area (ha)
Morocco	1,562,000	2,002,000
Algeria	573,800	515,000
Tunisia	90,200	99,150
Libya	80,000	170,00

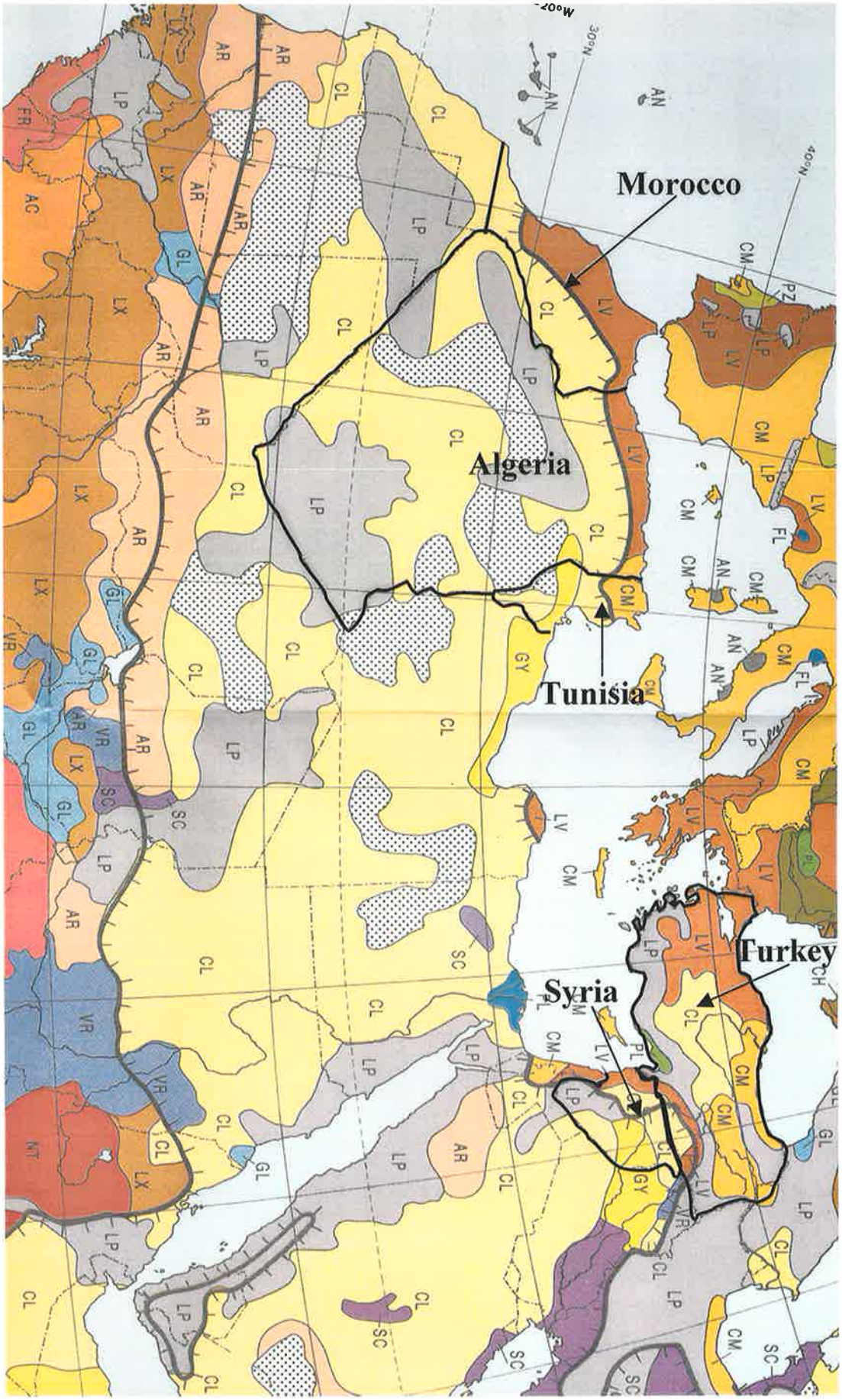


Figure 6.6: Map indicating the soil patterns of North Africa and West Asia. Map reproduced from World Soil Resources Reports No. 66 (FAO, 1993). Key:CL-Calcisols, Cambisols and Luvisols

