MANGANESE EFFICIENCY IN DURUM WHEAT
(*Triticum turgidum* L. var *durum*)

By

Hossein Khabaz-Saberi

M. Sc.

Tehran University

Tehran, Iran

This thesis submitted for the degree of Doctor of philosophy of the University of Adelaide

Department of Plant Science

Waite Campus
The University of Adelaide
March, 1999

Declaration

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

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

Hossein Khabaz Saberi

Table of contents

Chapter 6 Use of Amplified Fragment Length Polymorphism (AFLP) in the study of Manganese efficiency in durum wheat

Acknowledgments

I wish thank to Dr A.J. Rathjen and Professor Robin D. Graham for their valuable guidance, advice and constructive supervision throughout the course of this study.

I am grateful to Dr J. Ascher-Ellis and Dr J.G. Paull for their valuable comments and suggestions on an earlier version of this thesis and Dr Vivien Vanstone for her help in proof reading.

I would like to thank Dr P. Langridge and Dr S.J. Barker for providing the facilities for AFLP analysis. Special thanks due to J.M. Kretschmer and M. Pallotta for their advice and assistance with laboratory work.

Many thanks to members of the wheat breeding and trace element groups at the Waite Institute, especially C. Stone, M.C. Kroehn, N. Robinson, T. Fowles and all the staff of the Department of Plant Science for their help.

Financial support of a joint IRAN/ICARDA project for this research is highly appreciated.

Publications

Khabaz-Saberi H, Graham R D and Rathjen A J (1996) Screening for manganese efficiency in durum wheat (*Triticum turgidum* L. var. *durum*). In: Proceedings of 5th International Wheat Conference, pp 206-207. Ankara, Turkey, June 1996.

Khabaz-Saberi H, Graham R D and Rathjen A J (1997) Genotypic variation for manganese efficiency in durum wheat (*Triticum turgidum* L. var. *durum*). In: Plant Nutrition for Sustainable Food Production and Environment. Eds. T Ando *et al.* pp 289-290. Kluwer Academic Publishers, Tokyo, Japan.

Khabaz-Saberi H, Graham R D and Rathjen A J (1998) Inheritance of manganese efficiency in durum wheat (*Triticum turgidum* L. var. *durum*). *Journal of Plant Nutrition.* 22(1):11.

Khabaz-Saberi H, Barker S, Graham R D and Rathjen A J (1998) Potential of Amplified Fragment Length Polymorphism (AFLP) in breeding for Mn efficiency in durum wheat (*Triticum turgidum* L. var. *durum*). In: Proceedings of the 6th International Symposium on Genetics and Molecular Biology of Plant Nutrition. August 17-21, 1988, Elsinore, Denmark (In press).

Schlegel R, Ozdemir A, Cakmak I, K-Saberi H and Atanasova M (1998) Localisation of genes for zinc and manganese efficiency in wheat and rye. In: Proceedings of the 6th International Symposium on Genetics and Molecular Biology of Plant Nutrition. August 17-21, 1988, Elsinore, Denmark (In press).

Pallotta M, Khabaz-Saberi H, Graham R D and Barker S (1999) Breeding for tolerance to nutritional stress - Molecular mapping of loci for Mn efficiency in barley and durum wheat. In: Proceedings of 11th Australian Plant Breeding Conference. April 19-23, 1999, Adelaide, South Australia, Australia (In press).

Thesis Summary

A study was undertaken to investigate the genetic diversity for tolerance of durum wheat (*Triticum turgidum* L. var *durum*) to micronutrient deficient soils with an emphasis on manganese (Mn), a major micronutrient deficiency in South Australia. The objectives were to: identify genetic variation for the trait; develop efficient selection criteria for screening; identify tolerant genotypes, study the mode of inheritance; employing aneuploids to elucidate the location of genes conferring tolerance to Mn deficiency; the latter investigations involving the study of Amplified Fragment Length Polymorphism with bulk segregant analysis in a cross of Mn-efficient by Mn-inefficient genotypes for identification of closely linked molecular markers to the trait. The results of these studies are being employed in the durum breeding program for Mn deficient soils.

A poor adaptation of a range of durum wheats was observed on a site deficient in several micronutrients. Investigation of the causes of the leaf symptoms and low grain yields established the intolerant nature of durum wheat to soils with low availability of a range of micronutrients, especially zinc and Mn.

Genotypic variation for tolerance to Mn deficiency was observed both in the field and in controlled environment rooms. Screening durum wheat genotypes in a controlled environment correlated well with field results and led to identification of shoot Mn content of the seedling 35 days after sowing, as a selection criterion for screening and genetic studies.

Further screening allowed identification of a moderately Mn-efficient genotype (Stojocri 2) with a greater yield in deficient soil than a standard durum wheat cultivar, Yallaroi. Pedigree analysis of efficient and inefficient genotypes revealed the geographic source of Mn efficiency in durum as Algeria.

Through a study of a F_2 and F_3 segregating populations of the cross, Stojocri 2/Hazar (Hazar as a Mn-inefficient genotype) it was concluded tentatively that two major genes with additive effect controlled the segregation for Mn efficiency in this cross. The study of the F_1 hybrid of the same cross, compared to the parents, revealed that the Mn efficiency genes were incompletely dominant with no maternal effect in the reciprocal cross.

An attempt to elucidate the location of genes on chromosomes by a study of substitution lines (Langdon D-genome disomic substitution lines) was hindered, mainly due to confounding effects of variable seed Mn content arising from different sized seed of substitution stock, adversely effecting the substitution of 4B by 4D and either 7A or 7B by 7B. It appeared that gene(s) for Mn efficiency to be located on 6D and 1D; however, Chinese Spring, the source of D-genome was inefficient and not suitable for studying Mn efficiency.

The application of Amplified Fragment Length Polymorphism jointly with bulk segregant analysis to the same cross (Stojocri 2/Hazar) led to identification of two markers potentially linked to the Mn efficiency. If these markers co-segregate for Mn efficiency loci in a screening of the mapping population, they have potential of being used in marker assisted selection. Development of a PCR based assay, following cloning and sequencing of the markers could be the next approach to be undertaken.

The application of the results of this study is contributing to increasing the adaptation and establishing the area of cultivation of durum wheat into marginal lands with micronutrient deficient soil. Mn-efficient durum cultivars will enable higher durum wheat production to be maintained on Mn-deficient soils with less Mn fertiliser (which quickly reverts to unavailable forms).

Summary of outcomes

1) A higher critical concentration of Mn for durum wheat was observed in both shoots and youngest emerged blades compared to either barley (11 mg/kg dry weight) or bread wheat (10-12 mg/kg dry weight). This finding has application in the chemical diagnosis of both marginal or severe deficiencies. This may aid in investigations aiming at understanding of the internal Mn requirements of durum wheats compared to bread wheats.

2) Considerable genotypic variation was identified during screening for Mn-efficient genotypes. These Mn-efficient genotypes (Stojocri 2 and Zenati Bouteille) are currently being used in the durum wheat breeding program to incorporate Mn efficiency into advanced breeding lines.

3) The development of a reliable, efficient selection criterion (shoot Mn content of 35 days old seedlings) has made screening of a large number of genotypes for Mn efficiency in a relatively short time quite feasible.

4) The pedigree analysis of Mn-efficient genotypes has revealed Algeria as a geographic source of Mn efficiency. This could be further exploited to find other Mn-efficient genotypes for incorporation into the breeding program.

5) The study of mode of inheritance and number of genes controlling the trait has determined the minimum number of backcrosses (two backcrosses) and the size of the segregating backcross populations necessary in breeding for Mn efficiency.

6) Two primer combinations potentially linked to Mn efficiency loci were identified using an approach combining the bulk segregant analysis with the amplified fragmented length polymorphism technique. If these primers ((*Pst* I+ACA/*Mst* I+CAA) and (*Pst* I+ACC/*Mse* I+CAG)) are linked to Mn efficiency loci they will be used in marker assisted

selection. They could also be converted to simple PCR primers to be employed more efficiently in the breeding program, increasing the efficiency of breeding in terms of time, accuracy and reduced drudgery.

Thesis Introduction

Occurrence of Mn deficiency around the world has been reported on a diverse range of soil types, and in various crops over a wide range of climates (Reuter *et al.*, 1988a). In South Australia, single and multiple trace element deficiencies together with macronutrient deficiencies have been recognised and are widespread. Manganese deficiency on calcareous sands is the most severe micronutrient disorder in South Australia (Fig 1), though not as widespread as zinc deficiency. Manganese deficiency is commonly observed alone or in combination with other trace element deficiencies on alkaline and calcareous soils which dominate large tracts of the state's agricultural zone (Reuter *et al.*, 1988a). On these soils, it is potentially the main constraint for normal growth and development of a micronutrient-inefficient crop such as durum wheat.

Durum wheat is a new and rapidly expanding crop in South Australia. Commercial production of durum wheat in South Australia started with the importation of seed of the variety "Yallaroi" from New South Wales, which resulted in production of 500 tonnes in 1990 (Sharpe, 1993) and it was expanded 100-fold to 52,334 tonnes by 1996 (Sharpe, 1993). Parallel to the commercial production of durum wheat, Dr A.J. Rathjen at The University of Adelaide, Waite Campus commenced a breeding program for durum in South Australia , using multi-location testing and evaluation of entries introduced from the durum wheat breeding program in New South Wales and other sources (ICARDA, CIMMYT, Italy, Turkey, Algeria, North America and Canada). The yield of Australian commercial durums ranges from ~50% to equal or above the yield of the commercial bread wheat varieties Spear and Aroona. In general, the widely cultivated durum wheat Yallaroi which has resistance to leaf diseases (stem rust, leaf rust, stripe rust and *Septoria*

tritici) and cereal cyst nematode (CCN) has yielded well only on deeper and more fertile soils. Its performance in low fertility and low rainfall districts, where the dry finish to the growing season favours comparatively high protein levels, has often been very poor. The restriction of cultivation of current durum wheat varieties to fertile soil can be explained by their intolerance to toxic levels of boron and sodium, susceptibility to diseases (root lesion nematode and crown rot) and especially their poor performance on micronutrient-deficient soils (Mn, Zn, Cu and Fe) (Brooks, 1991; Brooks *et al.*, 1994). The fact that Yallaroi has been used as a susceptible check in studies of tolerance to high concentrations of B, and also in trials to determine trace element efficiency, to some extent exemplifies its lower relative yield in comparison to bread wheat and also its current restriction in cultivation predominantly to deep, fertile soils.

Broadening the adaptation of durum wheat to less fertile soils demands firstly a diagnosis of the major biotic and abiotic constraints limiting normal growth and development, exploring the sources of tolerance or resistance and finally the incorporation of identified tolerance traits into advanced lines and varieties through plant breeding. When agronomic approaches for tackling the problem fail, where there is evidence of genotypic variation for the trait, and where the soil has adequate supply of the micronutrient for efficient genotypes, breeding will be justifiable.

Research on genetics of B toxicity in wheat has been undertaken (Paull, 1990; Chantachume, 1995) and has been extended to barley (Jenkin, 1993), peas (Bagheri, 1994) and finally to durum wheat (Jamjod, 1996). Breeding for high yield, wide adaptation, high quality and boron tolerance are the current major objectives of the Waite Campus durum breeding program in South Australia. A number of studies will be described in this thesis that attempt to direct the breeding program toward the improvement of Mn efficiency in current durum wheats.

Identification of the cause of development of pale, yellow-green leaves in nearly all durum wheat genotypes grown at Coonalpyn (145 km south east of Adelaide), attributed to iron and Mn deficiency (Brooks, 1993) at a multiple micronutrient deficient site (King *et al.*, 1992) has been carried out. The diagnosis was severe Mn deficiency; its confirmation and preliminary screening in pots and in the field will be discussed in Chapter 2.

Because of the low effectiveness of Mn fertilisers, our preference was for a genetic solution. Chapters 2 and 3 deal with observations of genotypic variation from preliminary screening, confirmation of the extent of genetic variation, development of selection criteria and identification of sources of Mn efficiency (by pedigree analysis of efficient and inefficient genotypes).

The study of mode of inheritance of Mn efficiency in a cross of a relatively Mn-efficient genotype by an inefficient genotype, and its F_2 and F_3 segregating populations, is discussed in Chapter 4.

Chapter 5 reports on an attempt to elucidate the location of gene(s) conferring Mn efficiency in tetraploid wheat using Langdon D-genome disomic substitution lines; also discussed are constraints and limitations of aneuploids used to study durum wheat genetics.

Application of AFLP and bulk segregant analysis in the study of F_2 segregating populations and the potential of AFLP in marker assisted breeding for Mn efficiency is described in Chapter 6.

Chapter 7 is a general discussion of the results of this thesis.

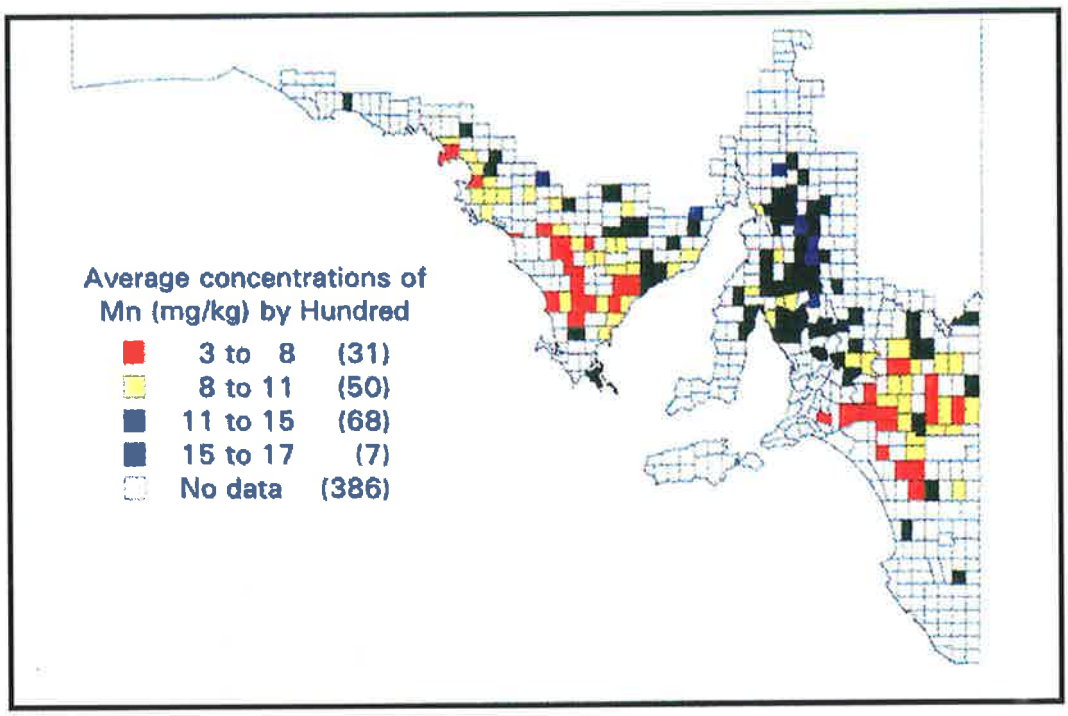

Fig 1. The area of potential Mn deficiency in the agricultural zone of South Australia determined by average Mn concentration in grain of the Mn-deficient barley genotype, Galleon. Source: Spouncer et al. 1989/90. CSIRO Australia, Divisional Report No120.

Chapter 1

Review of Literature

1.1 Introduction

Manganese deficiency was the first trace element deficiency recorded in South Australia (Samuel and Piper, 1928). Since then either severe or moderate deficiency has been reported in a wide array of cultivated crops and a range of soils. Graham (1988) defined a Mn-efficient genotype in an agronomic sense as "able to grow and yield well without added Mn fertiliser in a soil which is limiting in available Mn for another standard genotype". Cultivation of Mn-inefficient crops (barley cv. Galleon and narrow leafed lupins) has provided a tool for determining the full extent of areas of Mn deficiency. A wide range of genetic variation for Mn efficiency has been exploited, with efficient varieties of bread wheat and barley being more commonly grown on deficient soils.

Several areas of research on durum wheats including end use qualities, tolerance to boron toxicity and factors contributing to broad adaptation have received considerable attention considering the short time since its introduction to South Australia. Durum wheat is intolerant to micronutrient stresses (both toxicity and deficiency). Considering the importance of durum wheat as a new and promising crop to South Australia, where micronutrient deficiency is widespread in the state's agricultural zone, this study was carried out on the effect of Mn deficiency on growth and development of this crop. This study investigated the extent of genetic variation for tolerance to low levels of soil Mn, the

sources of genetic variation, the mode of inheritance, and potential for developing markers for accelerating breeding of Mn-efficient durum wheat. The following review of literature was undertaken to establish the knowledge base on which to construct the planned investigations.

1.2 Manganese in plants and soils

1.2.1 Manganese in plants

In plants, Mn plays a vital role in redox processes and a range of valances of Mn, primarily basic divalent (Mn II), can be found. The Mn^{2+}, with ionic radius of 0.075 nm, lies between Mg^{2+} and Ca^{2+} and could substitute or compete with either of these ions in a number of chemical reactions (Marschner, 1995). Manganese is directly or indirectly involved in many biochemical processes. Unlike most essential trace elements which are important components of enzymes, Mn has a vital role in only two Mn containing enzymes and otherwise acts as an activator of some other enzymes.

1.2.1.1 Manganese containing enzymes

Manganese is an integral part of a Mn protein in photosystem II (PSII) that functions in the process of water splitting, O_2 evolution and electron transfer associated with PSII. This is considered as the most crucial function of Mn in photosynthetic cells (Prince, 1986). This role, as well as its structural function in stacking of chloroplast lamellae, is impaired by Mn deficiency (Simpson and Robinson, 1984). Manganese is also part of Mn containing superoxide dismutase (SOD) and purple acid phosphatases, with the

former often found in the mitochondrial matrix, where it has a protective role in tissues against the deleterious effects of oxygen free radicals produced in some enzyme reactions. It catalyses the conversion of free oxygen radical (O^{2-}) to H_2O_2 for subsequent dismutation into H_2O and O_2 (Elstner, 1982).

1.2.1.2 Manganese as a cofactor

Manganese serves as activator of a variety of enzymes, including hydrogenases, transferases, hydroxylases and decarboxylases. It is also the major cofactor in oxidative and non-oxidative decarboxylation of tricarboxylic acid cycle intermediates (Burnell, 1988).

A number of key reactions involved in the synthesis of plant secondary metabolites (lignin synthesis) require Mn as a cofactor. Synthesis of a number of simple phenols (caffeic, coumaric, chlorogenic, ferulic, protocatachuic and quinic acids) derived from intermediates of the shikimic acid pathway or secondary metabolites which have a crucial role in plant defence systems is affected under Mn deficiency (Burnell, 1988). Manganese nutrition of plants affects the level of indole acetic acid oxidase production. Manganese deficiency favours increased production of peroxidase and indole acetic acid oxidase activity and considerable differences in chloroplast peroxidase content were observed in wheat genotypes differing in Mn efficiency (Kaur *et al.*, 1989). Manganese peroxidase oxidises Mn II and, as an extracellular peroxidase, is involved in lignin degradation and oxidation of phenol and phenolic compounds (Glenn *et al.*, 1986). Considering the structural role of lignin in sclerenchyma, wilting symptoms observed under Mn deficiency, as a result of dysfunction of lignin/phenol synthesis, can be attributed to lack of structural support (Campbell and Nable, 1988).