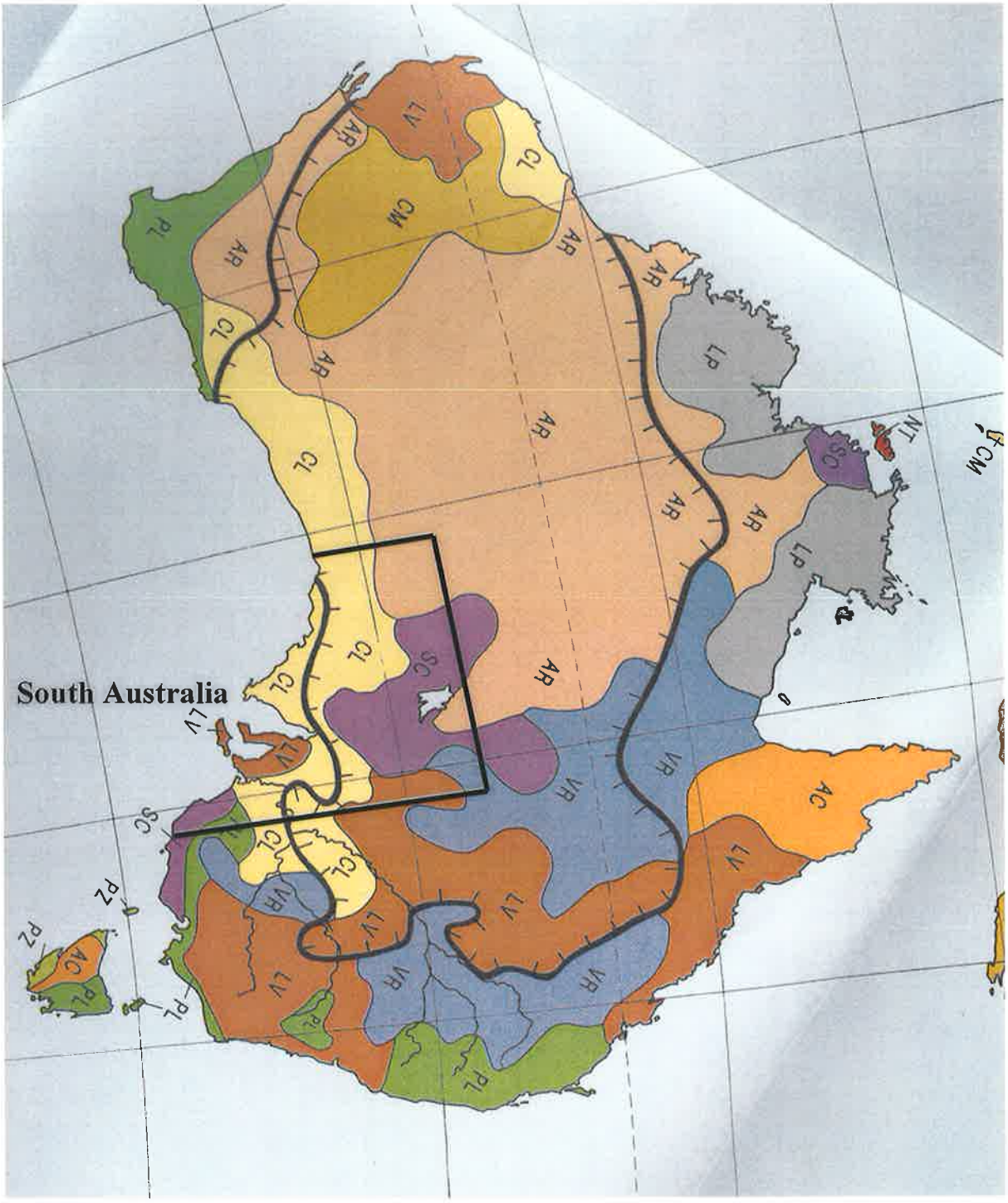


Figure 6.7: Map indicating the soil patterns of Australia. Map reproduced from World Soil Resources Reports No. 66 (FAO, 1993). Key:CL-Calcisols, Cambisols and Luvisols

The identification of populations of *Hordeum spontaneum* in North Africa, which have been suggested to be genetically distinct from populations of the Fertile Crescent, and evidence of separate domestication events, has lead to this region being proposed as one of the centres of origin of cultivated barley (Molina-Cano *et al.*, 2002). By definition a centre of origin is a region where domestication took place, and also where the wild ancestor and derived landraces and cultivated species co-exist (Molina-Cano *et al.*, 2002). The similarity of environment and soil type between North Africa and South Australia, and the designation of North Africa as a center of origin, suggests this region may offer the greatest potential for accessing novel germplasm with adaptation to sandy soils.

More specifically, the important barley growing area of central Tunisia (Rezgui *et al.*, 2000), which is coincident with a significant distribution of sandy soils (FAO Soils Bulletin No. 25, 1975), is one of the most promising regions to be targeted for germplasm that could be evaluated on sand in South Australia. Advice from ICARDA breeders also suggests Tunisian material, either landraces or advanced breeding lines, may be of value to improving the sand adaptation of South Australian germplasm, given that much of their barley production is on deep sands (Barr, *pers. comm.*).

Accessing a range of advanced breeding lines, developed from crosses between improved ICARDA lines, landraces and *Hordeum spontaneum* selections targeted for Tunisia, for field evaluation in South Australia would require a formalised collaboration between ICARDA, the Tunisia program and SABIP. Landraces directly selected on sandy soils could also be obtained for evaluation. An alternative resource of potential value is the Australian Winter Cereals Collection (AWCC). The AWCC contains a broad array of material from landraces to advanced breeding lines and cultivars, and a simple database search may uncover material sourced from locations with a predominance of sandy soils.

A possible strategy, involving ICARDA and landrace selections and material from AWCC, to improve the rate of genetic gain for sand adaptation could comprise three fundamental elements:

1. Material could be extensively evaluated in field trials and controlled environment experiments and assessed against local controls contrasting in adaptation on sand.

2. Mapping populations could be constructed, a linkage map developed, and assessed to characterise chromosome regions associated with key traits for sand adaptation. Through such a strategy, molecular markers linked to traits associated with sand adaptation could be identified and used to assist in the introgression of chromosome regions of benefit into South Australian germplasm. Three variants within this strategy are possible.