1.2.2 Manganese in soils

Manganese is the tenth most abundant element in the earth's upper continental crust, with an average concentration of 650 mg kg^{-1}. It is an essential constituent of many minerals, and most other minerals contain Mn as a minor substitute for Fe^{2+} and Mg^{2+} as structural ions. It may be present in rock and soil minerals in one or more of the three valance states, (Mn II, Mn III and Mn IV). While some of the Mn exists in soil solution or adsorbed to surfaces of mineral and organic matter or incorporated into organisms, it is mostly a constituent of primary and secondary minerals (Gilkes and McKenzie, 1988). Manganese participates in diverse reactions in soils, including oxidation and reduction (redox), ion exchange, specific adsorption and solubility equilibria.

The distribution of Mn between solution and solid phase is related to pH, redox conditions, and the characteristics of ligands and surfaces. Manganese forms complexes in soil solution with organic ligands produced by various organisms. Organic acids, amino acids, sugar acids, hydroxamate siderophores and phenols are the major biochemical ligands. The fully hydrated Mn II is the dominant inorganic form of Mn in soil solution with $SO_4^=$, HCO_3^- and Cl^- forming the major inorganic complexes. The Mn^{2+} complex with $SO_4^=$ is present in considerable concentration in many soils, while HCO_3^- is important under neutral and alkaline conditions (Norvell, 1988).

1.2.3 Acquisition and translocation of manganese in plants

1.2.3.1 Manganese acquisition

The availability of Mn to plants is governed by a redox process which is under control of soil chemical, microbial and plant factors (Marschner, 1988). The uptake of Mn (Mn^{2+}) is related to its external concentration and is a function of availability of other divalent cations (Ca^{2+}, Mg^{2+} and Zn^{2+}) and of H^{+} which plays a prominent role possibly as a source of diffusible electrons to facilitate reduction of oxide-Mn (Islam *et al.*, 1980). Its availability in neutral and alkaline soil, where Mn deficiency is prevalent, is affected by changes (mainly induced by roots) in chemical and microbial characteristics of the rhizosphere which in turn affect its further acquisition by roots. The change in redox status (as determined by rhizosphere pH (-log of proton activity) and pe (-log of electron activity)), root exudate, contact reduction and microbial activity in the rhizosphere are proposed to be the main variations which affect the solubility of Mn induced by roots under Mn deficiency (Marschner, 1988). Under Mn deficiency, poor shoot and root growth was observed in wheat (Marcar and Graham, 1986), but changes in root morphology and physiological function in response to Mn deficiency had not been reported by 1988 (Marschner, 1988). Webb and Dell (1990) studied the effect of withholding Mn on the structure and growth of roots in young bread wheat seedlings grown in water culture. The growth and development of bread wheat roots was affected by Mn deficiency through impairment of lateral root initiation, root elongation and lignification of cell walls (Webb and Dell, 1990). Lignification was depressed prior to the onset of foliar Mn deficiency symptoms.

1.2.3.2 Manganese translocation in plants

In xylem sap, Mn is present primarily as a hydrated divalent ion in equilibrium with unstable organic acid complexes (Loneragan, 1988). It moves freely in the transpiration stream and, when supplied adequately, accumulates in roots, stems and leaves in a pattern conventionally described as "phloem immobile". Consequently, in experimental systems, the phloem sap of such plants may provide an adequate amount of Mn to developing seeds only if the plant accumulates enough Mn prior to omission of Mn supply (Hannam *et al.*, 1985). At adequate and high levels of Mn, Mn concentrations are higher in roots than in leaves, higher in mature leaves than in young leaves, and higher in leaves than in stems, flowers and seeds (Nable and Loneragan, 1984a). Pearson *et al.* (1996) found the same pattern of distribution of Zn and Mn in leaves of wheat cv. Aroona where in 14 day old seedlings, the younger leaves accumulated less Mn and Zn than the older leaves. As a plant grows into deficiency, Mn concentrations decrease rapidly in roots, then in stems and young leaves, but remain high in the older leaves; changes result from the displacement of Mn from root and stem cells, its transport in xylem or phloem, and its poor remobilisation from leaves via the phloem (Nable and Loneragan, 1984a). The observation of high concentrations of Mn (Pearson *et al.*, 1996) in glumes and leaves of bread wheat pre- and post-anthesis, compared to grains, is also evidence for the poor remobilisation of Mn to the developing seeds of bread wheat.

1.3 Occurrence and diagnosis of manganese deficiency

1.3.1 Occurrence of manganese deficiency

Deficiency of Mn occurs when the concentration of Mn in the specific plant tissues falls below the level required for sustaining the metabolic function at a rate which does not limit plant growth (Loneragan, 1968). Manganese deficiency is associated with impoverished soils:

(i) with inherently low Mn in the parental material, or

(ii) from which Mn has largely been removed by leaching, and

(iii) with high pH and free carbonates (Dudal, 1976).

This deficiency is widespread around the world, and in South Australia both severe and moderate deficiency have been reported commonly on alkaline and calcareous soils and calcareous sands which dominate large tracts of the state's agricultural zone (Reuter *et al.*, 1988a).

1.3.2 Diagnosis of manganese deficiency

1.3.2.1 Symptoms

Deficiency symptoms can be observed either in plants growing in soil with parental material inherently low in Mn or on highly weathered soils, and is also common on soils of high pH containing free carbonates (Reuter *et al.*, 1988a). Whitish-grey spots, flecks or stripes on leaves are probably the best known deficiency symptoms of cereals in Australia and around the world. Also typical are the symptoms of interveinal chlorosis,

dark brown spots on leaves and premature senescence of older leaves (Campbell and Nable, 1988). Distinct symptoms of Mn deficiency will develop when the growth rate and yield have been highly depressed. The transient nature of Mn deficiency in some soils, confusion of Mn deficiency symptoms with deficiency of other nutrients (Fe, Mg and S) and its association with other stresses (biotic and abiotic) makes the use of visual judgement somewhat unreliable (Hannam and Ohki, 1988).

1.3.2.2 Chemical analysis

The relationship between growth response and Mn supply, usually measured by Mn concentration in the tissue, is the basis for diagnosis of deficiency by chemical analysis. The concentration of Mn in plant tissue for which the growth is depressed by 10% below maximum growth is known as the critical Mn concentration in that tissue, and has been adopted as a criterion to diagnose Mn deficiency in a wide range of crops (Reuter and Robinson, 1986). The critical Mn concentration has been determined for bread wheat grown in the field by Graham *et al.* (1985) and for barley by Hannam *et al.* (1987), and is in the range of 10-12 mg kg^{-1} Mn in dry matter of the youngest emerged blade (YEB). The Mn concentration determined by chemical analysis overestimates the specific Mn requirement, since it also includes the metabolically inactive portion. However, good correlation of this measurement with field performance and the convenience of the method has assured its wide application and acceptance (Reuter and Robinson, 1986).

1.3.2.3 Biochemical and physiological analysis

Changes in metabolic rates of Mn-specific enzymes or processes which respond directly to Mn supply provide useful indicies of the Mn status in plants. The function of Mn in the water splitting process of photosynthesis and in the evolution of O_2 has been employed in the determination of a critical Mn concentration (20 mg kg^{-1} YEB) for subterranean clover. In this work the critical Mn concentration in YEB was determined from a close correlation with photosynthetic O_2 evolution (Nable *et al.*, 1984).

Chlorophyll 'a' fluorescence has been used as an indicator of photosynthetic dysfunction to enable early diagnosis of Mn deficiency. The fluorescence in leaves at limiting concentrations of Mn depends on the association between Mn concentration and electron transport from water to photosystem II occurring on a thylakoid membrane. At a limiting Mn concentration, electron flow and light harvesting is affected, resulting in increased constant yield fluorescence (F_0) and decreased variable fluorescence (F_v). The F_0/F_v ratio, the relationship between constant fluorescence F_0 and variable (F_v = maximum - constant) fluorescence as a measure of the stronger fluorescence associated with Mn deficiency and leaf Mn concentration was employed to define the critical Mn concentration for bread wheat (Graham *et al.*, 1985) and barley (Hannam *et al.*, 1987), as 11 mg kg^{-1} and 14 mg kg^{-1}, respectively .

1.3.2.4 Soil analysis

Plant available forms of Mn are a reflection principally of the concentration in the soil solution. The fact that Mn behaves differently from other micronutrients makes the

prediction of available soil Mn by various chemical analyses difficult (Reisenauer, 1988). This is because:

(i) Manganese oxides along with soil solution phase Mn(II) can serve as direct sources to plants, and

(ii) there can be a wide variation in solution concentration of Mn within a short period of time.

Wide variation in Mn concentrations is a result of Mn being subject to inorganic (Norvell, 1988) and organic reactions (Bartlett, 1988) in the soil. The inability of chemical analysis to account for all these changes has made soil analysis an unreliable predictor of Mn deficiency in neutral and alkaline soils, and unsatisfactory for estimating the Mn requirement for various plant species and cultivars (Marschner, 1988).

1.4 Adverse effects of deficiency and its correction

1.4.1 Adverse effects and correction of Mn deficiency

Plant tissues which rely on nutrient supply of Mn from the phloem should be most sensitive to Mn deficiency. Cereal grain yield and yield components are sensitive to both low and high (toxic) levels of Mn supply and, consequently, plant reproduction was diminished when severe Mn deficiency was imposed over a period of a week during microsporogenesis (Campbell and Nable, 1988). Male sterility in cereals due to poor viability of pollen has been observed under moderate Mn deficiency (Kaur *et al.*, 1991). Nable and Loneragan (1984b) showed that Mn has specific functions in root growth of subterranean clover independent of Mn requirements for shoot growth. When Mn was

supplied externally to a split root system, it was shown that the root system with deficient internal supply of Mn decreased in growth 50% below the control, while shoot growth remained unchanged.

In soybean the percentage oil decreased from 21 % to 17 %, concomitant with a yield depression of >65%, under moderate Mn deficiency (Heenan and Campbell, 1980a; Wilson *et al.*, 1982). Manganese deficiency symptoms were more severe at low temperatures, and maturity was delayed: deficient barley plants took twice as long to reach booting stage compared to Mn-sufficient plants (Longnecker *et al.*, 1991a). The grain yield depressions in Mn deficient plants has been the consequence of poor pollen fertility (Sharma, 1992) and shortage of carbohydrate supply to the grain (Longnecker *et al.*, 1991a). Hence, Mn deficiency results in poor fertility and poorly filled endosperm. Consequently, grain yield is decreased.

1.4.2 Agronomic solutions to manganese deficiency

Severe Mn deficiency in plants leads to development of recognisable foliar symptoms; however, subclinical deficiency can be detected only by plant analysis (Walter, 1988).

Severe Mn deficiency in cereals can be prevented by banded soil applications of Mn (Mn sulphate) followed by two to three foliar applications of Mn during vegetative growth. In the case of mildly deficient soil, the deficiency can be corrected by one to two foliar sprays of Mn (Reuter *et al.*, 1988b; Walter, 1988). A high seed Mn content supplied naturally or artificially (coating with, or soaking in, $MnSO_4$) can improve plant growth and grain yield on Mn deficient soils (Marcar and Graham, 1986; Longnecker *et al.*, 1991b; Asher and

Graham, 1993). Evidence suggests none of these techniques completely eliminate Mn deficiency or allow the crop to reach its full yield potential (Graham, 1988).

1.4.3 Genetic solutions to manganese deficiency

1.4.3.1 Genotypic variation for Mn efficiency

The inadequate agronomic solutions to Mn deficiency, arising from low availability of both native and applied Mn in alkaline soil, the low residual value of applied fertiliser and the possibility of yield loss due to unrecognised subclinical deficiencies (Robson and Snowball, 1986), has made the genetic solution of breeding for Mn efficiency attractive. Tolerance of Mn-efficient genotypes to cereal cyst nematodes (*Heterodera avenae*), take-all disease (*Gaeumannomyces graminis*) (Wilhelm *et al.*, 1985; 1990) and powdery mildew (Jenkyn and Bainbridge, 1978; Graham, 1980) is one advantage of improving Mn efficiency. The development of a more extensive root system and, consequently, better tolerance to drought (Nable *et al.*, 1984), improved crop establishment and ultimately higher yield and quality of product (Fales and Ohki, 1982) are other factors of the agronomic case for breeding for Mn efficiency.

Genotypic differences in response to Mn have been observed since the 1920's, even before the element was recognised as being essential (Graham, 1988). Since then, considerable variation has been reported in diverse crop species (Graham, 1988). The most sensitive, and also the most tolerant, species to Mn deficiency have been reported to be either in the *Gramineae* or *Leguminosae* families (Graham, 1988). That intra-specific variation is as extensive as inter-specific variation in tolerance to Mn deficiency has made the generalisation of tolerance to Mn deficiency between plant families and also between

species difficult (Graham, 1988). Among cereals, rye, triticale, barley, wheat and durum wheat have been reported in descending order of efficiency to both Mn (Graham, 1988; Kaur *et al.*, 1989b) and Zn (Cakmak *et al.*, 1996).

Higher levels of variation for tolerance to Na (Shah *et al.*, 1987), B (Moody *et al.*, 1988; Chantachume, 1995) and Al toxicity (Foy and Da Silva, 1991) have also been reported in bread wheat compared to durum wheat, so greater potential exists for developing lines tolerant to mineral toxicity in bread wheat. However, no genotypic variation for tolerance to nutrient deficiencies has yet been confirmed for durum wheat. Brooks (1993) reported observations of genotypic differences in the development of Mn deficiency symptoms in a range of genotypes from the world durum growing countries sown at Marion Bay (Mn-deficient) and Coonalpyn (multiple-micronutrient deficient, dominantly deficienct in Mn) site, South Australia.

The fact that durum wheats have been employed as intolerant check genotypes in micronutrient studies is evidence of the intolerant nature of current durum wheat cultivars. The current poor tolerance of durum wheat to both micronutrient toxicities or deficiencies is due to the fact these breeding objectives (development of crops for less favourable environments) have not yet received adequate attention.

1.4.3.2 Mechanisms of manganese efficiency

Graham (1988) proposed five possible plant mechanisms for Mn efficiency:

(i) Superior internal utilisation or lower functional Mn requirement.

(ii) Improved internal redistribution.

(iii) Faster specific rate of absorption from the soil solution at low Mn concentrations (low Km, high Vmax).

(iv) Better root geometry.

(v) Greater root excretion of substances into the rhizosphere to mobilise insoluble Mn:

a) H^+,

b) reducing substances,

c) Manganese binding ligands,

d) microbial stimulants.

However, the following evidence argues that none of the mechanisms proposed above is likely to be the mechanism of Mn efficiency:

(i) Consistency of the critical Mn concentrations in YEBs of bread wheat genotypes differing in Mn efficiency; in addition to the fact that Mn-efficient genotypes absorb more Mn from the soil (Marcar and Graham, 1987a). This is the evidence argue against the importance of either superior internal utilisation or lower functional Mn requirements being factors in Mn efficiency.

(ii) Under Mn-deficient conditions, in which genotypic differences have been observed, Mn content of both efficient and inefficient roots have been low, so improved internal redistribution is unlikely to contribute to the very large differences in Mn content of shoots observed in those genotypes (Pearson and Rengel, 1995a; 1995b).

(iii) Munns *et al.* (1963) observed no genotypic differences in the rate of Mn absorption per unit of root length among six oat genotypes, chosen as the extremes in Mn efficiency. This is inconsistent with a faster specific rate of absorption at low Mn concentration being the mechanism for Mn efficiency.

(iv) Harbard (1992) did not observe significant differences in root system geometry of barley genotypes differing in Mn efficiency, nor did Graham (1984) notice any differences between a Cu-efficient 5R wheat translocation line and the Cu-inefficient parent line.

(v) The change of rhizosphere pH by root excretion of H^+, or the release of root exudates such as amino and organic acids and phenolics, directly (causing the dissolution of Mn oxides) or indirectly (serving as substrate or stimulant to microorganisms) is likely to affect the availability of nutrients. The efficiency of these processes is not favoured in soils of high pH where Mn deficiency is a major problem.

There has been an increased number of Mn reducers in the rhizosphere of Mn-efficient wheat genotypes under Mn deficiency than under control conditions (Marschener *et al.*, 1991; Rengel, 1997). Therefore, it is quite possible that genetic control of Mn efficiency is expressed through the composition of root exudates rather than through changes in root morphology or physiological functions in response to Mn deficiency. However, this may not imply that the other responses do not contribute, but that they have been difficult to measure (Marschner, 1988). Since no definitive mechanism has yet identified, screening for genetic efficiency must be undertaken empirically.

1.4.3.3 Screening for manganese efficiency

Graham (1984) discussed the principles governing development of screening techniques for selection of micronutrient-efficient genotypes. The complexity of screening techniques for micronutrient efficiency compared to those for micronutrient toxicity has also been discussed in detail (Vose, 1990). He emphasised higher efficiency (reflecting the field

response) and simplicity (ease of handling) of identifying the genotypes tolerant to micronutrient toxicity compared to micronutrient deficiency (Vose, 1990)

At the Waite Campus, a reproducible pot bioassay has been developed (Uren *et al.*, 1988). Calcareous soil (approximately 80% $CaCO_3$ with pH=8.5) from Wangary, a Mn-deficient site on the Eyre Peninsula of South Australia, is incubated at 25% gravimetric water content in a growth cabinet for three weeks before sowing. Basal nutrient can be added before or after incubation without affecting the availability of Mn to seedlings. Pre-incubation of soil (moistening the air dry soil at low temperature) favours the microbial oxidation of Mn and decreases the Mn uptake by plant, intensifying the Mn deficiency.

Longnecker *et al.* (1988) proposed a single level screening method (one level of Mn application to soil) and use of deficiency symptoms on a 1-5 scale as a selection criterion for segregating populations derived from crossing Mn-efficient by Mn-inefficient barley genotypes. They employed chlorosis score rather than any yield dependent criterion such as shoot Mn content. Huang *et al.* (1996) improved the screening technique by using a larger (6.5 cm diameter x 15 cm) pot size than that previously used (2.5 cm diameter x 16.5 cm) as Mn efficiency was better expressed in the larger pots. Furthermore, better ranking of genotypes for Mn efficiency was achieved by comparing shoot Mn concentration than using chlorosis score in barley genotypes studied under controlled environmental conditions. However, a completely reliable and accurate screening technique based on either direct gene product, or preferably on actual gene(s) differences, still remains an urgent need as the current methods have not been developed to a desirable level of effectiveness.

1.4.3.4 Confounding effects of seed reserves of manganese

The seed Mn content of barley genotypes grown in South Australia was reported to vary from 7.7 to 21.2 μg seed^{-1} (Uren *et al.*, 1988). Larger variation in seed Mn content (0.15-6.38 μg seed^{-1}) equivalent to seed Mn concentrations of 4.1 to 153.1 mg kg^{-1} was recorded for bread wheat genotypes (Marcar and Graham, 1986). Variation of seed Mn content of bread wheat cultivars grown at the same site was less than the observed variation between sites (Marcar and Graham, 1986). Soil types seemed to induce a larger effect on the variation in loading of Mn into the seed than differences in climatic conditions within and between sites (Uren *et al.*, 1988).

Seed Mn content affected early growth, particularly in Mn-deficient soil (Singh and Bharti, 1985; Marcar and Graham, 1986). Similarly, increasing seed Mn content improved the number of tillers and rate of phenological development of the crop (Longnecker *et al.*, 1991b), and the severity of Mn deficiency symptoms was dependent on the seed Mn content of wheat genotypes (Marcar and Graham, 1986). Therefore, in screening for Mn efficiency in pot studies, seed of genotypes obtained from different sources or the same source with different Mn contents can not be compared because of the confounding effect of seed Mn content through its effect on seedling vigour. Seed Mn content can affect the severity of deficiency symptoms, alter the yield and yield dependent characters, and therefore, alter the genotypic ranking for Mn efficiency. Sparrow *et al.* (1983) proposed that the problem of seed Mn content could be overcome, to a certain extent, by cutting the plant back at early tillering and scoring the regrowth. The cutting treatment removed much of the effect of seed reserves, equalised the Mn status of the genotypes and restored the ranking order. However, genotypic variation for regrowth unrelated to Mn efficiency made this approach unreliable.

1.4.3.5 Mode of inheritance of manganese efficiency

The development of Mn-efficient varieties would be facilitated if the mode of inheritance of Mn efficiency was well understood. Graham *et al.* (1983) undertook a pedigree analysis of 72 barley genotypes from a world collection in an attempt to understand the genetics and define the source of Mn efficiency in barley. It was observed that one efficient parent was common in the parentage of most of the efficient types, and one inefficient parent was common in parentage of most of the inefficient genotypes. Hence they proposed that a simple genetic system controlled Mn efficiency in barley.

In a study of 100 F_2 individuals from a cross of Mn-efficient (Weeah) and Mn-inefficient (Galleon) barley, transgressive segregation was observed using Mn content of vegetative tissue as a criterion, while in another study (a cross of Weeah by Mn-inefficient WI 2585) a 3:1 ratio was observed when chlorosis score was employed (McCarthy *et al.*, 1988). The latter observation and the result of the earlier pedigree analysis led to the conclusion that control of Mn efficiency in barley was governed by a single, major, dominant gene (Graham, 1988). Further study and the observation of close linkage of Mn efficiency to one group of RFLP markers on chromosome 4HS supported the hypothesis that control of Mn efficiency in barley is by one gene (M. Pallotta, personal communication), though there appear to be modifying minor genes.

The comparison of observed variances to expected variances for seedling Mn concentration of 85 F_2 single plants from a cross of Haruna nijo (Mn-efficient) x WI2585 (Mn-inefficient) was in agreement with the single gene model rather than a two gene model (M. Pallotta, personal communication). The genctics of Mn efficiency in soybean was investigated under field conditions by Graham *et al.* (1995). In a study of F_2 and F_2

derived F_3 families of a cross of Mn-efficient by Mn-inefficient soybean genotypes, a digenic mode of inheritance was proposed.

1.5. Durum wheat

1.5.1 Durum wheat classification and adaptation

Wheats belong to the genus *Triticum*, a member of the Gramineae family and Horedeae tribe, evolved from wild grasses found growing in the Eastern Mediterranean, Western Asia and Central Asia in places where other similar cereal crops were domesticated. They can be classified into three groups, characterised by having genomes of a diploid (14 chromosomes, seven pairs) with a polyploid series of tetraploid and hexaploid (Bozzini, 1988). On the basis of having genomic constitution AA, AABB or AABBDD, wheats are classified into three species: *T. monococcum* (AA), *T. turgidum* (AABB) and *T. aestivum* (AABBDD), with an additional polyploid series of the tetraploid *T. timopheevi* (AAGG) and hexaploid *T. zhukovskyi* (AAAAGG) having a G instead of a B genome. The source of A and D are *T. monococcum* and *T. tauschii* (*Aegilops squarrosa*) respectively, while the genome B is probably from *T. speltoides* and *T. urartu* (Kimber and Sears, 1987). The wild types of current tetraploid wheat, *T. dicoccoides* type, are mostly found in Palestine, Syria and Lebanon. Domestication resulted in types resembling *T. dicoccum* , which spread from West Asia to Egypt and to Ethiopia; and later, the more advanced type (*T. turgidum*) which spread to Europe, West and Central Asia and North Africa (Bozzini, 1988). All domesticated and wild types of tetraploid wheat having genome AABB have been grouped into one valid species, *T. turgidum* L. , which in turn is subdivided into several subspecies and botanical varieties: *T. carthlicum, T. dicoccoides, T. dicoccon, T. dicoccum, T. durum* and *T. polonicum* (Kimber and Sears, 1987). The *T. turgidum* L.

var *durum* is the most important cultivated form of the tetraploids. It is adapted to semiarid environments, occupying 10% of the wheat-cultivated area (Nachit, 1996). The area of adaptation of bread wheat overlaps the durum wheat area; however, inferior bread making quality, lower tolerance to harsh environments (lower tolerance to cold and abiotic stresses), lower ploidy level, separate evolutionary pathway and smaller breeding effort account for the narrower adaptation of durum wheat compared to bread wheat (Joppa and Williams, 1988). The Mediterranean basin, West Asia and North America are leading production regions, while the first of these remains the major consumer and importer of durum wheat (Nachit, 1996).

1.5.2 Aneuploidy in durum wheat

Bread wheat (*T. aestivum* L.) has a close cytogenetic relationship to durum wheat: both have A-and B-genome chromosomes. Individual homoeologous chromosomes in three genomes (A, B and D) of wheat have been found to contain genes for similar characteristics, and so they are able to compensate by homoeologous substitution for one another (Sears, 1966). The removal (aneuploidy) or addition of a chromosome can be used to determine and study the effect of gene(s) on that chromosome, locate a gene to a chromosome, map genes to the centromere, to transfer chromosomes from one cultivar or species to another and to identify chromosomal homoeologies. Genes located to chromosomes or chromosome arms in hexaploid wheat by use of these aneuploid lines include: those controlling morphological and physiological traits, pest and disease resistance and DNA marker loci (McIntosh, 1987). The loci controlling characters on a chromosome are usually represented by homoeologous loci on the corresponding chromosomes of the other two genomes (Gale *et al.*, 1989; Hart, 1994); however, there

have been some exceptions (e.g. the pest resistance and some mutant characters) (Hart, 1994). Some alleles of interest may be found in a specific variety within a species, but may not necessarily be homologous to the alleles controlling a similar character in another species (Moore *et al.*, 1993).

Aneuploidy has not been extensively used in genetic studies of tetraploids (*T. turgidum* L.), mainly because reduction of chromosome number in tetraploids is more deleterious than an increase in chromosome number (Joppa and Williams, 1988). However, a complete set of disomic substitution lines has been produced in durum wheat, by substituting a pair of D-genome chromosomes from Chinese Spring hexaploid wheat into Langdon durum wheat, where they replace a pair of A or B genome homologous chromosomes (Joppa and Williams, 1983a). The D-genome substitution lines in Langdon can be used for determination of the chromosomal location of genes, producing homologous disomic substitutions of chromosomes from one line into another and inducing translocations between homoeologous chromosomes. They can also be used in appropriate crosses to determine the chromosomal location of genes in the A or B genomes by methods similar to those in monosomic analysis in hexaploid wheat (Joppa, 1987). The location of gliadin structural genes was detected using D-genome substitution lines (Joppa and Williams, 1983b). Jamjod (1996) also used this stock in her study determining the location of genes conferring tolerance to high and toxic concentrations of boron in durum wheat.

1.6 Marker assisted selection

Marker assisted selection (MAS) is probably the most commonly claimed application for molecular markers in plant breeding. The other important applications of molecular markers in cereal breeding such as pyramiding genes, backcross analysis, analysis and selection of quantitative traits, analysis of alien chromosome segments and varietal identification through DNA fingerprinting, and other uses, have been discussed by Langridge (1994).

Development of markers has gone through various evolutionary phases. From the morphological markers that are derived from mutated characters which provided the basis of modern genetics, interest evolved to the detection of isoenzyme and protein variations which demonstrated the usefulness of markers in genetic analysis and breeding. The morphological and isozyme markers are based on the difference between expressed functional genes, and as such represent a small part of a whole genome (Langridge, 1994). Current interest in molecular markers derived from a much larger proportion of the genome that has the potential for detecting polymorphic sequences with or without plant phenotype differences. The complexity of the wheat genome has been the major limitation for application of molecular marker techniques to wheat breeding (Langridge, 1994).

1.6.1 Restriction Fragmented Length Polymorphism (RFLP)

RFLP is a DNA based marker technique based on the detection of sequence variation in genomic DNA by a combination of restriction endonuclease enzymes (which recognise specific sequences in DNA strands) and sequence-specific DNA probes. Different sized DNA fragments derived from a restriction digest are visualised on X-ray films by either

chemical or radioactive labelling (Guesella, 1986). In some plant species, RFLPs are able to detect high levels of polymorphism and are useful for map based cloning. The low levels of detected polymorphism between wheat varieties for the RFLP markers, and the fact that it is technically difficult to perform RFLP analysis, limits its application in MAS to wheat (Langridge, 1994).

1.6.2 Polymerase Chain Reaction (PCR) based markers

PCR based markers are an alternative to RFLPs. PCR, utilising specific or random primers, can be used for amplification of specific sequences of DNA and the detection of polymorphisms. The Randomly Amplified Polymorphic DNA (RAPDs) method is a PCR based finger printing technique which uses random primers, but has been considered unreliable because results have not been adequately reproducible. The other PCR based methods (Simple Sequence Repeat markers (SSRs) or micro satellite, Inter-Simple Sequence Repeat Amplification (ISA) and Amplified Fragmented Length Polymorphisms (AFLPs)) have not been adequately tested in wheat (Langridge, 1994).

1.6.3 Amplified Fragmented Length Polymorphism

AFLP is a new DNA finger printing technique developed by Vos *et al.* (1995). Finger printing is produced by AFLP technique, without prior knowledge of the sequence, using a limited set of generic primers. The reliability of RFLP and power of PCR are combined in this technique. The technique resembles RFLP in the detection of genomic DNA, with the major difference that PCR amplification instead of Southern hybridisation is used for

detection of restriction fragments (Vos *et al.*, 1995). The potential for analysis of a large number of markers is a major positive feature of this technique.

Milbourne *et al.* (1997) investigated the value of three techniques (AFLPs, SSRs and RAPDs) by examining the genetic relationships within primary north-western European cultivated potato gene pools. All three approaches discriminated between the sixteen cultivars. The usefulness of each technique was examined in terms of number of loci (effective multiplex ratio) and the amount of polymorphism detected (diversity index). AFLPs had the highest effective multiplex ratio and the SSRs the highest diversity index. The superiority of AFLPs compared to RAPDs in terms of reproducibility and number of polymorphic loci per PCR reaction was emphasised by Akerman *et al.* (1996) in a study of European white birch, using two curly birch (*Beta pendula f. carelica*) trees and their full-sib progeny. The higher efficiency of AFLPs compared to RFLPs was demonstrated in a study of soybean germplasm by Lin *et al.* (1996). The latter technique is cumbersome as it requires Southern blotting and probe hybridisation. The failure of nearly 50% of RFLPs in distinguishing even a single polymorphic band was notable. Vogel *et al.* (1996) compared the utility of four marker systems (RFLPs, RAPDs, SSRs, and AFLPs) in genome analysis of twelve soybean genotypes. The AFLP was the most satisfactory marker system, with its utility being apparent in its effective multiplex ratio and expected heterozygosity.

The potential of AFLP analysis in detection of a large number of independent loci has made it suitable for a wide array of genetic investigations including:

(i) Assessing the extent of variation in cultivated and wild species and determination of genetic relationship between accessions and varieties.

Paul *et al.* (1997) employed AFLP markers to detect diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinesis* L. *O. Kuntze*) of different origins which could not be distinguished on the basis of morphological and phenotypic traits. It was also used in studies of diversity, evaluating and analysing the genetic structure between and within the gene pool of a wild bean (*Phaseolus vulgaris*) core collection (Tohme *et al.*, 1996). DNA finger print patterns of 114 genotypes were analysed, leading to the recognition of major gene pools in the different geographical sources of origin of the wild bean. The data produced permitted a greater insight into the genetic structure of the wild bean than any other methods of analysis (Tohme *et al.*, 1996). Investigating the genetic diversity among *Vitis vinifera* L. ecotypes and identification of clones has been another application of AFLP and Inverse Sequence-Tagged Repeat (ISTR) analysis. The two techniques were employed successfully in the study of genetic biodiversity in two *Vitis vinifera* L. *Sangiovese* and *Colorino* genotypes (Sensi *et al.*, 1996).

The study of ecogeography and the genetics of salt tolerance in accessions of wild barley (*Hordeum spontaneoum C. koch*) was another area of investigation using AFLP techniques (Pakniyat *et al.*, 1997). They demonstrated that genotypes from the same site of origin can exhibit very similar AFLP profiles and sharp genetic differences could be detected between genotypes separated by relatively short distances. Twelve AFLP markers were found to be associated with shoot Na^+ content and shoot carbon isotope composition, and that these were associated with site of origin ecogeographic data, particularly longitude. The markers were partitioned into groups, with significant association within groups but no significant association between groups. Using multiple regression analysis three AFLP markers from separate groups accounted for more than 60% of observed variation for shoot Na+ content. The study of phylogenetic

relationships and analysis of diversity among *Lactuca* spp (Hill *et al.*, 1996) and lentil accessions (Sharma *et al.*, 1996) have been other applications of AFLP techniques. (ii) AFLPs as genetic markers and construction of linkage maps.

AFLP marker systems have been the most useful approach for generating high density genetic maps via their integration into pre-existing RFLP maps. This has been carried out for barley (Becker *et al.*, 1995; Powell *et al.*, 1997), sugar beet (Schondelmaier *et al.*, 1996), soybean (Maughan *et al.*, 1996; Keim *et al.*, 1997) and rice (Mackill *et al.*, 1996). The outcomes were an extended linkage map in sugar beet, and filling in of gaps on the maps of barley chromosomes 2HL, 4HL and 6H, to which few RFLP loci had been mapped, giving greater genome coverage and the revelation of some new quantitative trait loci (QTL) locations as result of sampling different regions of the genome by AFLP.

1.7 Conclusions

Manganese deficiency is widespread throughout the world, and possibly the most severe micronutrient disorder in South Australia. It is a major limitation to growth and to the development of durum wheat as a new crop in South Australia. The current poor tolerance of durum wheat to nutritional disorders has confined cultivation to highly fertile soils and prevented expansion to low fertility duplex soils and low rainfall districts where comparatively high grain protein level could be attained.

The preference of a genetic solution to the problem of intolerance to Mn deficiency, rather than agronomic solutions, has been discussed. Considerable genetic variation for tolerance to Mn deficiency has been recognised in a wide range of crop species, but genetic variation has not been established for durum wheat.

Considering the importance of durum wheat as a promising and rapidly expanding crop, this project began with the following objectives to tackle the problem of intolerance to Mn deficiency in durum wheat:

(i) To identify and determine the extent of genotypic variation in durum wheat for tolerance to Mn deficient soils.

(ii) To develop selection criteria.

(iii) To carry out genetic analysis to determine the source of Mn efficiency in durum wheat.

(iv) To study the mode of inheritance in a cross of a Mn-efficient by a Mn-inefficient genotypes.

(v) To identify the location of genes on chromosomes, and attempt to develop markers for breeding for Mn efficiency using AFLP techniques.

Chapter 2

Screening for manganese efficiency in durum wheat (*Triticum turgidum* L. var *durum*)

2.1 Introduction

In South Australia, single and multiple micronutrient deficiencies have been recognised and are widespread (Reuter *et al.*, 1988a). The commercial cultivation of a durum wheat variety Yallaroi in South Australia began in 1990, but has been confined to deeper, fertile soils. Its performance in low fertility and low rainfall districts is considered poor (Brooks, 1991; Brooks *et al.*, 1994). The fact that Yallaroi has been used as a susceptible check genotype in screening for tolerance to soils with high concentrations of B and low availability of micronutrients, confirms its poor performance as compared to bread wheats, and partly explains its adaptation mainly to fertile soils.

The poor tolerance of durum wheat varieties to micronutrient deficiency is due to the fact that breeding for less favourable environments has not received attention. To broaden the adaptation of durum wheat to micronutrient-deficient soil demands:

(i) diagnosis of the extent and nature of the constraint and determination of whether a genetic approach is justifiable,

(ii) presence of adequate genotypic variation within the species to mitigate the problem,

(iii) development of a precise screening technique and selection criteria, and

(iv) incorporation of genetic variation for tolerance into current germplasm and selection of superior genotypes through plant breeding.

Manganese deficiency was the first trace element deficiency recorded in South Australia (Samuel and Piper, 1928), and is one of the most severe micronutrient disorders in a large area of calcareous soils of the state's agricultural zone. It is a serious problem, restricting the expansion of durum wheat, which is intolerant to Mn deficiency. Considering the inadequate agronomic solutions to the problem, the genetic alternative remains the best approach. Although variation in the expression of Mn efficiency has been reported in an array of cultivated crops and on a range of soils (Graham, 1988), durum wheat has been ranked as least tolerant among the cereals to deficiency of Zn (Graham *et al.*, 1992; Cakmak *et al.*, 1996) and Mn (Graham *et al.*, 1983).

Genetic variation for tolerance to nutrient deficiency traits had not been reported for durum wheat prior to the work beginning in South Australia.

Brooks (1993) reported the observation of differences in development of Mn deficiency symptoms in a range of twenty genotypes from different sources (world durum growing countries) planted at Marion Bay (lower Yorke Peninsula, a Mn-deficient) and Coonalpyn (145 km south east of Adelaide) of South Australia, a multiple-micronutrient deficient site. Further preliminary screening of 69 genotypes (including accessions and advanced lines from exotic sources) at both sites as an observation nursery revealed (Table 2.1):

(i) generally higher expression of leaf chlorosis symptoms of Mn deficiency in durum compared to bread wheat,

(ii) presence of severe to moderate Mn deficiency at Marion Bay and at Coonalpyn in terms of the expression of deficiency symptoms, and

(iii) Observation of a wide range in the severity of symptoms between genotypes from CIMMYT and ICARDA origins. Genotypes from the Mediterranean basin were moderate in expression of symptoms and genotypes from Australia, Canada and North Dakota expressed the most severe deficiency symptoms.

Longnecker *et al.* (1988) proposed screening at a single Mn level and the use of chlorosis scores in genetic studies of barley. The screening technique was further improved by use of a larger pot size through which better genotypic discrimination was obtained (Huang *et al.*, 1996). Further, better ranking of barley genotypes for Mn efficiency was reported on the basis of shoot Mn concentration (Huang *et al.*, 1994). However, in the large-scale evaluation of durum wheat genotypes or in studies of segregating populations, reliable and accurate seedling-based selection criteria remained an urgent need.

This chapter describes a series of experiments aimed at investigating the presence of genetic variation for Mn efficiency in durum wheat cultivars, and development of a selection criterion for screening the genotypes and genetic material required as a basis for improvement of Mn efficiency in durum wheat. The objectives of the work in this chapter were :

(i) to confirm variation among durum genotypes in the expression of Mn deficiency symptoms observed in the field through a study of reactions to different levels of applied Mn in a controlled environment.

(ii) to determine the critical concentration of Mn for diagnosis of Mn deficiency in the seedlings of three durum genotypes.

(iii) to evaluate a range of selection criteria for their value in subsequent screening and genetic studies through a parallel study in the field and in controlled environment conditions.