   2.1 Construct a population derived from a cross between a superior ICARDA breeding line, based on field and controlled environment evaluation, and a local cultivar with superior adaptation response on sand.

   2.2 Develop a population between two agronomically adapted cultivars for sand (e.g. cross an ICARDA improved breeding line well adapted to the Tunisian environment and a landrace from Tunisia selected on sand). A precedent has been set for this approach to identifying QTL for key agronomic traits. The Arta/Harmal population was constructed to determine the underlying genetic control, and identify novel alleles for specific drought tolerance mechanisms (Eglinton *et al.*, 2001). Both parents are well adapted to low rainfall conditions and are characterized by high yield stability. Importantly, they

also represent significant genetic diversity (Eglinton, 2001), critical for maximising genetic gain.

2.3 Alternatively, a population derived from a Tunisian landrace (or wild type) and an agronomically adapted cultivar from South Australia could form the basis for determining the genetic control of specific adaptation traits.

The Advanced Backcross-Quantitative Traits Loci ("ABQTL") population (*H. spontaneum* x Barque) developed to characterise chromosome regions associated with grain yield potential and yield stability under terminal drought is a notable example of this approach (Coventry, *pers. comm.*). The underlying principle of this strategy is to map new and favourable alleles which could be used as a genetic resource for genetic improvement and improve the level of genetic diversity, and introgress these new alleles into the recurrent parent (adapted elite line) through backcrossing to BC_2 (number of backcrosses) or BC_3, in one process (Pillen *et al.*, 2003). By BC_2 or BC_3, the genetic makeup of progeny is essentially the recurrent parent, but with small insertions of the donor parent. In addition to facilitating the mapping of new alleles, such BC lines are also suitable as advanced parents to utilize the alleles, detectable by molecular markers, in breeding populations.

3. Initiate a crossing program to introgress adaptation traits from overseas germplasm into agronomically suited genetic background(s) employing marker assisted selection.

Chapter 7. General Discussion

7.1 Traits associated with improved growth and grain yield of barley on sandy soils of low fertility

The studies presented in this thesis have shown that the adaptation response of barley on sandy soils of low fertility is a complex inter-relationship between a number of essential traits. In view of this, it is doubtful whether any one variety will possess the optimum level of expression of all traits, rather a balanced package of traits is likely to be the key. In this context, Mundah exhibits the most effective combination of traits, despite a lack of suitable resistance to economically significant diseases and a response to drought stress that is inferior to varieties such as Keel (Table 7.1).

The basis of the superior adaptation and grain yield of Mundah on sandy soils was found to be based largely on the ability to set yield potential through greater early vigour, both in terms of biomass production and leaf area development. It is also likely that increased early vigour encourages faster root growth and therefore a deeper system (Turner and Nicolas, 1987; Richards et al., 2002). The variety comparison field experiments, described in Chapter 2, clearly demonstrated that Mundah had superior early vigour across all environments, and that at three (Lowbank in 1999 and 2000, Minnipa in 2000) of the six site by year locations, Mundah also achieved the greatest yield potential. The ranking of varieties at these sites were highly correlated and were defined as a 'sand' response because the ranking of varieties followed expectation based on the long term results from SARDI (Chapter 2, Rob Wheeler, *pers comm.*). At sites demonstrating the typical 'sand' response the higher grain yield of Mundah was related to superior early vigour. These results in general follow several other reports that have shown superior grain yield in low yield potential environments to be associated with superior early vigour (Turner and Nicolas, 1987; Brown *et al.*, 1987;

Ceccarelli, 1987; Acevedo *et al.*, 1991; Whan *et al.*, 1991; Annicchiarico and Pecetti, 1995; El Hafid *et al.*, 1998; Simane *et al.*, 1998). The findings of these studies demonstrate that early vigour is a very important component of adaptation to these soil types.

Table 7.1: Ratings for specific traits associated with improved growth and grain yield on sandy soils for four varieties of differing adaptation response.

Trait	Mundah	Keel	Barque	Galleon
Large seed	***	**	**	**
Long coleoptile	**	**	***	***
Deep roots	***	*$^{1/2}$	***	***
Early vigour-biomass	***	**	**	*$^{1/2}$
Early vigour-leaf area	***	*$^{1/2}$	*$^{1/2}$	*$^{1/2}$
Erect growth habit	***	**	**	*
Phosphorus utilisation efficiency	***	**	**	**
High assimilate transfer	***	*	**	**
Early flowering	***	***	**	*
Resistance to leaf diseases	*	***	***	**
Drought stress response	*	***	*	*
Grain yield potential	***	**	**$^{1/2}$	*
Overall rating	***	**	**$^{1/2}$	*

Key: *** = high, ** = moderate, * = low

In contrast, early vigour may not be as important for crops grown on heavier textured soils with superior fertility and water holding capacity. The more favourable climatic conditions typical of the environments in which they exist in SA is likely to better sustain grain development, such that greater biomass production may be less essential to yield potential. Studies on the effect of seed size on adaptation to sand showed that inferior early vigour was related to sowing small seed, and that sowing small seed was also likely to impact on tillering ability.