2.2 Study of the response of three durum wheat genotypes to increasing levels of manganese application to soil

An experiment was conducted to investigate the response of three genotypes representative of the range in expression of leaf chlorosis symptoms observed at the field sites (Marion Bay and Coonalpyn, 1994) (Table 2.1). These were grown at nine levels of soil applied Mn and replicated three times in a controlled environment chamber.

2.2.1 Materials and methods

Genotypes

This pot bioassay used three durum wheat genotypes (Re/Dac//Teal, Senatore Cappelli and Cando) that had expressed a gradient of Mn deficiency symptoms at Coonalpyn and Marion Bay in 1994 (1.5-2.5, 2.0-3.0 and 3.5-4.0, respectively, based on 1 to 5 visual score, where 1 represents nil and 5 the greatest expression of deficiency symptoms) (Table 2.1). Seed of each genotype with similar Mn content (0.10 μg Mn per seed) was obtained from the obscrvation nursery of 69 genotypes (including accessions and

advanced lines from exotic sources) planted at Marion Bay in 1994. The origin and parentage/pedigree of these lines is given in Table 2.1.

Soil and pots

A calcareous sand of approximately 80% $CaCO_3$ and pH 8.5 (Huang *et al.*, 1994) was collected from a severely Mn-deficient site at Wangary, South Australia in 1994. Air-dried top soil (0-10 cm) and subsoil (10-20 cm) were sieved through a 1 mm stainless steel sieve and stored separately in plastic bags. The topsoil and sub-soil (1:1, w/w) were well mixed and water added to 20% (w/w). The wet soil was then incubated at 15°C day/10°C night for three weeks as described by Huang *et al.* (1994). Prior to potting and planting, the following basal nutrients were added in solution and mixed thoroughly with the incubated soil (Longnecker *et al.*, 1988) to maintain optimum fertility for all nutrients except Mn: (mg/kg soil) $Ca(NO_3)_2.4H_2O$, 918; K_2SO_4, 114; KH_2PO_4, 144; $MgSO_4.7H_2O$, 140; $FeSO_4.7H_2O$, 17.2; H_3BO_3, 5.6; $ZnSO_4.7H_2O$, 26.4; NaCl, 12.8; $CuSO_4.5H_2O$, 3.0; $CoSO4.7H_2O$, 0.9; $H_2MoO_4.H_2O$, 0.9. Clear plastic pots covered with aluminium foil to exclude light and lined with polythene bag were filled with 450 g dry soil.

Manganese levels

The genotypes were evaluated at nine levels of Mn supply: 0, 10, 30, 60, 90, 120, 160, 240 and 300 mg Mn/kg dry soil (pure $MnSO_4.4\ H_2O$ added in solution).

Durum wheat seeds were surface sterilised (30% ethanol for 30 seconds, rinsed in milli Q water, immersed in 5% sodium hypochlorite for 1 min, and again rinsed with milli Q water) (Wilhelm *et al.*, 1988), soaked overnight in aerated water at 4°C and left 24 hr in

Petri dishes on moistened filter paper in an incubator (20°C) for uniform germination. The germinating seeds were sown five to a pot and grown in a controlled environment chamber with a 10 hour light/14 hour dark photoperiod at 15°C day/10°C night. The photon flux density at the surface of the pots was maintained at 500 mol/m^2/s by adjustment of the light height. On day 7, the seedlings were thinned to three per pot.

The pots were watered daily and the moisture content was maintained as near as possible to 20% (w/w). Thirty five days after sowing (DAS) the seedlings were harvested, separated into youngest emerged leaf blade (YEB), shoots (severed at ground level) and roots. Roots were washed with reverse osmosis water (RO) and three times with milli Q ultra pure water. All plant material was oven-dried (70°C), digested in 70% nitric acid and analysed for mineral elements by ICP spectrometery (Zarcinas *et al.*, 1987).

The presented data are the means of three replicates, subjected to analysis of variance using Super Anova (statistical package) installed on Macintosh computers.

Critical level of Mn deficiency

For determination of the critical level of Mn deficiency a modified Mitscherlich response equation (Ware *et al.*, 1982) represented by

$$Y = \beta(1-\gamma e^{-\alpha x})$$

was calculated to quantify critical Mn deficiency levels by characterising plant growth as a function of tissue nutrient concentration.

Mitscherlich established a growth law model for plant species by quantifying the relationship between yield and nutrient supply in soils from both field and pot experiments

(Ware *et al.*, 1982). Mitscherlich's plant growth model was based on the rate equation given by

$$dy/dx = \alpha(\beta - y)$$

In applying this model for determining critical nutrient values for plant tissue, y denotes plant yield at a tissue concentration of x, β asymptotic maximum yield as x approaches infinity, $(\beta - y)$ the decrement from maximum yield, and α the constant of proportionality. Integrating the above equation, with the assumption that y equals zero at tissue nutrient concentration zero, gives the Mitscherlich growth model written as

$$y = \beta(1 - e^{-\alpha x})$$

The assumption that at $x = 0$, $y = 0$ is too restrictive. Under an initial assumption that at $x = 0$ there is some yield, y_0, results in a modified model written as

$$y = \beta(1 - \gamma e^{-\alpha x})$$

where $\gamma = (\beta - y_0)\beta$. The parameters α, β and γ of the above equation can be estimated from the observed data. To calculate the tissue nutrient concentration corresponding to 90% of maximum yield let

$$y/\beta = 1 - \gamma e^{-\alpha x} \quad \text{where} \quad y/\beta = 0.9$$

Solution of the above equation for x, the critical nutrient concentration, gives

$$x = \ln(0.1/\gamma)/\alpha$$

This model was applied in calculating the critical level of Mn deficiency for the seedlings of three durum genotypes (35 DAS) grown at nine levels of soil Mn supply in a controlled environment chamber. The result was compared with the critical level obtained from the

regression of relative shoot dry weight against Mn concentration of YEB. In utilising the Mitscherlich model for solving the critical nutrient deficiency level, careful attention must be given in applying the growth model to nutrient calibration exhibiting toxicity or Steinberg effects (Ware *et al.*, 1982). In attempting to fit the model, the experimental points giving rise to these effects (reduction of yield due to toxicity) must be omitted from the data set.

2.2.2 Results

Manganese concentration of the YEBs of the three durum wheat genotypes increased with increasing soil applied Mn from 0 to 360 mg/kg soil, and the genotypes did not interact statistically ($P<0.01$) with Mn supply (Fig 2.1) (see Appendix A, Table A1 for analysis of variance). The shoot Mn concentration also tended to increase as soil Mn supply increased but the genotypes, in contrast with the results for the YEBs, interacted with Mn level (Fig 2.2) (see Appendix A, Table A2 for analysis of variance). The differences between the genotypes were statistically significantly for YEBs Mn concentration up to 240 mg/kg of soil applied Mn (Fig 2.1), but was no longer statistically significant at 360 mg/kg dry soil, and there was clear discrimination of genotypes throughout the whole range on the basis of shoot Mn concentration (Fig 2.2). However, while Senatore Cappelli had the lowest YEBs concentration, Cando had the lowest shoot concentration.

The Mn concentration of roots responded positively to addition of Mn and the genotype by Mn interaction was significant ($P<0.01$). There were no significant difference between genotypes in root Mn concentration at low Mn supply up to 30 mg/kg dry soil and again Cando had the lowest concentration (Fig 2.3) (see Appendix A, Table A3 for analysis of variance).

The shoot dry weight of the genotypes responded positively but differently ($P<0.01$) to applied Mn and reached a plateau around 160 to 240 mg/kg and then decreased. Senatore Cappelli had the lowest shoot dry weight in all treatments except 0 mg Mn/kg dry soil (Fig 2.4) (see Appendix A, Table A4 for analysis of variance). The root dry weight and seedling dry weight (shoot + root) also showed the same trend as shoot dry weight upon addition of soil Mn (Fig 2.5) (see Appendix A, Tables A5 and A6 for analysis of variance).

The shoot Mn content (shoot dry weight x shoot Mn concentration) and root Mn content tended to increase throughout the range of applied Mn (Figs 2.7 and 2.8) (see Appendix A, Tables A7 and A8 for analysis of variance). The trend in seedling Mn content (shoot Mn content + root Mn content) of genotypes to the addition of Mn was the same as shoot Mn content and root Mn content (Fig 2.9) (see Appendix A, Table A9 for analysis of variance). Senatore Cappelli and Rea/Dac//Teal had similar uptake, except at 360 mg Mn/kg dry soil.

Genotypic discrimination on basis of the chlorosis score was observed up to 30 mg/kg; then only the inefficient genotype, Cando, had visual deficiency symptoms at 60 mg/kg (Fig 2.10) (see Appendix A, Tables A10 for analysis of variance).

The relative shoot dry weight (dry wt at Mn_x x 100/max shoot dry wt) increased up to 160 mg/kg, levelled around 160-240 mg/kg (Fig 2.11) (see Appendix A, Tables A11 for analysis of variance). In contrast, seedling Mn uptake (Fig 2.9) increased over the whole range of applied Mn.

The correlation coefficient matrix between the measured growth parameters (chlorosis score, YEBs Mn concentration, shoot Mn concentration, root dry weight, root Mn concentration, root Mn content, shoot dry weight, shoot Mn content, seedling dry weight,

Mn uptake and relative shoot dry weight) was determined at all levels of applied Mn (see Appendix B; Table 2.2 D-I). As the greatest genotypic differences (in measured parameters) were to be observed at the lowest level of applied Mn, emphasis will be given to the correlation coefficient matrix for 0, 10 and 30 mg Mn/kg (Table 2.2 A-C).

At the lowest Mn application rate (Mn=0.0 mg kg/dry soil) significant correlations were observed (Table 2.2 A) for:

(i) Root dry weight versus root Mn content (0.80**), where smaller genotypic differences were observed, compared to higher level of Mn application, rates, in root growth and parallel to it in root Mn content.

(ii) Seedling dry weight versus relative shoot dry weight (0.85**) as a reflection of both the inherent correlation between a parameter and a ratio derived from it and the genotypic variation in shoot dry weight at severe Mn deficiency being in agreement with relative shoot dry weight ((shoot dry weight at deficient level of soil applied Mn x 100)/ (shoot dry weight at sufficient level of applied soil Mn)).

(iii) Relative shoot dry weight versus chlorosis score (-0.63*) which reflects the negative relationship between shoot growth and the development of chlorosis.

(iv) Relative shoot dry weight versus the YEBs (0.83**) as an indication of genotypic variation in terms of shoot dry weight parallel to shoot Mn concentration and YEBs Mn concentration.

(v) Mn uptake versus shoot Mn content (0.97**) as would be expected as most of the Mn taken up is located in the shoot.

By increasing the supply of Mn (Mn=10 mg/kg dry soil), in addition to the above mentioned parameters, correlation between the following also became statistically significant (Table 2.2 B).

Shoot dry weight versus shoot Mn content (0.63*),

Shoot Mn content versus YEBs concentration (0.90**), shoot Mn content versus chlorosis score (-0.61*), and root Mn content versus chlorosis score (-0.79**),

Shoot Mn content versus seedling dry weight (0.71*).

This indicates the variation in genotypic response to applied Mn expressed in terms of root and shoot growth, also reflected in higher shoot and root Mn content which were in agreement with development of chlorosis and YEBs.

At the higher level of Mn (Mn=30.0 mg/kg dry soil) the correlation coefficients between all criteria became statistically significant, except shoot Mn concentration which correlated significantly with only the YEBs concentration and shoot content (Table 2.2 C). The genotypes mostly responded to addition of Mn by increasing shoot and root dry weights rather than shoot Mn concentration, and these were reflected by higher shoot and root Mn contents.

The critical Mn concentrations in YEBs corresponding to 90% relative shoot dry weight were calculated for all three genotypes using the modified Mitscherlich plant growth model (Ware *et al.*, 1982), and these were compared with the regressions of shoot dry weights as a function of YEBs Mn concentrations (Table 2.3). It seemed that using the Mitscherlich plant growth model led to over estimation of the critical levels. The critical levels of Mn deficiency for the three genotypes (Rea/Dac//teal, Cando and Senatore Cappelli) were marginally different from each other (Table 2.3). The hand fitted Mn level

for each genotype is given in Figs 2.12, 2.13 and 2.14 and these are less than the values estimated by the modified Mitscherlich plant growth model.

2.2.3 Discussion

In this experiment significant genotype by Mn interactions ($P<0.01$) for nearly all measured parameters (except YEBs) confirmed that there are genotypic differences in response to availability of Mn (see Appendix A, Tables A2-A11 for analysis of variance). In other words, Mn-efficient genotypes had the highest concentration of Mn in shoots, produced the most extensive root systems and the highest shoot dry weights, had the highest uptake of Mn and expressed the lowest chlorosis scores. These results demonstrate genetic variation in response to soil available Mn in durum wheat not previously reported.

The use of chlorosis score in screening was employed by Longnecker *et al.* (1988); however, its application is restricted to comparison of genotypes with low seed Mn reserves, since the expression of chlorosis symptoms is confounded by seed content (Longnecker *et al.*, 1991b).

Clear genotypic variation for root Mn concentration was not observed at low levels of Mn supply (0.0 to 30 mg Mn/kg dry soil). Shoot Mn concentration (Fig 2.2), the proposed criterion for screening barley genotypes for Mn efficiency (Huang *et al.*, 1994), appears to be inconclusive for durum wheat (Fig 2.2).

Genotypic differentiation was clearly observed ($P<0.01$) for shoot Mn content and uptake at deficient (10 to 30 mg/kg) levels of Mn supply (Figs 2.7 and 2.9). Higher uptake of Mn has already been observed in Mn-efficient barley (Huang, 1996) and wheat (Marcar

and Graham, 1987a); considering that efficient genotypes mobilise and accumulate more Mn than inefficient genotypes, it seemed that shoot Mn content is the most discriminating criterion for single level screening. Screening at both low and high levels of Mn and measurement of efficiency as relative shoot dry weight (Fig 2.11) or Mn uptake (Fig 2.9), as described by Graham (1984), provides information about the performance of genotypes at optimum fertility as well as at deficient levels of soil Mn.

Clear discrimination of genotypes under Mn deficiency (especially at 30 mg Mn/kg soil treatment) was observed for shoot and root dry weight, seedling dry weight, shoot Mn content (eg. at nil Mn it was; 0.15 µg, 0.10 µg, 0.50 µg and at 10 mg Mn/kg; 0.50 µg, 0.30 µg and 0.70 µg per pot was for three genotypes), root Mn content (eg. at nil Mn it was; 0.15 µg, 0.10 µg, 0.50 µg and at 10 mg Mn/kg; 0.50 µg, 0.30 µg and 0.70 µg per pot was for three genotypes) and chlorosis score (Figs 2.4, 2.5, 2.6, 2.7, 2.8 and 2.10) in this experiment. The highest coefficient of correlation between growth parameters (YEBs Mn concentration, shoot Mn concentration, shoot Mn content, chlorosis score and relative shoot dry weight) was obtained at Mn=30 mg/kg dry soil rather than at lower levels of Mn supply (Table 2.2 A-C). Therefore, Mn=30 mg/kg dry soil was selected for both the single level screening and as the lower level in a two level screening.

The adequate (+Mn) level of Mn for screening seemed to be around 160-240 mg Mn/kg soil, while the higher level (360 mg/kg soil) seemed deleterious to Rea/Dac//Teal (Fig 2.6).

The critical Mn concentration in YEBs for three durum genotypes (29-33 mg Mn/kg dry matter, Table 2.3) based on the modified Mitscherlich plant growth model, was higher than obtained (18-19 mg/kg dry weight) from hand fitted curves (Figs 2.12, 2.13 and 2.14). The Mitscherlich estimates were much higher than the critical level for field grown bread wheat (10-12 mg/kg) (Graham *et al.*, 1985) or barley (11 mg/kg) (Huang, 1996). This has not previously been reported. It appears that durum wheat has a higher internal

Mn requirement than bread wheat and barley, and this must be taken into account when diagnosing deficiency based on the chemical analysis of YEBs.

The result of this experiment confirmed that the variation observed in the field between genotypes in terms of both the visual chlorosis score and Mn concentration of YEBs at Marion Bay and at Coonalpyn was due to Mn deficiency, and reflected different responses of genotypes to this deficiency.

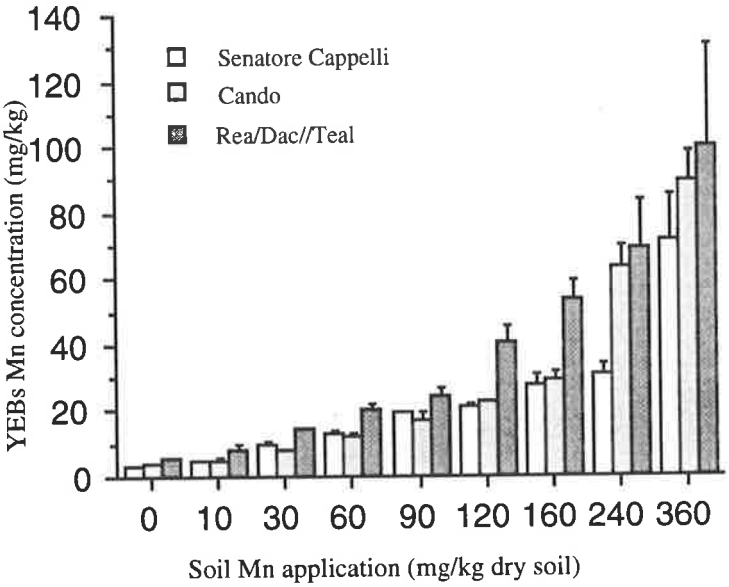

Fig 2.1 Manganese concentration (mg/kg) in YEB of three durum genotypes 35 DAS at nine levels of added Mn (mg/kg dry soil). Plants grown in Mn-deficient Wangary soil in pots in a controlled environment chamber. Error bars show standard error of means.

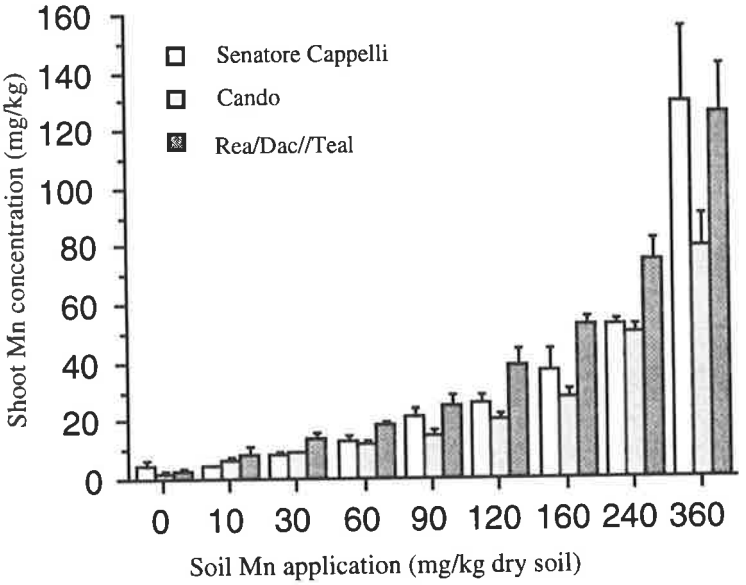

Fig 2.2 Shoot Mn concentration (mg/kg) of three durum genotypes 35 DAS at nine levels of added Mn (mg/kg dry soil). Plants grown in Mn-deficient Wangary soil in pots in a controlled environment chamber. Error bars show standard error of means.