At sites where growing season rainfall was significantly lower than average, the prevailing adaptation response appeared to mostly be a function of moisture stress rather than an effect of soil type. Early vigour in this situation was not an important determinant of high yield potential. For example, consider the performance of Keel at Minnipa in 1999. Despite a lower biomass at early tillering, Keel out yielded Mundah. However, the yield response of Mundah was relatively stable over environments with a 'sand' effect (not affected by terminal drought) and those displaying a 'drought' effect. In contrast, the adaptation response of Keel was predominantly determined by prevailing environmental conditions, with a greater grain yield potential than Mundah at sites characterized by terminal drought stress.

Both early vigour and a deep root system have implications for water use and water use efficiency (WUE) on sand. High early vigour will contribute towards reducting moisture loss from from soil surface evaporation, increasing the amount of moisture potentially available to the plants through evapotranspiration. A rapidly developing canopy is also important in crop photosynthesis as it will provide for greater light interception. The greater early vigour of varieties such as Mundah is likely to provide assimilate for biomass production, and grain yield by generating a larger potential sink through ear and spikelet formation and development (Richards, 2000). Although increased light interception favours greater evapotranspiration, this does not necessarily translate into better tranpiration efficiency. The erect growth habit of Mundah suggests a compromise, balancing high evapotranspiration with

high crop photosynthesis, and moisture conservation. It follows then that the superior biomass production of Mundah implies better water use efficiency relative to the other varieties evaluated in this study (Chapter 2).

A deep root morphology, rather than a shallow and dense system, is an advantage on sandy soils to counterbalance the characteristically uneven distribution of moisture and the rapid drainage of water down the soil profile. This study has shown that Mundah has a rapidly extending and deeper, but low density, root system in the early stages of growth (Chapter 3). This evidence supports theoretical predictions in recent studies by Asseng (2002) and Dreccer *et al.* (2002). These authors found, through crop simulation models, that fast early root growth and a greater maximum rooting depth would have a large impact on wheat yields on light textured (sandy) soils.

The vagaries of the moisture relations of sandy soils (*i.e.* rapid drainage, uneven distribution through the soil profile because of water repellency) means that plants in the early stages of growth could enter moisture stress very rapidly even after significant rainfall events. In contrast, seedlings growing in heavier soils can maintain early growth in non-moisture stress conditions using very little water, and can set yield potential on smaller rainfall events, because of the greater water holding capacity of these soils. Therefore the superior performance of Mundah on sand is likely to be a function of overall plant growth (*i.e.* root and shoot). The greater early vigour of Mundah protects the soil surface in the early stages of growth and sets yield potential, and the more rapid and deeper root morphology provides a significant advantage in 'chasing' the depleting moisture through the soil profile. The genotype x environment interaction for grain yield observed with Keel may be explained by Keel possessing traits more important in tolerance to moisture stress at late plant growth stages.

The deep root system of Mundah could also be important in terms of the availability of essential nutrients, particularly nitrogen in the form of nitrate (NO_3^-). Nitrate, a plant

available form of nitrogen, is easily lost below the root zone through leaching, and rapid root growth, and a deep rooting behaviour, will allow nitrogen to be 'chased' down the soil profile.

Seed size was shown to be an important factor in the growth and grain yield of barley on sandy soils (Chapter 4). It has been demonstrated that adjusting seed size can modify the response of a variety that displays inherent adaptation to sandy soils (*e.g.* Mundah). Plants grown from large seed were found to produce longer coleoptiles, produced a greater level of emergence and establishment, greater early vigour, and superior grain yield than plants grown from small seed. Previous evidence, both in this thesis (Chapter 2) and by other authors (Turner and Nicolas, 1987; Brown *et al.*, 1987; Ceccarelli, 1987; Acevedo *et al.*, 1991; Whan *et al.*, 1991; Annicchiarico and Pecetti, 1995; El Hafid *et al.*, 1998; Simane *et al.*, 1998), has related grain yield potential to early vigour. Results from the seed size study showed that grain yield improvement on sandy soils was determined predominately by seed size. Even though early vigour was higher with sowing larger seed, covariate statistical analysis showed that early vigour was not a factor in determining grain yield. The only exception was at Lowbank in 2001, where seed size was not significantly related to grain yield. Nevertheless, and for the underlying principles relating to water use, WUE and crop photosynthesis discussed above, early vigour is an essential feature of adaptation to sandy soils. Improving early vigour through variety selection, altering seed size, and improving nutrition could also lead to improved competitiveness with weeds, a reduction in erosion, and possibly a reduction in the damaging effects of sand "blasting". An additional implication from these experiments (Chapter 4) is that genetic improvement in grain size could also lead to improved performance on sand.

A significant relationship between coleoptile length, plant establishment, and early vigour could not be directly verified in this study (Chapters 2 and 4). However several studies have shown a positive relationship between coleoptile length and plant establishment (Whan, 1976;

Bacaltchuk and Ulrich, 1990; Hughes and Mitchel, 1987; Radford, 1987; Radford and Wildermuth, 1987; Sharma, 1990), and coleoptile length and early vigour (Gul and Allan, 1976; Gorny and Patyna, 1981; Redona and Mackill, 1996; Rebetzke and Richards, 1996; Rebetzke *et al.*, 1999). Intuitively, a long coleoptile should be an important component of a variety adapted to sandy soils, precisely because seeding depth is typically highly variable on these soils. Endosperm reserves (starch and nutrients) are also important in sustaining early seedling development (*e.g.* coleoptile and root growth). However seed nutrient reserves are limited and short term, such that the rapid development of roots into the soil profile is essential for plants to acquire nutrients and moisture to sustain early growth.