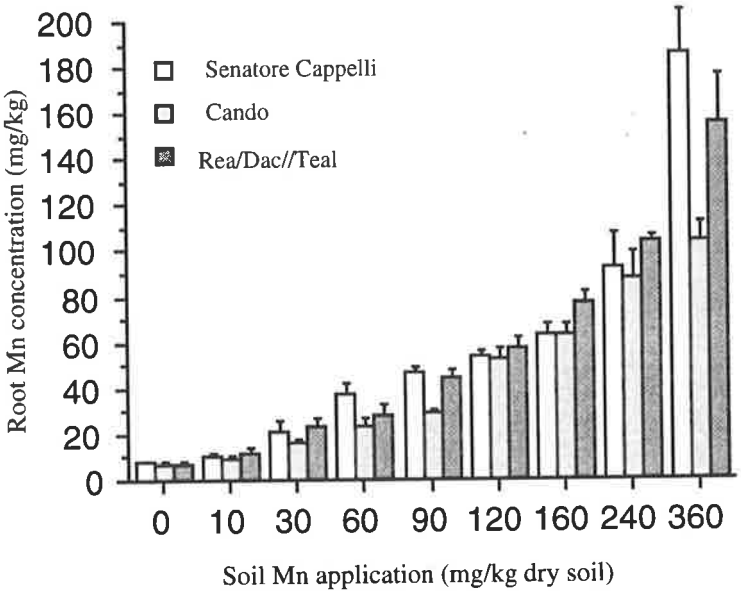

Fig 2.3 Root Mn concentration (mg/kg) of three durum genotypes 35 DAS at nine levels of added Mn (mg/kg dry soil). Plants grown in Mn-deficient Wangary soil in pots in a controlled environment chamber. Error bars show standard error of means.

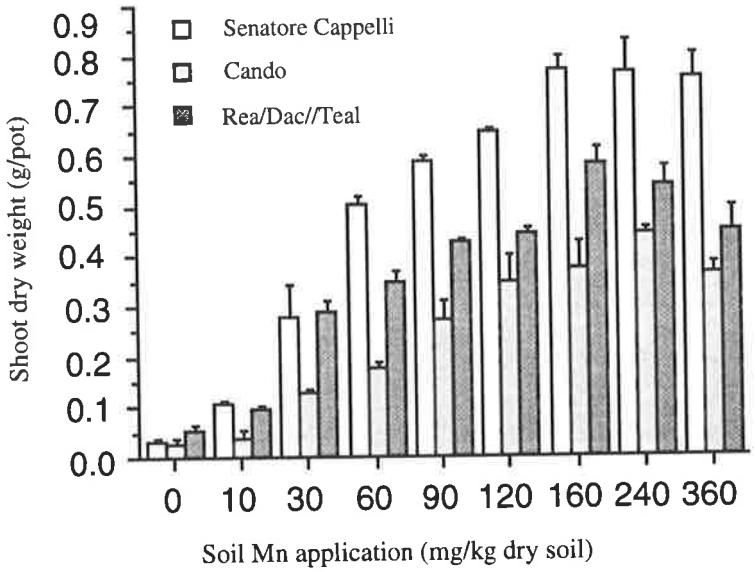

Fig 2.4 Shoot dry weight (g/pot) of three durum genotypes 35 DAS at nine levels of added Mn (mg/kg dry soil). Plants grown in Mn-deficient Wangary soil in pots in a controlled environment chamber. Error bars show standard error of means.

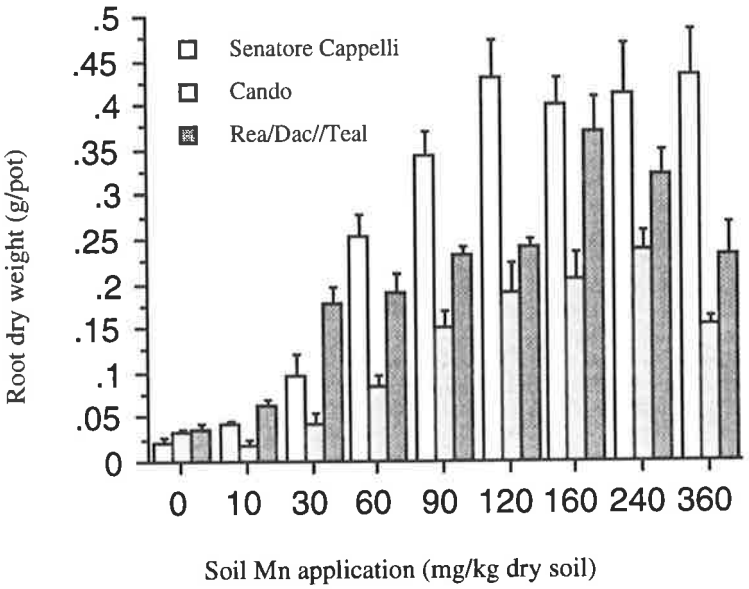

Fig 2.5 Root dry weight (g/pot) of three durum genotypes 35 DAS at nine levels of added Mn (mg/kg dry soil). Plants grown in Mn-deficient Wangary soil in pots in a controlled environment chamber. Error bars show standard error of means.

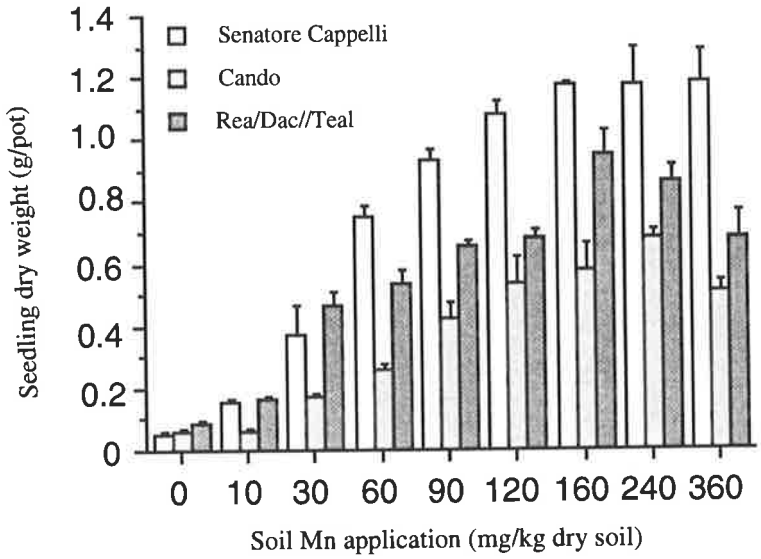

Fig 2.6 The seedling (shoot + root) dry weight (g/pot) of three durum genotypes 35 DAS at nine levels of added Mn (mg/kg dry soil). Plants grown in Mn-deficient Wangary soil in pots in a controlled environment chamber. Error bars show standard error of means.

NOTE:
Page 45 is missing from the print copy of
the thesis held in the University of Adelaide Library.

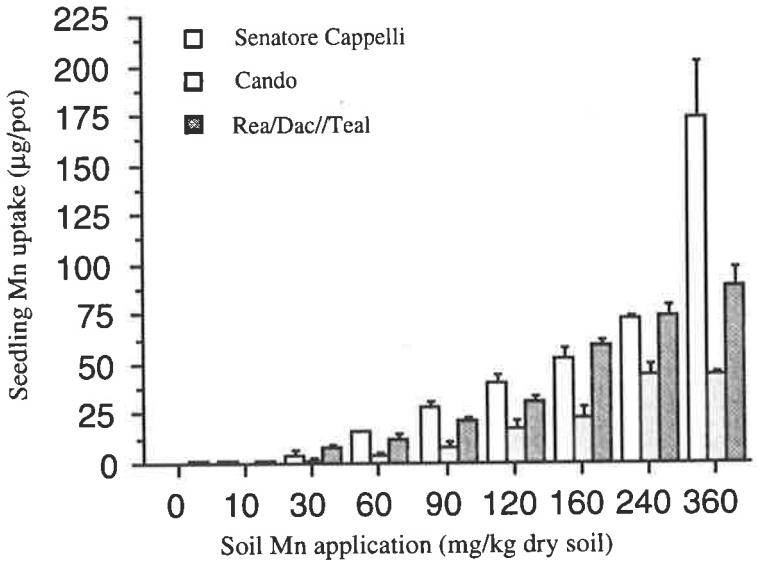

Fig 2.9 The seedling Mn uptake (µg/pot) of three durum genotypes 35 DAS at nine levels of added Mn (mg/kg dry soil). Plants grown in Mn-deficient Wangary soil in pots in a controlled environment chamber. Error bars show standard error of means.

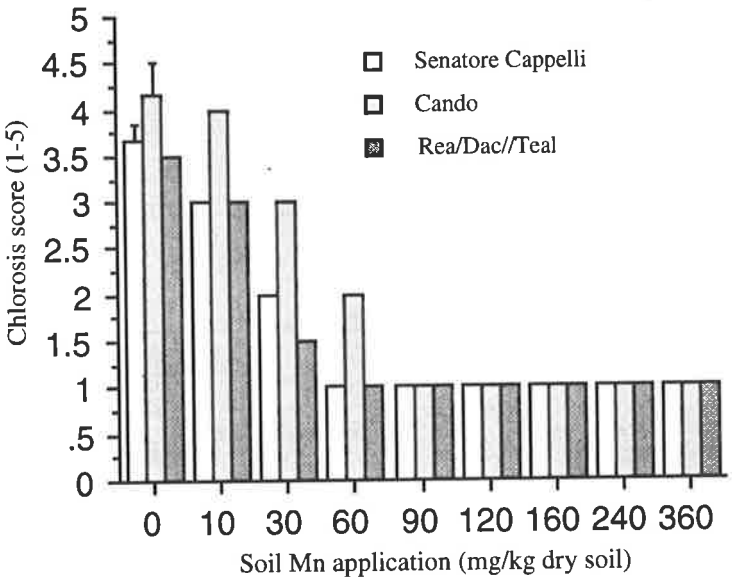

Fig 2.10 The chlorosis score (1-5) of three durum genotypes 35 DAS at nine levels of added Mn (mg/kg dry soil). Plants grown in Mn-deficient Wangary soil in pots in a controlled environment chamber. Error bars show standard error of means.

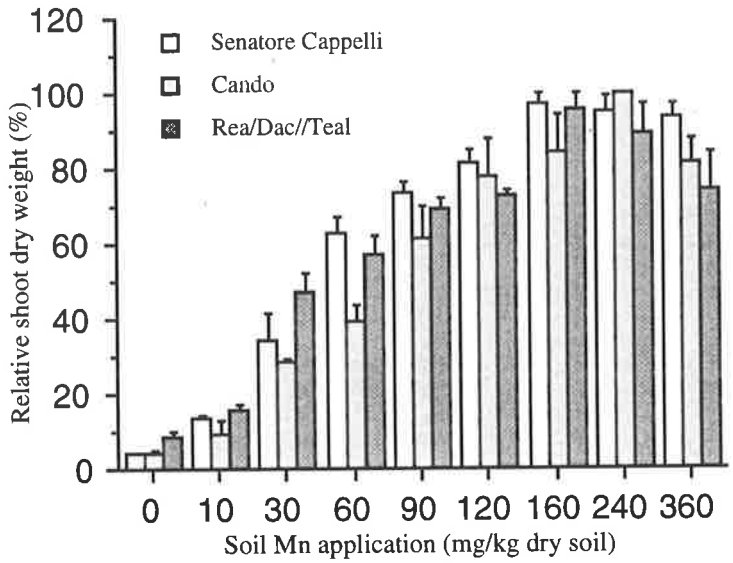

Fig 2.11 Relative shoot dry weight (shoot dry weight at Minimum soil applied Mn x 100/maximum shoot dry weight, %) of three durum genotypes 35 DAS at nine levels of added Mn (mg/kg dry soil). Plants grown in Mn-deficient Wangary soil in pots in a controlled environment chamber. Error bars show standard error of means.

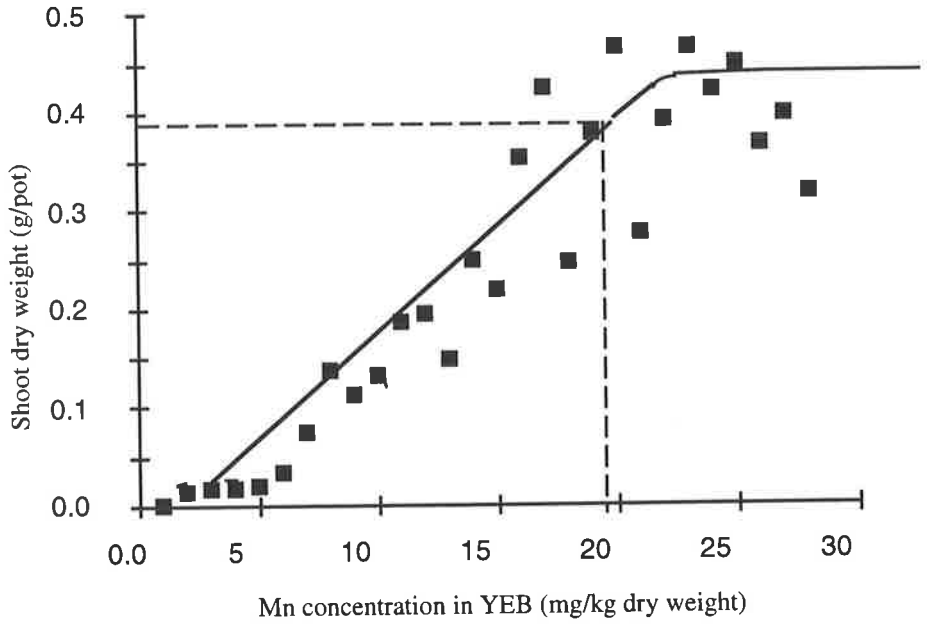

Fig 2.12 Shoot dry weight (g/pot) as function of YEBs Mn concentration (mg/kg dry weight) in durum wheat genotype Cando.

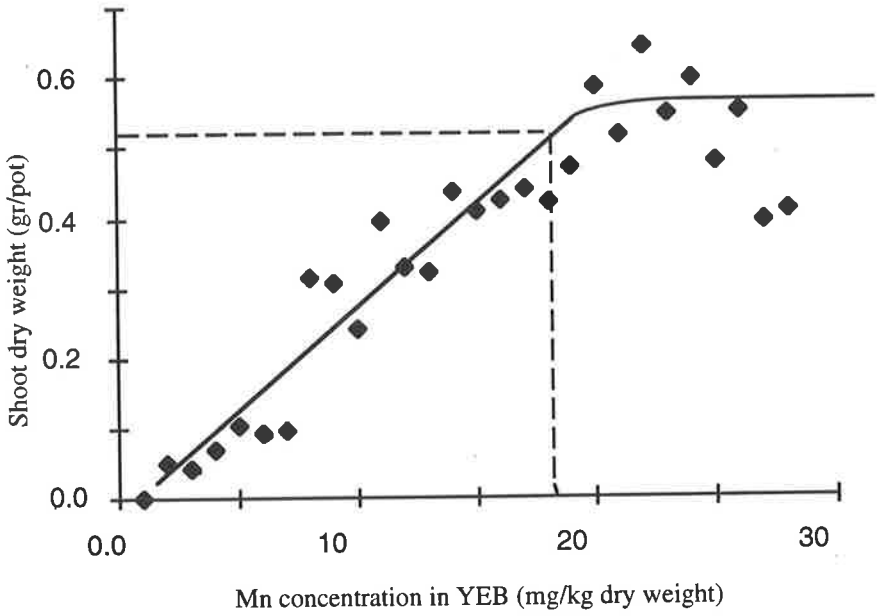

Fig 2.13 Shoot dry weight (gr/pot)) as function of YEBs Mn concentration (mg/kg dry weight) in durum wheat Rea/Dac//Teal.

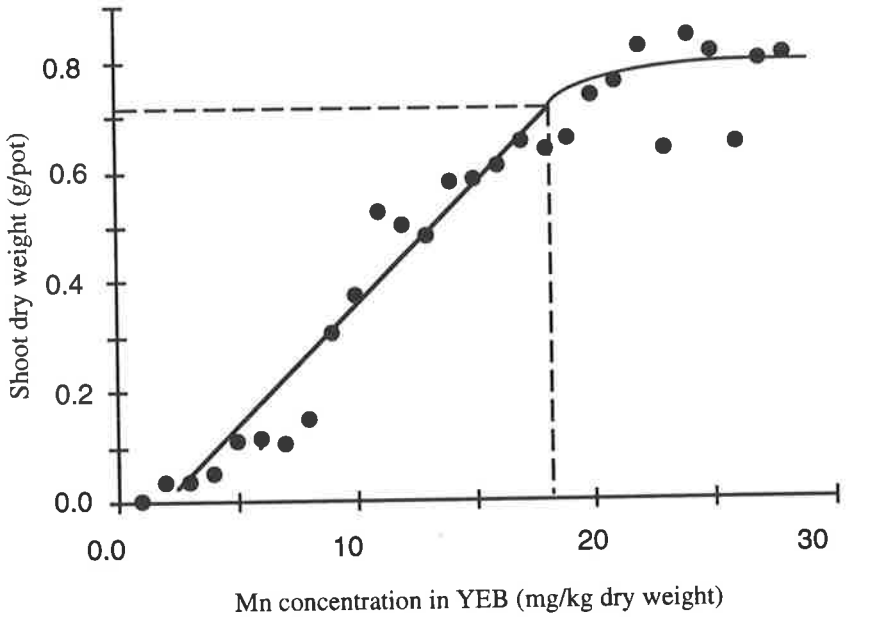

Fig 2.14 Shoot dry weight (g/pot) as function of YEBs Mn concentration (mg/kg dry weight) in durum wheat Senatore Cappelli.

Table 2.1 The world collection of durums. Pedigree, origin and Mn deficiency chlorosis score (1-5; 1 least severe and 5 most severe) in an observation nursery (69 durum and one bread wheat genotype) planted at Coonalpyn and Marion Bay, 1994.

Name	Parentage/Pedgree	Origin	Score (1-5)
Ain Arous 2	Fg/Magh L0559-0L-2AP-1AP-0AP	Lebanon/Syria	3-4
Ain Arous 4	Fg/Magh L0559-1L-2AP-2AP-3AP-0AP	Lebanon/Syria	3-4
Alsin	Rabi/3/Gs/AA//Plc L0603-5L-1AP-0AP	Lebanon/Syria	3-4
Asi	D.Dwarf S-15/Cr//Br/Al ICD77-0019-1AP-0SH-0AP	Syria	3-4
Awalbit 1	Aw12/Bit ICD84-0322-5AP-0TR	Syria/Lebanon	4-5
Awali 1	Cit//D.dwarf S15/Cr L412-3L-1AP-0AP	Lebanon/Syria	3-4
Awali 2	Cit//D.dwarf S15/Cr L412-3L-1AP-2AP-0AP	Lebanon/Syria	4-5
Awali 3	Cit//D.dwarf S15/Cr L412-3L-1AP-2AP-0AP	Lebanon/Syria	3-4
Awali 4	Cit//D.dwarf S15/Cr L412-3L-1AP-2AP-0AP	Lebanon/Syria	3-4
Belikh 1	Cr/Stk L92-6AP-2AP-0AP	Lebanon/Syria	3-4
Belikh 2	Cr/Stk L92-6AP-1AP-1AP-0AP	Lebanon/Syria	3-4
Cando	Seln. of D65152/D6148	North Dakota State University	5-5
Creso	Italy		4-5
Daki=Cyn	Dack/Gediz//USDA575 CD 19606-D-5Y-3M-1Y-1B-1Y-0AP	Mexico	2-3
Deraa	Can 2101/Mag//Stk/3/Wlls/65150 CD 15111-3S-2AP-2AP-1AP-1SH-0AP	Mexico/Jordan/ Syria	4-5
Furat 1	Snipe/3/Jo/Cr/gs/AA L74-0119-2L-0AP	Lebanon/Syria	3-4
Furat 2	Snipe/3/Jo/Cr//Gs/AA L616-0L-1AP-1AP-1AP-0AP	Lebanon/Syria	4-5
Furat 3	Snipe/3/Jo/Cr/gs/AA ICD74-0119-2L-1AP-0AP	Syria	3-5
Hagla	Cr//T. dic. V. Vernum/G11/3/Jo L0583-3L-1AP-0AP	Lebanon/Syria	4-5

Table 2.1 Continued

Name	Parentage/Pedgree	Origin	Score (1-5)
Harmel	AA/Ld537E//2*Tc/3/Gll/4/Rabi/31810 L606-0L-3AP-OAP	Lebanon/Syria	3-5
Hazar	T.dur.T.Sph-Ram/Gll//M.Sadova/3/AA/Cr/4/21563// 61-130/Lds 1AP-2AP-0AP	Lebanon/Syria	4-5
Heider	Can 2109//Jo/AA/3/S15/Cr CD 10535-D-1M-1Y-1M-2Y-0M-0AP	Mexico/Syria	4-5
Jabbul	Cr//T. Dic. V. Vernum/G11/3/Stk L126-2AP-2AP-0AP	Lebanon/Syria	4-5
Janz (Breadwheat)	3Ag3/4*Cndr/3/Tg/Cndr//Cndr QT 3685	Australia	1-2
Jordan	GdoVZ469/Plc/5/21563/3/LK/Ld390//Ch67/4/Cit71 ICD77-0032-1AP-0SH-0AP	Jordan/Syria	4-5
Karasu	Gs/AA//Plc/3/Cit71 L576-2L-1AP-2AP-0AP	Lebanon/Syria	4-5
Khabur 1	D. Dwarf S15//Cr/Stk L96-1AP-1AP-0AP	Lebanon/Syria	4-5
Lahan	Shwa/3/21563/AA//Fg CD 20626-1AP-2AP-1AP-0KE-0AP	Mexico/Syria	4-5
Lattino	Land race	Italy	3-4
Mesaoria	Aa"s"/Volunteer D31728	Cyprus	4-5
Nabrada	Unknown	India	3-4
Omeguer-4	Mrb16/Guerou 1 ICD85-0988-6AP-TR-2AP-0TR	Syria/Lebanon	4-5
Omguer 2	Mrb16/guerou 1 ICD85-0988-15AP-TR-9AP-0TR	Syria/Lebanon	4-5
Omrabi 16	Jori c69/Hau L0589-4L-2AP-3AP-2AP-0AP	Lebanon/Syria	3-4
Omrabi 17	Jori c69/Hau L0589-1L-1AP-2AP-1AP-0SH-0AP	Lebanon/Syria	4-5
Omrabi 20	Jori c69/Hau L0589-3L-1AP-2AP-0AP	Lebanon/Syria	3-4
Omrabi 23	Jori c69/Hau L0400-1L-1AP-2AP-3AP-0AP	Lebanon/Syria	3-4
Omtel 2	Mrb19/THD83 No 10 ICD-BM-ABL-405-0AP	Syria/Lebanon	4-5

Table 2.1 Continued

Name	Parentage/Pedgree	Origin	Score (1-5)
Omtel 4	Mrb19/THD83 No 10 ICD-BM-ABL-408-0AP	Lebanon/Syria	3-4
Omtel-5	Mrb19/THD83 No 10 ICD83-0587-AP-6AP-TR-4AP-0TR	Syria/Lebanon	4-5
Oronete 6	Cit71/Mexi//Shwa CD 21884-2AP-1AP-1AP-OAP	Mexico/Syria	4-5
Oronte 7	Cit71/Mexi//Shwa Cd 21884-4AP-3AP-0AP	Mexico/Syria	4-5
Pinguino	Cr"s"/4/Tace/4*Tc60/3/Zb/Wls D 28984	Mexico	4-5
Rea/Dac//Teal	Seln. of Rea/Dac//Teal	Mexico	2-3
Rubio De Cordoba	Land race from	Spain	4-5
Rufom 6	Ru/Mrb15 ICD84-1257-7AP-TR-5AP-OTR	Syria/Lebanon	4-5
Rufom 7	Ru/Mrb18 ICD84-1255-14AP-TR-17AP-0TR	Syria/Lebanon	4-5
Rufom-2	Ru/Mrb18 ICD84-1255-14AP-TR-16AP-OTR	Syria/Lebanon	4-5
Rusmar 4	Ru/3/Snipe//Ovi/Amarelejo ICD-BM-ABL-411-0AP	Syria	4-5
Rusomar 3	Ru/3/Snipe//Ovi/Amarelejo ICD-BM-ABL-319-0AP	Syria	3-4
Rusomar 5	Ru/3/Snipe//Ovi/Amarelejo ICD84-0069-2AP-1AP-TR-3AP-0TR	Syria	4-5
Sabil 1	Ibis/Fg//Cando ICD79-1437-14AP-1AP-0AP	Syria	3-4
Sabil 3	Ibis/Fg//Cando ICD79-1437-14AP-1AP-0AP	Syria	3-4
Sabil 5	Ibis/Fg//Cando ICD79-1437-14AP-2AP-0AP	Syria	4-5
Sabil 6	Ibis/Fg//Cando ICD79-1437-28AP-1AP-1AP-4AP-0JB-0AP	Syria	2-3
Sajur	Snipe/TH unk L617-0L-3AP-1AP-0AP	Lebanon/Syria	4-5
Sebou	Cr/T.Polonicum L0559-1L-2AP-2-AP-3AP-0AP	Lebanon/Syria	3-4

Table 2.1 Continued

Name	Parentage/Pedgree	Origin	Score (1-5)
Senatore Cappelli	Seln. of Jeanah Rhetifah	Italy	2-3
Sham 1	Plc/Ruff//Gta/Gtte CM 17904-B-3M-1Y-1Y-OSK-OAP	Mexico/Syria	2-3
Souri Ac 60	Aus# 13170	Tunisia	2-3
Stojocri 2	Stk/4/Jo/3/Jo/Cr//Cit71 ICD-BM-ABL-311-0AP	Syria	2-3
Syrica 1	Shwa/Pt1 CD 20632-2AP-3AP-0AP	Mexico/Syria	3-4
Syrica 3	Shwa/Ptl CD 20632-2AP-3AP-0A	Mexico/Syria	3-4
Tigris	Fg/Pales//Mex/3/Ruff/Fg CD 10445-1Y-1M-1Y-1M-0Y-0AP	Lebanon/Syria	4-5
Vic	Seln. of Edmore /Ward CI 17789	North Dakota State University	4-5
Yallaroi	Guillemont Seln. No.3/Kamilaroi sib	Australia	4-5
Yamuna 1	BYE*2/Tace//AA/3/Plc/Ruff//Gta/D671 5 ICD77-0156-4AP-0SH-0AP	Lebanon/Syria	5-5
Yarmuk	Jo/Gll//61-130/Lds/3/Jo/Cr//Gs/AA L74-0128-1L-2AP-0AP	Lebanon/Syria	3-4
Yavarous	Jo"s"/Aa"s"//Fg"s"=Bit"s" CM 9799	Mexico	3-4
Zeroud 3	Snipe/Magh L74-122-2L-1AP-0AP	Lebanon/Syria	3-4

Table 2.2 Correlation coefficient matrix between chlorosis score (CS), youngest emerged blade Mn concentration (YEB), root dry weight (Rwt), root Mn concentration (RMnc), root Mn content (RMnct), shoot dry weight (Shtwt), relative shoot dry weight (RShtwt), shoot Mn concentration (ShtMnC), shoot Mn content (ShtMnCt), seedling dry weight (Sdwt) and seedling Mn uptake (Mnup) for the three durum wheat genotypes differing in Mn efficiency at 0.0, 10.0 and 30.0 mg Mn/kg soil dry weight.

Table 2.2 A Mn=0.0 mg/kg dry soil

	CS										
YEB	-0.29	YEB									
Rwt	0.13	0.52	Rwt								
RMnc	-0.11	0.09	-0.04	RMnc							
RMnct	-0.12	0.51	0.80**	0.52	RMnct						
Shtwt	-0.78 **	0.60*	0.06	0.14	0.23	Shtwt					
Rshtwt	-0.63*	0.83**	0.19	0.06	0.26	0.92**	Rshtwt				
ShtMnc	-0.14	0.05	-0.37	0.16	-0.32	-0.27	-0.13	ShtMnc			
ShtMnct	-0.37	0.49	0.03	0.17	0.13	0.45	0.62*	0.13	ShtMnct		
Sdwt	-0.69*	0.70*	0.47	-0.04	0.49	0.88**	0.85**	-0.48	0.41	Sdwt	
Mnup	-0.37	0.59	0.22	0.30	0.37	0.49	0.65*	0.05	0.97**	0.50	Mnup

*, ** significant at P<0.05 and 0.01, respectively. Data were average of three genotypes at three replications (n=9).

Table 2.2 B (continued), Mn=10.00mg

	CS										
YEB	-0.36	**YEB**									
Rwt	-0.81**	0.61*	**Rwt**								
RMnc	-0.26	-0.10	0.03	**RMnc**							
RMnct	-0.79**	0.37	0.84**	0.54	**RMnct**						
Shtwt	-0.89**	0.29	0.59	0.30	0.601*	**Shtwt**					
Rshtwt	-0.62*	0.52	0.41	0.44	0.50	0.83**	**Rshtwt**				
ShtMnc	-0.03	0.79**	0.20	-0.49	-0.14	-0.05	0.17	**ShtMnc**			
ShtMnct	-0.61*	0.90**	0.65*	-0.06	0.42	0.63*	0.73*	0.69*	**ShtMnct**		
Sdwt	-0.94**	0.44	0.81**	0.23	0.76*	0.95**	0.77*	0.05	0.71*	**Sdwt**	
Mnup	-0.82**	0.78**	0.87**	0.29	0.82*	0.73*	0.74*	0.37	0.87**	0.87**	**Mnup**

*, ** significant at P<0.05 and 0.01, respectively. Data were average of three genotypes at three replication (n=9).

Table 2.2 C (continued), Mn=30.00.mg

	CS										
YEB	-0.81**	**YEB**									
Rwt	0.86**	0.87**	**Rwt**								
RMnc	-0.48	0.62*	0.72*	**RMnc**							
RMnct	-0.77*	0.81**	0.96**	0.86**	**RMnct**						
Shtwt	-0.76*	0.62*	0.84**	0.73*	0.82**	**Shtwt**					
Rshtwt	-0.67*	0.74*	0.91**	0.74*	0.93**	0.84**	**Rshtwt**				
ShtMnc	-0.50	0.81**	0.51	0.23	0.38	0.30	0.40	**ShtMnc**			
ShtMnct	-0.85**	0.89**	0.90**	0.65*	0.82**	0.86**	0.83**	0.73*	**ShtMnct**		
Sdwt	-0.82**	0.73*	0.93**	0.75*	0.91**	0.98**	0.92**	0.40	0.91**	**Sdwt**	
Mnup	-0.84**	0.89**	0.98**	0.81**	0.97**	0.87**	0.93**	0.55	0.94**	0.95**	**Mnup**

*, ** significant at P<0.05 and 0.01, respectively. Data were average of three genotypes at three replication (n=9).

Table 2.3 Estimated parameters and critical Mn concentration in YEBs calculated from the modified Mitscherlich model characterising the relationship between YEBs Mn concentration (mg/kg) and relative shoot dry weight (%) for three durum wheat genotypes, compared with those developed from hand fitted curves.

					Critical Mn concentration in YEB	
Genotype	α	β	γ	r^2	model	hand-fitted
Cando	0.08	92.84	1.42	96	32	19
Rea/Dac//teal	0.08	85.02	1.46	92	33	18
Senatore Cappelli	0.09	100.00	1.32	96	29	18

2.3 Development of selection criteria by concurrent studies under field and controlled conditions for Manganese efficiency in durum wheat

In the previous experiment (Section 2.2) genotypic variation for Mn efficiency in durum wheat was demonstrated. Further screening had two objectives:

(i) to determine the extent of this genotypic variation, and

(ii) to develop a selection criterion which could be employed in a genetic study and later in a breeding program.

The following experiments were conducted on 24 durum wheat genotypes, at two levels of applied Mn, replicated three times. Plant were grown in the field at Marion Bay and in a controlled environment chamber in 1995.

2.3.1 Materials and methods

Genotypes

Twenty four genotypes (including the bread wheat, Janz) (Table 2.1) expressing a wide range of deficiency symptoms were selected for this study from the observation nursery of 69 entries (including advanced breeding lines and introduced materials from diverse sources) sown in the field at Coonalpyn in 1994. The seed of the selected genotypes from the observation nursery had Mn contents in the range 0.3-0.5 μg/seed. The selected genotypes were evaluated on Mn-deficient soil at Marion Bay and in a controlled environment at two levels of Mn supply.

Mn level

Pot bioassay

Two levels of Mn (30 and 160 mg/kg Mn dry soil) were used in the pot bioassay. The experiment was arranged as a completely randomised factorial replicated three times (24 genotypes x 2 Mn levels x 3 replications = 144 pots) . The pots were watered daily and re-randomised every second day. Soil preparation, experimental conditions and harvest procedures were the same as described in Section 2.2.

Field experiment

The Mn deficient site Marion Bay, South Australia, was selected for field screening in 1995, and the genotypes evaluated as paired plots (split, ± Mn), replicated three times, using a randomised block design. Plots were four rows with 15 cm row spacing, 30 cm between plots and 4.2 m in length. Seed of the 23 durum and one bread wheat genotypes were sown on 7 May 1995 at a density of 300 seeds/m^2 (250 seeds/m^2 for the bread

wheat variety, Janz). Each -Mn plot received basal fertiliser at 150 kg/ha: mono-ammonium phosphate (MAP,15.1% N: 17.7% P) incorporated with 1.16% Cu, 1.18% Zn, 0.145% Mo, 1.28% S and 0.14% Co by bulk blending granulated trace elements with the basic high analysis MAP (carried out by HI-FERT PTY. LTD). The +Mn treatment received the same rate of basal MAP with Cu, Zn, S, Mo and Co. Manganese (60 kg/ha Mn oxysulphates, corresponding to 16.8 kg pure Mn/ha) was applied as granules mixed with the seed. A foliar spray of liquid Mn fertiliser (Mangasol, TOP AUSTRALIA. LTD) at the recommended rate of 6.5 L/ha (173 g/litre Mn as $MnSO_4$) was applied at tillering, Feekes scale 7 (Large, 1954), to the + Mn treatments.

The plots were scored visually three times for the development of Mn deficiency symptoms as described in section 2.2 (at Feekes scale 5, 7 and 10 corresponding to 80, 115 and 140 DAS). Plots were sampled at tillering (Feekes scale 5) by cutting at ground level (2 rows x 0.5 m). At maturity, the plots were harvested by machine (20 December 1995). Plant samples and grain were analysed by ICP spectrometer as described previously. Grain yield, grain Mn concentration, shoot Mn concentration, chlorosis score, and dry matter at tillering were subject to analysis of variance as described previously.

Correlation Analysis

In an attempt to find a reliable selection criterion, and also to study the effect of the seed Mn reserve on the measured parameters, the correlation coefficient matrix for the parameters from the genotypes in both experiments (pot bioassay and field screening) was calculated using Stat View 4.02.

2.3.2 Results

Pot bioassay

The genotypes expressed significant variation ($P<0.01$) in terms of chlorosis score (Table 2.4) (see Appendix A, Tables A12 for analysis of variance). Least chlorosis was expressed by the bread wheat genotype (Janz) while the durum genotypes generally demonstrated greater deficiency symptoms (Table 2.4). Significant genotype by Mn interaction was observed for the development of chlorosis symptoms (Appendix A, Table A12).

Genotypic differences ($P<0.01$) was also observed in terms of YEBs, shoot Mn concentration, shoot dry weight, shoot Mn content and relative shoot dry weight (Tables 2.4) (see Appendix A, Tables A13, A14, A15, A116 and A17 for analysis of variance). Durum wheat genotypes had generally lower YEBs Mn concentration, shoot Mn concentration, shoot Mn content, shoot dry weight and relative shoot dry weight as compared to the bread wheat (Table 2.4). However, considerable variation was observed within durum wheat genotypes.

Field screening

Considerable genotypic variation ($P<0.05$) in terms of development of Mn deficiency chlorosis symptoms, above ground biomass at tillering, grain yield and relative grain yield was found among entries in the field experiment (Table 2.5) (see Appendix A, Tables A18, A19, A20 and A21 for analysis of variance). The efficient bread wheat genotype, Janz, developed the least deficiency symptoms, greatest above ground biomass at tillering and had the highest relative yield compared to durum genotypes (Table 2.5). However,

significant variation ($P<0.05$) was observed among the durums (Table 2.5). Genotypes responded differently (significant genotype by Mn interaction) to application of Mn in terms of chlorosis symptoms, above ground biomass at tillering, grain yield and relative grain yield (Table 2.5).

Correlation coefficients between measured parameters from field screening, pot bioassay and original seed Mn content (Table 2.6) showed that:

(i) the measured parameters from genotypes in the pot bioassay (chlorosis score, shoot Mn concentration and shoot Mn content relative to shoot dry weight) correlated highly ($P<0.01$) with each other.

(ii) the same trends as in the pot bioassay were observed between the measured parameters from the genotypes in the field, where chlorosis symptoms, grain yield and relative grain yield correlated highly with each other ($P<0.01$).

(iii) the correlations of all measured parameters from the pot bioassay against field screening were significant ($P<0.05$), except that YEBs and shoot Mn concentration correlated insignificantly with relative yield.

(iv) Mn content of the seed used for sowing in the experiments correlated significantly ($P<0.05$) with development of Mn deficiency chlorosis symptoms in both pot and field screening, and to some extent affected other parameters.

The ranking order ($P<0.01$) of the genotypes on the basis of chlorosis score, shoot Mn content, relative shoot dry weight versus grain yield are shown respectively in Figs 2.15, 2.16 and 2.16.

Table 2.4 Growth measurements from 23 durum and one bread wheat genotypes at two levels of soil Mn supply (30 and 160 mg/kg dry soil) 35 DAS in a controlled enviroment chamber. The values are means of three replications. Parameters are: Chlorosis score (CS, 1-5 scale), Mn concentration of youngest emerged blade (YEB, mg/kg dry weight), shoot Mn concentration (Shtc, mg/kg dry weight), shoot dry weight (Shtwt, g/pot), relative shoot dry weight (Rshtwt, %) and shoot Mn content (Shtct, µg/pot).