The efficient utilisation of phosphorus was found to be a component of the overall 'ideotype' that defined the superior adaptation of Mundah (Chapter 2). Phosphorus nutrition is a key component of successful cropping in South Australia, and many soils are below levels critical to plant health. This was evident from the levels of available phosphorus in the soil in our trials. In general the level of phosphorus in the top 10 cm soil fraction was between 9 and 15 mg P kg^{-1} soil, and up to 10 mg P kg^{-1} soil at a depth of 80 cm. These levels were well below the critical value for phosphorus (18 mg P kg^{-1} soil, Reuter *et al.*, 1995). Phosphorus has a relatively low mobility in the soil, such that effective use and/or an extensive plant root system to capture the available phosphorus are important. The low root density of Mundah shown in these experiments suggests that the total available phosphorus that can be exploited from the soil may be limited, and that efficiency of use of the available phosphorus arises from high biomass production per unit of phosphorus taken up by the plant.

The superior adaptation of Mundah is also related to an ability to convert the potential into improved grain yield and grain yield stability. This is achieved through earlier flowering to maximize the duration of grain filling, and the capacity to support the development of larger grain. In addition to the inherently poor moisture relations of sandy soils throughout the growing season, conditions post-anthesis in South Australia (SA), in general, become

increasingly unfavourable for grain development (*e.g.* leaf senescence, moisture and heat stress, increase evaporation) as climatic conditions become hotter and drier. Under these constraints fructan and ethanol soluble carbohydrates (ESC) (Chapter 2), stored in the stem prior to flowering, and the deep root system of Mundah (Chapter 3), is likely to assist in sustaining grain filling and preventing premature physiological maturity under the environmental conditions often encountered in SA. Further research is required to elucidate completely the contribution of fructan and ESC to grain filling on sandy soils. Measuring the rate and duration of grain filling, and changes in fructan and ESC in the stem during this period should be components of any future study.

The experimental results presented in this thesis confirm and refine the barley 'ideotype' for sand adaptation (Figure 7.1) postulated from the review of literature (Chapter 1). In addition, the findings of this study conclude that terminal drought stress response, phosphorus utilisation efficiency and nitrogen availability should also be integrated into the barley 'ideotype'. Drought stress is a component of adaptation since the superior performance exhibited by Keel (moderately poor adaptation on sand) on sand where terminal drought was an issue, illustrates that adaptation response on sand requires some 'flexibility' to accommodate prevailing environmental conditions (*i.e.* drought) that easily override the 'sand' effect (Chapters 2 and 5). This study did not directly relate nitrogen utilisation efficiency or availability to adaptation on sand. While it is tempting to suggest this is a component of sand adaptation, because leaching below the root zone is a problem on highly permeable soil types with low water holding capacity (sand), a detailed investigation into nitrogen availability, uptake and utilisation, and the variation between varieties of differing adaptation is required.