Genotype	CS	YEB (-Mn)	YEB (+Mn)	Shtc (-Mn)	Shtc (+Mn)	Shtwt (-Mn)	Shtwt (+Mn)	Rshtwt	Shtct (-Mn)	Shtct (+Mn)
	1-5	mg/kg	mg/kg	mg/kg	mg/kg	gr/pot)	gr/pot	(%)	µg/pot	µg/pot
Harmel	3.3	9.4	33.7	7.3	30.9	0.2	0.8	21	2	24
Oronete 6	2.5	8.6	39.3	8.1	36.1	0.3	1.0	26	2	35
Sham 1	2.0	8.4	54.6	9.7	39.9	0.6	0.9	56	5	38
Asi	2.2	10.8	49.1	9.7	49.8	0.4	1.0	42	4	51
Sabil 3	2.8	6.9	40.2	8.7	42.3	0.4	1.1	41	4	48
Hazar	3.5	9.3	38.8	7.4	33.9	0.1	1.1	12	1	36
Omrabi 20	3.0	5.5	32.7	7.2	30.9	0.2	0.7	22	2	22
Omtel 4	2.7	6.9	34.8	8.5	36.6	0.3	0.9	31	3	32
Stojocri 2	2.0	8.9	39.3	9.5	41.4	0.4	1.1	43	4	44
Rusomar 3	2.7	8.2	33.2	7.7	34.9	0.4	1.1	36	3	35
Senatore Cappelli	1.8	7.2	46.5	9.4	38.5	0.7	1.3	69	6	50
Daki=Cyn	2.5	7.9	33.6	9.2	40.2	0.5	1.2	47	4	47
Vic	3.5	6.9	30.7	7.7	30.9	0.2	0.9	19	1	29
Janz (bread wheat)	1.2	15.6	56.9	14.1	56.4	0.8	0.9	84	12	49
Yarmuk	2.5	7.9	40.5	8.1	37.8	0.3	1.0	29	2	37
Yallaroi	2.5	11.9	40.4	8.5	35.3	0.3	1.6	35	3	56
Rea/Dac//Teal	2.5	9.45	46.6	9.5	47.8	0.4	1.1	38	4	50
Cando	3.5	7.0	30.3	6.9	29.9	0.2	1.1	18	1	31
Belikh 1	3.2	8.1	42.6	7.7	37.5	0.5	1.1	47	4	39
Sebou	2.3	7.6	39.4	9.3	38.3	0.4	1.0	44	4	38
Alsin	2.0	7.8	46.9	9.4	39.6	0.5	0.9	46	4	45
Syc3	2.9	7.2	36.7	6.4	33.1	0.3	1.1	27	2	35
Awali 4	3.5	7.8	38.0	6.3	32.8	0.3	0.9	30	2	30
Ain Arous 2	2.3	8.3	31.5	9.4	35.2	0.6	1.1	63	6	33
LSD5% genotype	0.2		4.5		2.7		0.2	4	7	
LSD5% Mn level	0.1		1.3		0.8		0.1	1	2	
LSD5% interaction	0.3		ns		ns		0.3	ns	ns	

Table 2.5 Growth measurements from mid-season and maturity harvests of 23 durum and one bread wheat genotypes at two levels of soil Mn supply (0 and 16.8 kg Mn/ha) at Mn deficient site (Marion Bay South australia, 1995). The values are means of three replications. Parameters are: Chlorosis score (CS, 1-5 scale), over ground dry mater production at tillering (BioT, g/pot), grain yield (Gyld, g/pot) and relative grain yield (Rgyld, %).

Genotype	CS (-Mn)	BioT (-Mn)	BioT (+Mn)	Gyld (-Mn)	Gyld (+Mn)	Rgyld
	1-5	g/pot	g/pot	g/pot	g/pot	(%)
Harmel	4.3	33	82	46	477	11
Oronete 6	4.2	17	60	88	551	16
Sham 1	3.2	69	85	543	648	83
Asi	3.2	48	87	478	691	71
Sabil 3	3.5	45	74	253	410	61
Hazar	4.8	6	56	14	382	4
Omrabi 20	4.0	21	78	81	641	13
Omtel 4	3.8	36	63	390	720	54
Stojocri 2	3.0	98	148	388	598	68
Rusomar 3	4.2	31	107	120	754	16
Senatore Cappelli	3.2	80	131	252	526	47
Daki=Cyn	3.2	32	81	255	735	34
Vic	4.5	58	89	50	468	11
Janz (bread wheat)	1.4	67	84	791	827	92
Yarmuk	3.8	23	77	243	611	41
Yallaroi	4.0	48	162	140	638	21
Rea/Dac//Teal	3.3	96	154	474	679	70
Cando	4.5	35	96	62	286	18
Belikh 1	3.8	133	185	293	600	49
Sebou	3.8	75	179	262	540	50
Alsin	3.5	73	144	343	744	46
Syc3	4.0	64	131	205	453	46
Awali 4	3.7	76	129	172	429	45
Ain Arous 2	3.8	73	211	314	537	57
LSD 5% genotype	0.4		41	96		11
LSD 5% Mn level	0.1		8	24		3
LSD 5% interaction	0.6		23	218		29

Table 2.6 Correlation coefficients between measured parameters from 23 durum and one bread wheat genotypes evaluated at two levels of soil Mn supply (0 and 16.8 kg Mn/ha) and (30 and 160 mg/kg dry soil) respectively at a Mn deficient site (Marion Bay South Australia, 1995) and 35 DAS in a controlled enviroment chamber. Parameters from controlled enviroment experiment are: Chlorosis score (CS, 1-5 scale), Mn concentration of youngest emerged blade (YEB, mg/kg dry weight), shoot Mn concentration at 30 mg/kg dry soil (Shtc, mg/kg dry weight), shoot Mn content at 30 mg/kg dry soil (Shtct, µg/pot), relative shoot dry weight (Rshtwt, %) and seed Mn content of the original seed (µg/seed) used for planting. Parameters from field experiment are: Chlorosis score (CS, 1-5 scale), grain yield (Gyld, g/plot) and relative grain yield (Rgyld, %).

			P	O	T				
P		YEB							
O	CS	-0.56**	CS						
T	Shtc	0.61**	-0.80**	Shtc					
	Shtct	0.68**	-0.79**	0.93**	Shtct		FIELD		
F	Rshwt	0.64**	-0.80**	0.82**	0.95**	Rshwt			
I	CS	-0.51**	-0.80**	-0.83**	-0.86**	-0.83**	CS		
E	Gyld	0.41*	-0.76**	0.84**	0.82**	0.78**	-0.89**	Gyld	
L	Rgyld	0.19	-0.49*	0.39	0.43*	0.57**	-0.56**	0.58**	Rgyld
D	Seed Mn	0.36	-0.44*	0.30	0.29	0.29	-0.44*	0.31	0.31

*,** significant respectively at P<0.05 and 0.01, data mean of three replication of 24 genotypes (n=24) from pot bioassay and field experiment (n=6).

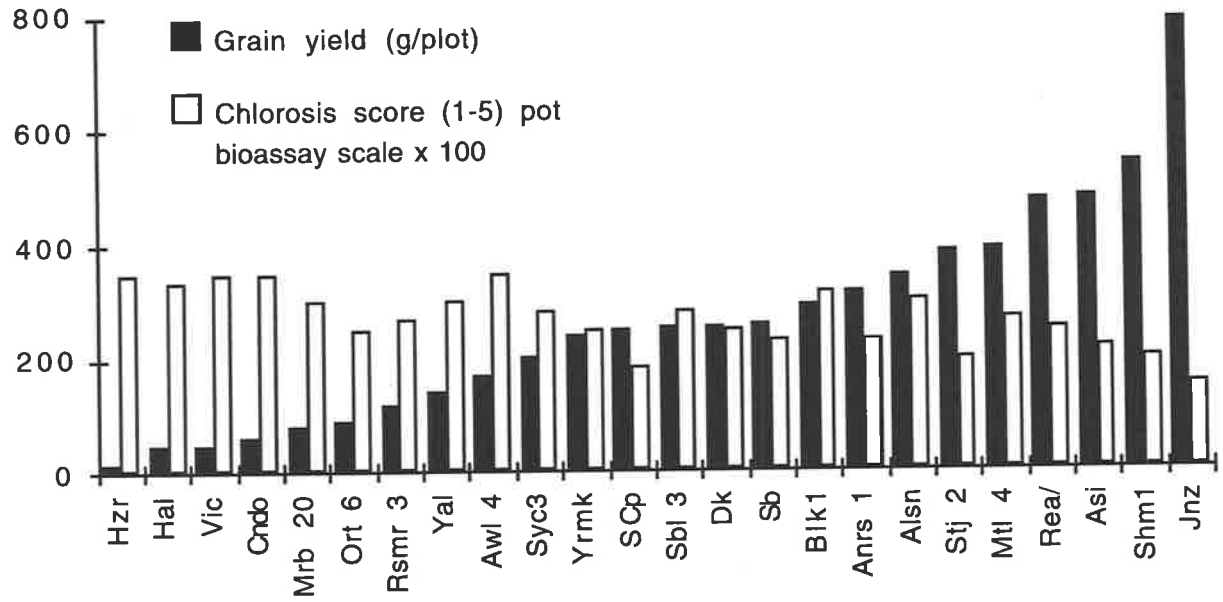

Fig 2.15 Ranking of 23 durum and one bread wheat genotypes on the basis of grain yield (g/plot) from -Mn field plot vs chlorosis score (1-5 scale x 50) from the 30 mg of soil applied Mn pot bioassay ($r=-0.76^{**}$ at $P<0.01$).

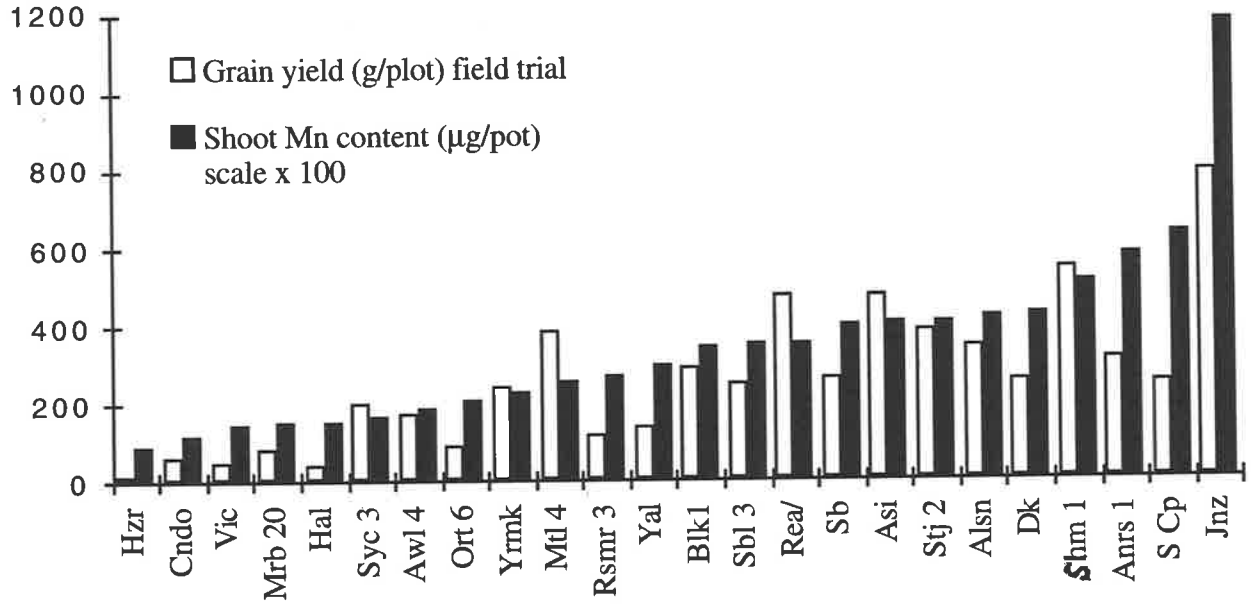

Fig. 2.16 Ranking of 23 durum and one bread wheat genotypes on the basis of grain yield (g/plot) from-Mn field plot (Yld) vs shoot Mn content pot $^{-1}$ (µg x 10 $^{-1}$) from the 30 mg of soil applied Mn pot bioassay (r=0.82** at P<0.01).

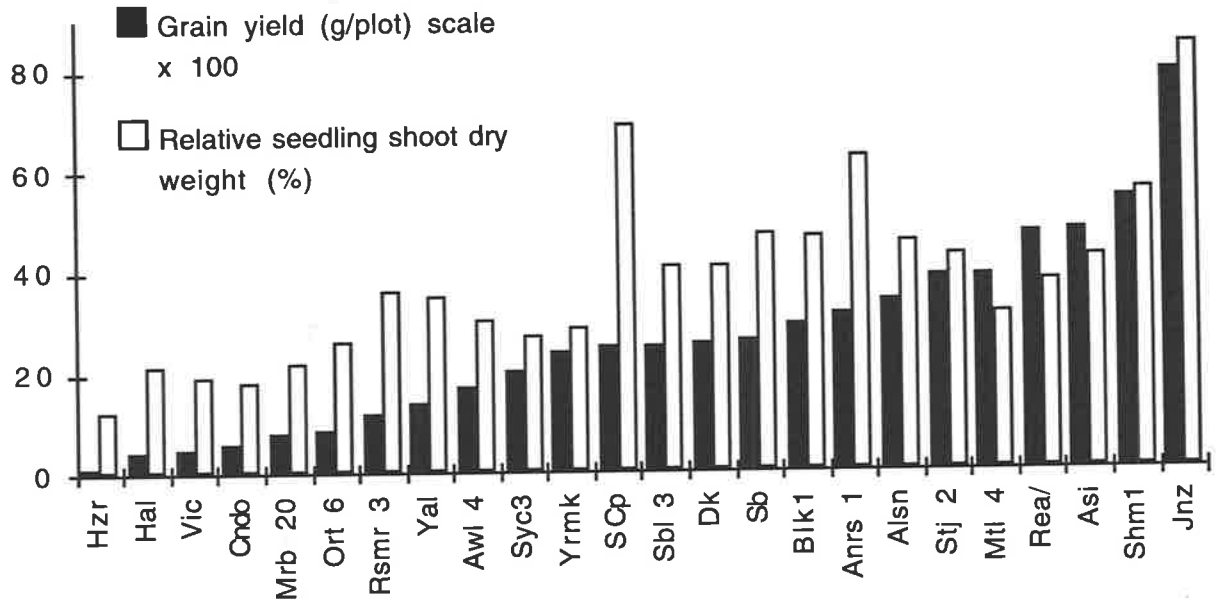

Fig. 2.17 Ranking of 23 durum and one bread wheat genotypes on the basis of grain yield (gr/plot) from the -Mn field plot vs relative seedling shoot dry weight (%) from the 30 mg of soil applied Mn pot bioassay ($r=0.78^{**}$ at $P<0.01$).

2.3.3 Discussion

The genotypes for this study were selected on the basis of expression of a wide range of Mn deficiency symptoms in an observation nursery at Coonalpyn in 1994. The entries originated from the Mediterranean basin, Italy, Syria, Lebanon, Algeria, Turkey, ICARDA, CIMMYT, Australia, Northern Dakota and Canada. The unadapted accessions either from Turkey (facultative to winter growth habit) or northern USA and Canada (mainly very Mn-inefficient) had already been excluded and highly mixed entries from Tunisia and Algeria were also discarded.

The efficient bread wheat genotype, Janz ranked the highest for Mn efficiency defined agronomically as:

Relative grain yield = (grain yield at -Mn treatment) * 100/ (grain yield at +Mn treatment)

Janz was ranked the lowest for chlorosis score both in the field screening and in the pot bioassay, and the highest for YEBs Mn concentration, shoot Mn concentration, shoot Mn content, shoot dry weight and relative shoot dry weight in the pot bioassay (Tables 2.5 and 2.6). Considerable variation in durum wheat genotypes in terms of either relative grain yield, chlorosis score, seedling Mn concentration and seedling Mn content was observed (Tables 2.5 and 2.6). Genotype(s) from Australia (Yallaroi) and North Dakota (Vic and Canada) demonstrated the lowest tolerance, while those from the Mediterranean basin (Senatore Cappelli) and the CIMMYT breeding program (Rea/Dac//Teal) showed moderate tolerance to Mn deficiency. The entries, Hazar and Stojocri 2, originating from the same breeding program (ICARDA), responded quite differently to Mn deficiency (Tables 2.5 and 2.6).

Ranking of genotypes for chlorosis score from the pot bioassay was negatively correlated with grain yield at the Mn-deficient field site and with relative grain yield, r=-0.76** and -0.49* respectively (Table 2.6; Fig 2.15). This evidence confirmed the validity of using chlorosis score in durum as a selection criterion as proposed for barley by Longnecker *et al.* (1988). However, its applicability in screening seedlings grown from seed with high Mn content still remains unproven. Ranking genotypes for Mn efficiency on the basis of shoot Mn content, relative shoot dry weight or shoot Mn concentration as proposed for barley by Huang *et al.* (1994) (Table 2.6; Figs 2.16 and 2.17) are other promising alternatives for durum (Table 2.6; Figs 2.16 and 2.17). However, considering the higher uptake of Mn by Mn-efficient genotypes grown in deficient soil, the higher observed correlation coefficient (between shoot Mn content from the pot bioassay with grain yield and relative grain yield from the field study) and the lower variance of shoot Mn uptake, make the application of the shoot Mn content potentially the most acceptable.

Variation in Mn content of seed sown in the field and pot bioassay influenced ($P<0.05$) the expression of chlorosis symptoms both in the field screening and the pot bioassay, and also the other measured parameters were affected to some extent (Table 2.6). The confounding effects of seed Mn reserve made the decision on choosing a suitable selection criterion dependent on further confirmation of the results presented here. This involved comparing genotypes with the same seed Mn content which is done in Chapter 3.

Chapter 3

Source of manganese efficiency and confirmation of selection criteria for screening durum wheat (*Triticum turgidum* L. var *durum*)

3.1 Introduction

Knowledge of centre of origin, evolutionary pattern and geographical distribution of durum wheat provides complementary information regarding the events in the process of domestication, and can be employed in the search for sources of tolerance or resistance to factors affecting the growth of durum wheat, specifically Mn efficiency. The evolution of durum wheat from wild grasses and its centre of origin has been discussed in Section 1.5.1.

In the preliminary assessment of genotypes from various sources (planted at Coonalpyn and Marion Bay in 1994, Section 2.3.2), genotypes from Turkey were late maturing (winter and facultative growth habit) and unadapted. Genotypes from Tunisia (Souri Ac 60) and Algeria (Medeah and Flameen) expressed average to good tolerance to Mn deficiency, respectively, but were highly mixed genetically and were therefore excluded. Australian cultivated durum wheat (Yallaroi and Kamilaroi) and entries from Northern USA and Canada were ranked as the least Mn efficient, while one from Italy (Senatore Cappelli) was moderately good. Expression of tolerance to deficiency was highly varied among the entries from CIMMYT and ICARDA: genotypes such as Rea/Dac//Teal,

Stojocri 2 and Sham 1 were ranked the best, while the worst (Hazar) was from the same source. It seems that tolerance to deficiency already exists in primary and secondary sources of origin of durum wheat, but its initial cultivation on non-calcareous soils during the expansion of the crop to North America and Australia explains the current poor level of tolerance to micronutrient deficiency among Australian (New South Wales), North Dakota and Canadian germplasm. The probable explanation for wide variation observed in Mn efficiency among genotypes developed by ICARDA, mainly for West Asia and North Africa (WANA), where alkaline soils with high pH dominate the rainfed cereal growing area, could be either :

(i) an emphasis on development of genotypes for favourable environments (high rainfall area or rainfed with supplementary irrigation) on comparatively fertile soils, or

(ii) linkage of Mn efficiency with an agronomic trait of interest.