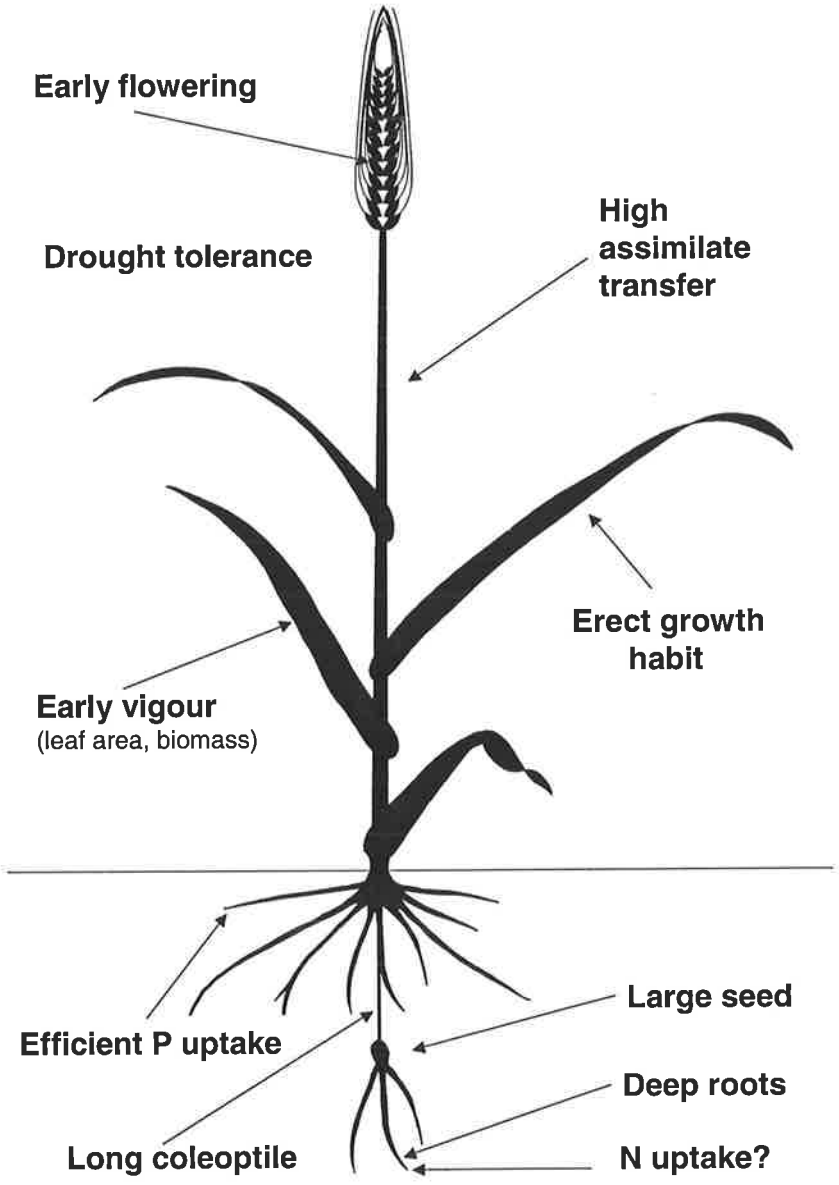


Figure 7.1: A putative barley 'ideotype' for sand adaptation devised from this thesis

(cereal plant graphic adapted from Araus *et al.*, 2001)

7.2 Mapping of chromosome regions associated with improved growth and grain yield on sandy soils of low fertility

To elucidate chromosome regions associated with traits conferring improved adaptation on sandy soils a mapping population was constructed from a cross between Mundah (very good sand adaptation) and Keel (moderately poor sand adaptation). This population was developed as F_3 derived recombinant inbred lines (RILs) through single seed descent from an F_2 bulk population. Originally 50 random lines were multiplied and phenotyped for traits 'desirable' for adaptation on sandy soils in 1999 and 2000. An additional 60 random lines from the F_2 bulk were multiplied over summer 2000/01 and added to the original 50 lines. In the 2001 sand screening trials 95 of the possible 110 lines were phenotyped.

Overall, the construction of the Mundah/Keel linkage map with the number of markers used was constrained by the low level of marker allele polymorphism between the parents. The linkage map that was produced had an inadequate marker density and distribution of molecular markers. However, significant QTLs associated with grain yield, grain weight, low screenings and early vigour (dry matter production) were identified. Many of the QTL for yield related traits were coincident with major genes controlling time of flowering, although a putative QTL for yield *per se* was identified. While further phenotyping and a more detailed linkage map is necessary, the detection of QTL for yield *per se* in this high yielding parent by high yielding parent population could be very valuable to barley breeding programs.

Typically, parents selected for mapping population construction contrast for specific traits of interest or in adaptation response to specific environments. This is considered fundamental to the efficacy of detecting QTL-marker associations. The QTL analysis of the Mundah/Keel RIL population demonstrated that alleles contributed by Keel can contribute positively to adaptation response on sand. This observation, supported by the performance of Keel in the variety comparison experiments (Chapter 2), confirms that traits contributing to the apparent

superior moisture stress tolerance of Keel may also contribute positively to adaptation on sand. Likewise, Mundah produced high grain weight and low screenings at sites where moisture stress was prevalent. It was also clearly evident that the variation in the adaptation response displayed by Keel, in terms of grain yield, was influenced by prevailing environmental conditions, verifying the observations made in the variety comparison trials (Chapter 2) that terminal drought confounds the 'sand' effect.

The prospective value of the Mundah/Keel RIL population will be contingent on more markers being integrated into the current linkage map. This is imperative for further QTL analysis to detect alternate markers with significantly better association with QTL for adaptation on sand, and better define the chromosome regions associated with key adaptation traits. In addition, the putative markers detected in this study will need to be validated in current breeding populations to ascertain the viability of marker-assisted selection for these QTL as a selection tool for sand adaptation. A high quality linkage map with a greater density of markers will also be of value to identify QTL associated with agronomic traits relating to other abiotic and biotic stresses, for which the parents differ.

The evidence presented in this thesis raises the notion that superior progeny should possess 'flexible' adaptation to accommodate the environmental pressures (terminal drought) that override the 'sand' effect. As was evident by the superior performance of Keel on sand where moisture stress was apparent. The SA Barley Improvement Program is currently evaluating germplasm from the cross Mundah/Keel//Barque on sandy and other soil types. The exceptional overall performance of many of the lines in 2002 (Chapter 6), and more specifically on sandy soils, suggests that the alleles from Keel for superior moisture stress tolerance, the positive alleles from Mundah for sand adaptation and the high yield potential of Barque may have been successfully combined. The provisionally good resistance of these lines to spot form of net blotch (*Pyrenophora teres* f. sp. *maculata*), net form of net blotch (*Pyrenophora teres* f. sp. *teres*), cereal cyst nematode (cereal eelworm, CCN) (*Heterodera*

avenae) and leaf scald (*Rhynchosporium secalis*) infers that the superior disease resistance properties of Keel and Barque have also been successfully combined.

7.3 Management of barley on sandy soils

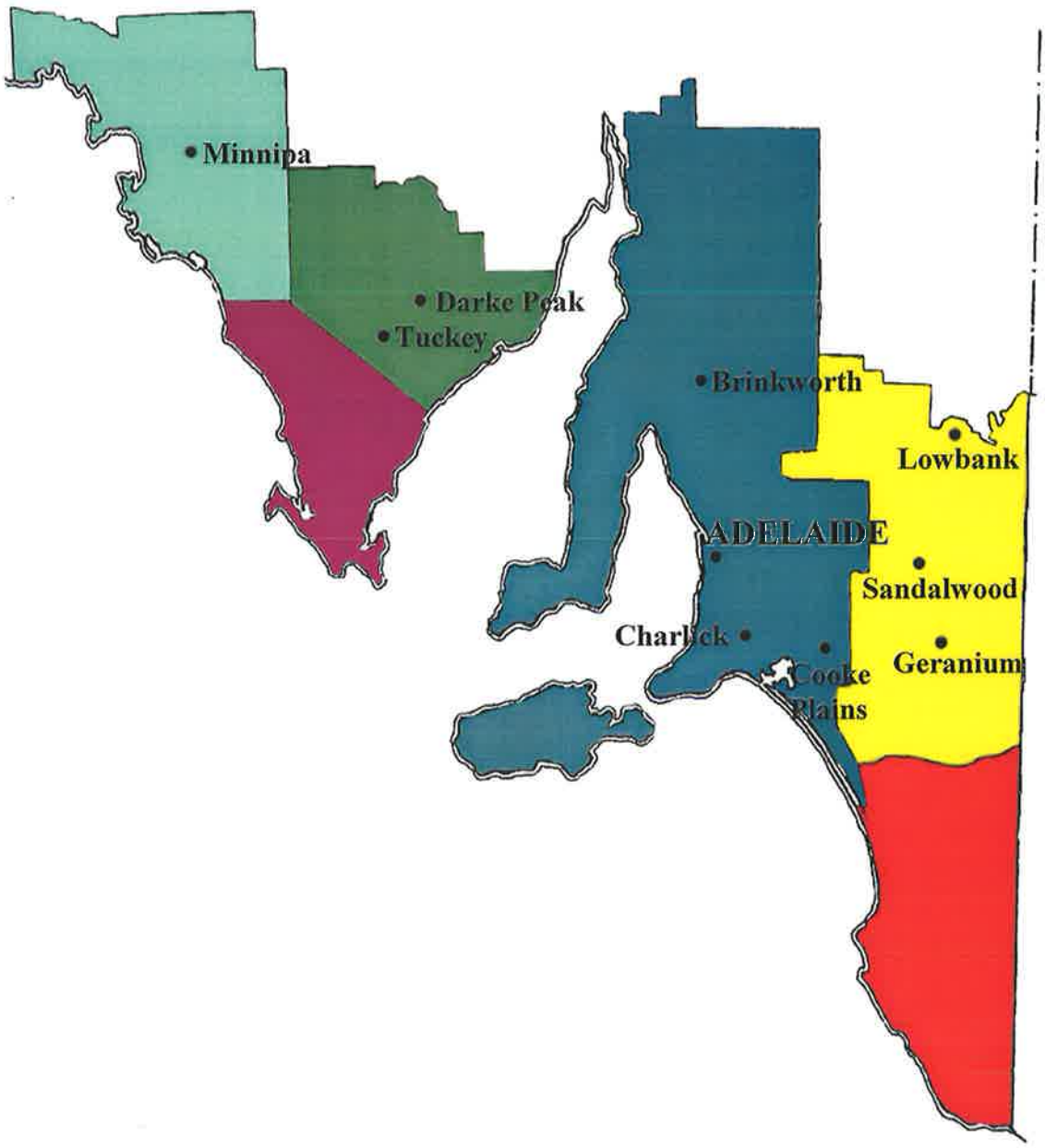
Progress towards the breeding and selection for superior adaptation to sandy soils has been demonstrated in current breeding germplasm (WI3806, WI3804 and WI3802) developed from the cross Mundah/Keel//Barque. In the interim Mundah, and possibly Barque, remain the best options for growers on sandy soils. The performance of adapted varieties can be improved through a strategy of managing seed size. Early vigour and grain yield on sandy soils can be improved by sowing seed of large average size through;

1. Selecting seed from the plumpest crop,
2. Selecting seed from favourable environments and soil types that produce seed of higher average size, weight and nutrition or,
3. Screening seed heavily and selecting the large grain fraction for sowing.

The higher nutrient status and starch content of large seed is likely to better sustain early development (*i.e.* coleoptile and root growth), thereby improving emergence potential and establishment, and seedling growth, until the roots can extract sufficient resources to maintain adequate plant growth.

Crop response to tillage practices was not a subject addressed in this study, because the focus was clearly directed to identifying traits associated with genotypic differences in adaptation to sand. Suffice to say, crop productivity is also likely to be affected by the type of tillage system employed. In early work by Hamblin *et al.* (1982) on a loamy sand, a conventional tillage system (disc ploughing) provided a more favourable seedbed, by reducing soil strength through greater soil disturbance, compared with the no-till system (direct-drilling). Under disc ploughing they observed a more rapid wetting of the subsoils, wheat roots extended

deeper into the soil profile, and earlier, and dry matter production and grain yield was improved. In general, the rate of crop establishment and early vigour is reduced under direct-drill, and may relate to limited soil disturbance causing reduced seed-soil contact. The greater soil strength under direct-drilling observed by Hamblin *et al.* (1982), may provide a physical barrier that could impede the rate of coleoptile and root growth. This is critical, since poor establishment and reduced early vigour are major factors limiting productivity on sandy soils. In recent times reduced tillage and no-till sowing systems have been gaining favour, and have been adapted to suit sandy soils. This is primarily because these tillage systems markedly reduce the risk of erosion, can lead to improved soil structure and aid in the build up of soil organic matter. Imperative to the success of reduced till or no-till farming is controlling seeding depth, optimising seedbed tilth and seed-soil contact to improve establishment and early vigour (Rainbow, 2003). The use of press wheels is an effective way of controlling the depth of soil cover (seeding depth) and improves seed-soil contact (Rainbow, 2003) to optimise germination and emergence, and therefore early vigour. It is likely that breeders will need to locate selection nurseries on deep sands sown by direct drill seeding systems to provide the best varieties for future barley production on these soils. This will also require the adoption of more sophisticated experimental designs and analysis to manage the inherent variation in these soils.



Appendix 1: Map of South Australia showing field experiment locations.
(adapted from SAFCEP Annual Reports (Wheeler, R.D. *et al.*, 1988-2000))

Eastern Eyre Peninsula

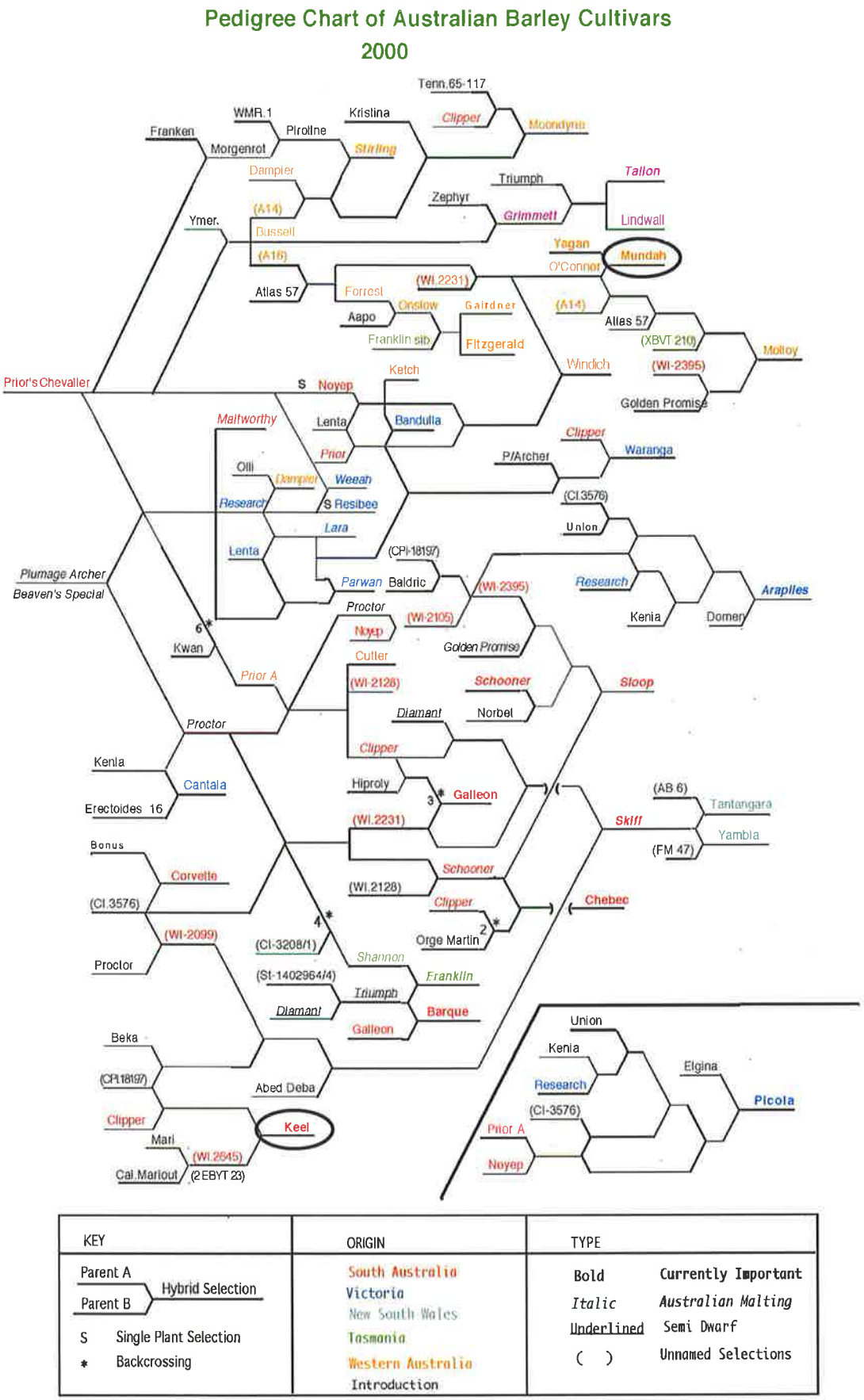
Upper Western Eyre Peninsula

Lower Eyre Peninsula

Yorke Peninsula, Mid North, Murray Basin, Kangaroo Island

Murraylands

South East



Appendix 2: Pedigree chart for Australian barley varieties
(Sparrow *et al.*, 2000)

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