The issues dealt with in this chapter, identifying a selection criterion and determining the source of suitable genotypic variation, are important as prerequisites for a plant breeding approach to resolving Mn deficiency. An additional issue, variation in seed Mn content of the durum genotypes, which confounded the screening for Mn efficiency and has already been described (Section 2.3), is investigated thoroughly in this chapter. Experiments presented in this chapter are aimed at:

(i) establishing the selection criterion and the extent of genotypic variation using genotypes with seeds of similar Mn content, and

(ii) locating the geographical source of efficiency through pedigree analysis.

3.2 Reassessment of the selection criterion for manganese efficiency

A limited number of durum wheat genotypes representing the range of observed variation in tolerance to Mn deficiency, but with similar seed Mn contents (0.20-0.23 µg/seed), were selected from the field experiment (Section 2.3) to confirm the extent of observed genotypic variation and the validity of the selection criterion (shoot Mn content of seedling 35 DAS). Using fewer genotypes and seed from ± Mn field plots, compared to seed sourced from a single observation plot as used in earlier screening (Section 2.3.2), made selection of seed with similar Mn reserves possible, avoiding this confounding effect.

3.2.1 Materials and methods

Six selected durum genotypes, representing the full spectrum of observed variation in Mn efficiency reported previously (Chapter 2), and with similar seed Mn reserves (0.20 to 0.23 µg/seed), were evaluated concurrently in two experiments: a pot bioassay in a controlled environment chamber, and a field experiment at a Mn-deficient site.

Genotypes

Six durum genotypes, Stojocri 2, Sham 1, Rea/Dac//Teal, Harmel, Hazar and Yallaroi (Australian durum wheat check cultivar), were grown in this study (Table 2.1). Their Mn efficiency in terms of relative yield from Table 2.5 were: 68%, 83%, 70%, 11%, 4% and 21%, respectively.

Selection of seed with even seed Mn reserve

In selection of seed with similar Mn reserves the following precautions were taken into account to maintain both purity of the genotypes and the same Mn content.

(i) Prior to machine harvesting of the field trial, off types were excluded (by roguing twice before maturity) and twenty or more random heads from main tillers were collected by hand.

(ii) Heads were threshed manually or by a single-head thresher, shrivelled seed discarded, and thousand kernel weight (TKW) determined.

(iii) Seeds were classified into very small, small, average, large and very large seeds initially by size (visually) and later by weight. Five to ten seeds from the average size were analysed by ICP spectrometer to determine the Mn content.

(iv) Variation in Mn content (seed weight x seed Mn concentration) of the entries was addressed by selecting entries from either + Mn or - Mn plots. Seed for the efficient genotypes generally came from the -Mn treatment and that for the inefficient genotypes from the +Mn treatment.

(v) Minor variation in Mn reserves for the entries was compensated for by selecting either larger or smaller seed size groups, provided that Mn concentration was the same in the other size classes.

Seeds of six durum genotypes with the same Mn content (Table 3.1) were selected for this study from the field experiment at Marion Bay, 1995. By reducing the variation in seed Mn reserves, confounding effects on screening and expression of efficiency in terms of chlorosis score, shoot Mn content and shoot Mn concentration were kept to a minimum.

Pot Bioassay at two levels of applied Mn

Two levels of Mn (30 and 240 mg/kg dry soil), representing an appropriate level of deficiency for genotypic discrimination (as determined in Chapter 2) and adequate Mn

supply, respectively, were prepared in the calcareous soil as described in Chapter 2 for the pot bioassay. The lower level of soil applied Mn (160 mg/kg dry soil) in the previous pot bioassay (Section 2.3) than that used in the current assay (240 mg/kg dry soil) was to prevent yield depression in bread wheat. Soil preparation, conditions of growth, measured characters and harvest were the same as described in Section 2.2. Plants were harvested 35 DAS.

Field screening at three levels of applied Mn

A manganese-deficient site (Marion Bay, South Australia, as described in Section 2.3) was selected in 1996 for the field screening. Genotypes were evaluated at three levels of Mn in split plots, replicated four times in a randomised block design. Plots consisted of six rows with 15 cm row spacing and were 6 m in length.

Plots were sown on 26 June 1996 at a density of 300 seeds/m^2, with each 0.0 Mn plot receiving monoammonium phosphate (MAP, 15.1% N:17.7% P) incorporated with 1.16% Cu, 1.18% Zn, 0.145% Mo, 1.28% S and 0.14% Co at the rate of 150 kg/ha as basal nutrients. The +Mn treatments received the same rate of basal nutrients and either 11.2 kg or 22.4 kg of Mn/ha (as 40 or 80 kg Mn oxysulphate/ha, 28% Mn) added in furrow with the seed, representing the moderate and adequate level of soil applied Mn, respectively. The higher level (22.4 kg/ha) of soil Mn application was used because in Section 2.3, plots with 16.8 kg Mn/ha still had symptoms of Mn deficiency.

Plots with the higher level of soil Mn application also received a foliar application of Mangasol at the recommended rate of 6.5 l/ha (173 g/litre Mn as MnSO4) at FS 7, 107 DAS . The plots were scored visually for the development of Mn deficiency symptoms at FS 5 and 7 corresponding to 85 and 107 DAS, respectively. YEBs samples were collected at tillering (FS = 5). The above ground biomass was collected from two rows x

0.5 m at tillering (FS = 5) and at maturity (184 DAS) by cutting at ground level, and grain was harvested by machine.

The above ground biomass at tillering and at maturity, chlorosis score, YEBs Mn concentration, grain yield, relative grain yield, grain Mn concentration and content, shoot Mn concentration at maturity, shoot Mn content at maturity, total above ground Mn uptake (grain Mn content + straw Mn content) were subject to analysis of variance as described previously (Chapter 2). A correlation matrix was conducted for the data collected from field and controlled environment chamber experiments, and correlation coefficients were compared in order to determine the utility of the alternative selection criteria.

Table 3.1 Seed Mn concentration (mg/kg), content (mg/seed) and weight for six durum wheat genotypes differing in Mn efficiency.

Genotype	Mn concentration (mg/kg)	Seed weight (g/seed)	Mn content (µg/seed)
Stojocri 2	4.74	0.05	0.23
Cham 1	5.41	0.04	0.23
Rea/Dac//Teal	3.80	0.05	0.20
Harmel	4.53	0.04	0.20
Hazar	4.91	0.04	0.22
Yallaroi	4.20	0.05	0.22

3.2.2 Results

Field screening

Genotypic differentiation (P<0.05%) was observed for chlorosis score at each level of Mn supply, and the efficient genotype(s) (Stojocri 2 and Sham 1) developed less severe deficiency symptoms at nearly all levels of Mn supply than the other genotypes (Table 3.2) (see Appendix A, Table B1 for analysis of variance). The genotypes responded to application of Mn differently (genotype by Mn, P<0.05) in terms of less severe development of chlorosis (Table 3.2). Deficiency symptoms were also observed at the higher level of Mn supply, 22.4 kg Mn /ha (Table 3.2).

Significant genotypic variation existed at all levels of soil Mn supply for YEBs Mn concentration, where either Hazar or Harmel maintained lower levels compared to other genotypes, but clear discrimination between other genotypes (Stojocri 2, Sham, Rea///Teal and Yallaroi) was not observed (Table 3.2) (see Appendix A, Table B2 for analysis of variance). The YEBs Mn concentration of genotypes generally increased with Mn supply (Mn, P<0.05) but relationships among genotypes were maintained (Table 3.2).

Genotypic variation was observed in above ground dry matter production at tillering at all levels of Mn supply, and Stojocri 2 produced significantly higher dry matter compared to other genotypes (Table 3.2) (see Appendix A, Table B3 for analysis of variance). Although the dry matter production of all genotypes increased upon supply of Mn, Stojocri 2 responded differently compared to other genotypes (genotype by Mn, P<0.05) to Mn supply (Table 3.2) with a large increase between the two added Mn treatments.

Stojocri 2 and Sham 1 produced higher straw biomass at 0.0 kg Mn/ha than the other genotypes, but not necessarily at other levels where a greater response to addition of Mn

was observed in Rea/Dac//Teal and Yallaroi (Table 3.2) (see Appendix A, Table B4 for analysis of variance). The production of straw increased with Mn supply, and Stojocri 2 and Sham 1 responded differently (genotype by Mn, $P<0.05$) to supply of Mn in terms of straw biomass compared to other genotypes (Table 3.2).

Rea/Dac//Teal had the highest grain yield across all levels of Mn supply, and Stojocri 2 and Sham 1 produced significantly higher grain yields at 0.0 and 11.2 kg Mn/ha, but not at 22.4 kg Mn/ha (Fig 3.1 and Table 3.2) (see Appendix A, Table B5 for analysis of variance). Grain yield of genotypes generally increased but differently (genotype by Mn, $P<0.05$) in response to Mn supply: Stojocri 2 and Sham 1 responded less to Mn supply than other genotypes (Fig 3.1 and Table 3.2).

Stojocri 2, Sham 1 and Rea/Dac//Teal had higher relative grain yields than Yallaroi, Hazar and Harmel at 0.0 kg Mn/ha (Table 3.2) (see Appendix A, Table B6 for analysis of variance). In terms of relative grain yields, the genotypes responded significantly but differently (genotype by Mn, $P<0.05$) to Mn supply (Mn, $P<0.05$); Stojocri 2 demonstrated the lowest response (Table 3.2) in relative grain yield.

Although significant genotypic variation for grain Mn concentration existed between genotypes at all levels of Mn supply, the differences were comparatively small compared to those other measurements. Stojocri 2 and Sham 1 had higher grain Mn concentration across the Mn levels (with the exception of Yallaroi at 11.2 kg Mn/ha) than the other genotypes (Table 3.2) (see Appendix A, Table B7 for analysis of variance). The grain Mn concentration generally remained unchanged, with the exception of Sham 1 which showed a decline and Yallaroi which expressed sharp increase due to the addition of 11.2 kg Mn/ha. Further increase in the supply of Mn (22.4 kg Mn/ha) significantly increased grain Mn concentration of all genotypes, with the exception of Yallaroi and Harmel which

remained unchanged. However, no significant genotype by Mn interaction was observed (Table 3.2).

Significant genotypic variation was observed for grain Mn content at all levels of Mn supply: Stojocri 2, Sham 1 and Rea/Dac//Teal had higher grain Mn contents than other genotypes at all levels, with the exception of Yallaroi at 11.0 kg Mn/ha (Table 3.2) (see Appendix A, Table B8 for analysis of variance). The grain Mn content generally increased with increasing Mn supply, especially at 22.4 kg Mn/ha, and significant genotype by Mn interaction was observed (Table 3.2).

Straw Mn concentration showed no significant variation at 0.0 kg Mn /ha and 12.2 kg Mn /ha, except at 22.4 kg Mn/ha where Stojocri 2, Sham 1 and Yallaroi had higher concentrations resulting in a significant genotype by Mn interaction (Table 3.2) (see Appendix A, Table B9 for analysis of variance). Genotypic variation was observed for straw Mn content only at 22.4 kg Mn/ha where Stojocri 2, Sham 1 and Yallaroi had higher ($P<0.05$) Mn contents compared to other genotypes (Table 3.2) (see Appendix A, Table B10 for analysis of variance) resulting in a significant genotype by Mn interaction.

Genotypic variation was observed for above ground biomass production at all levels of Mn supply. Stojocri 2, Sham 1 and Rea/Dac//Teal produced higher above ground biomass at both 0.0 kg Mn/ha and 11.2 kg Mn/ha, with the exception of Yallaroi, but not necessarily at 22.4 kg Mn/ha (Table 3.2) (see Appendix A, Table B11 for analysis of variance). Genotypes responded to supply of Mn by higher above ground biomass production, but showed a genotype by Mn interaction, $P<0.05$): Stojocri 2 and Sham 1 were less responsive to Mn supply than other genotypes (Table 3.2).

Stojocri 2, Sham 1 and Rea/Dac//Teal had higher Mn uptake at all levels of Mn supply, with the exception of Yallaroi, which had the highest uptake at 11.2 kg Mn/ha and 22.4 kg

Mn/ha (Table 3.2) (see Appendix A, Table B12 for analysis of variance). Genotypes responded to supply of Mn in terms of higher Mn uptake but the genotype by Mn interaction was significant ($P<0.05$) with Hazar and Harmel being less responsive (Table 3.2).

Summary of field results

(i) Genotypic variation was found ($P<0.05$) in all growth characteristics measured (development of chlorosis, YEBs Mn concentration, above ground biomass at tillering and at maturity, grain yield, relative grain yield, straw dry weight, straw Mn concentration, straw Mn content, shoot Mn, grain Mn concentration, grain Mn content, above ground biomass and Mn uptake).

(ii) Differentiation of genotypes was more distinct at 0.0 kg Mn/ha rather than at 11.2 kg Mn/ha or 22.4 kg Mn/ha.

(iii) Significant genotype by Mn interactions ($P<0.05$) occurred for all the measured parameters except for YEBs Mn concentration, grain Mn concentration and content.

(iv) The correlation study of the measured parameters for genotypes from the field experiment indicated a higher correlation ($P<0.05$) for grain yield versus grain Mn content, Mn uptake, above ground biomass, and relative yield at 0.0 kg Mn/ha than at 11.2 or 22.4 kg Mn/ha (Table 3.4). The differences in response of genotypes to applied Mn were also observed visually in development of chlorosis and growth at tillering 107 DAS (Fig. 3.2).

Pot bioassay

Genotypic variation in chlorosis score was observed at 30 mg Mn/kg dry soil, whereas at adequate Mn supply (240 mg Mn/kg dry soil) genotypes developed no chlorosis symptoms (Table 3.3) (see Appendix A, Table B13 for analysis of variance). Stojocri 2 developed the least chlorosis symptoms while Hazar expressed the greatest, so clear discrimination of genotypes on the basis of chlorosis symptoms was observed (Table 3.3). Although genotypes generally responded by developing less severe chlorosis as the Mn supply increased, there was a significant interaction (genotype by Mn, $P<0.05$). Stojocri 2 developed no symptoms either at 30.0 Mn/kg dry soil or at 240 Mn/kg dry soil (Table 3.3).

Significant genotypic variation was not observed for YEBs Mn concentration at 30 mg Mn/kg dry soil (Table 3.3) (see Appendix A, Table B14 for analysis of variance). The genotypes responded to supply of Mn with higher YEBs Mn concentration, and Rea/Dac//Teal ranked the highest and Sham 1 the lowest in YEBs Mn concentration but there were no significant differences between the other genotypes (Table 3.3).

Genotypic variation in shoot Mn concentration was not statistically significant at 30 mg/kg dry soil (Table 3.3) (see Appendix A, Table B15 for analysis of variance). The genotypes responded to supply of Mn in terms of higher shoot Mn concentration so Rea/Dac//Teal had the highest shoot Mn concentration and Harmel the lowest but no significant genotypic difference was observed between the other genotypes (Table 3.3).

Significant genotypic variation was observed for shoot dry weight at 30 mg Mn/kg dry soil. Stojocri 2 had the highest and Yallaroi and Hazar the lowest shoot dry weight (Table 3.3) (see Appendix A, Table B16 for analysis of variance). The genotypes responded to supply of Mn with increased shoot dry weight production (genotype by Mn, $P<0.05$), and

Sham 1 and Hazar produced significantly higher shoot dry weight than other genotypes which were not significantly different from each other (Table 3.3).

Genotypic variation in shoot Mn content was observed at both levels of Mn supply: Stojocri 2 having the highest shoot Mn content and Hazar the lowest at 30 mg Mn/kg dry soil; clear discrimination was observed between the genotypes (Fig 3.3 and Table 3.3) (see Appendix A, Table B17 for analysis of variance). The Mn content increased with increased supply of Mn and Hazar, Sham 1 and Rea/Dac//Teal had significantly higher shoot Mn content at 240 mg Mn/kg dry soil compared to the other genotypes (Fig 3.3 and Table 3.3).

Genotypic variation was observed for relative shoot dry weight and Stojocri 2 had highest relative shoot dry weight while Hazar the lowest; Significant discrimination was also observed among the other genotypes. Genotypes also responded differently (genotype by Mn interactions, $P<0.05$) to addition of Mn (Table 3.3).

Genotypic discrimination was observed for seedling Mn uptake at 30 mg/kg dry soil, and the genotypes ranked in the same order as for shoot Mn content with Stojocri 2 having the highest and Hazar the lowest seedling Mn uptake (Table 3.3) (see Appendix A, Table B19 for analysis of variance). Genotypes responded to supply of Mn by higher Mn uptake, and Hazar and Sham 1 had significantly higher uptake than the other genotypes (Table 3.3).

Summary of pot bioassay results

(i) The genotypes in the controlled environment chamber were clearly differentiated ($P<0.05$) on the basis of chlorosis score, shoot Mn content, Mn uptake, relative shoot dry weight and shoot dry weight at 30 mg Mn/kg dry soil.

(ii) The genotype by Mn interactions was significant ($P<0.05$) for all measured parameters.

(iii) The correlation coefficient matrix of parameters for the 30 mg Mn /kg dry soil showed significant correlation ($p<0.05$) between all combinations of parameters except YEBs Mn concentration, which correlated only with chlorosis and shoot Mn concentration (Table 3.4).

Correlation study

The correlation coefficient matrix of parameters measured from field screening at 0.0 and 11.2 kg Mn/ha compared to parameters measured at 30 mg Mn/kg in the controlled environment chamber is shown in Table 3.4. Parameters from the pot studies measured at 30 mg/kg dry soil correlated better with those from field measurements at 0.0 kg Mn/ha than with field measurements at 11.2 kg Mn/ha so, emphasis will be given to the former. The objective was to compare the ranking order of the measured parameters from the controlled environment with those from the field, in order to evaluate selection criteria that could be effectively used in further study and to reconfirm the observations made in Chapter 2. The ranking order of the measured parameters from the controlled environment compared with those from the field (Table 3.4) revealed that:

(i) YEBs Mn concentration from pot studies did not correlate significantly with any of the parameters from the field except straw Mn concentration,

(ii) Chlorosis score from pot studies correlated significantly (r=0.76*) only with development of chlorosis score in the field,

(3) shoot Mn concentration from pot studies correlated with the development of Mn deficiency chlorosis symptoms (r=-0.77*), grain yield (r=0.80*), relative grain yield (r=0.75*) and straw Mn concentration (r=-0.76*) in the field,

(4) shoot Mn content from pot studies had a high level of correlation with the development of Mn deficiency chlorosis symptoms (r=-0.85*), grain yield (r=0.81*) and relative grain yield (r=0.80*) (Fig 3.4),

(5) Mn uptake showed a high correlation with the development of Mn deficiency chlorosis symptoms (r=-0.82*), relative yield (r=0.78*) and above ground biomass at tillering (r=0.76*),

(6) shoot dry weight showed a significant correlation with relative grain yield (r=0.76*), above ground biomass at tillering (r=0.80*) and straw yield (r=0.78*), and

(7) relative shoot dry weight did not show any significant correlation with any of the parameters from the field.

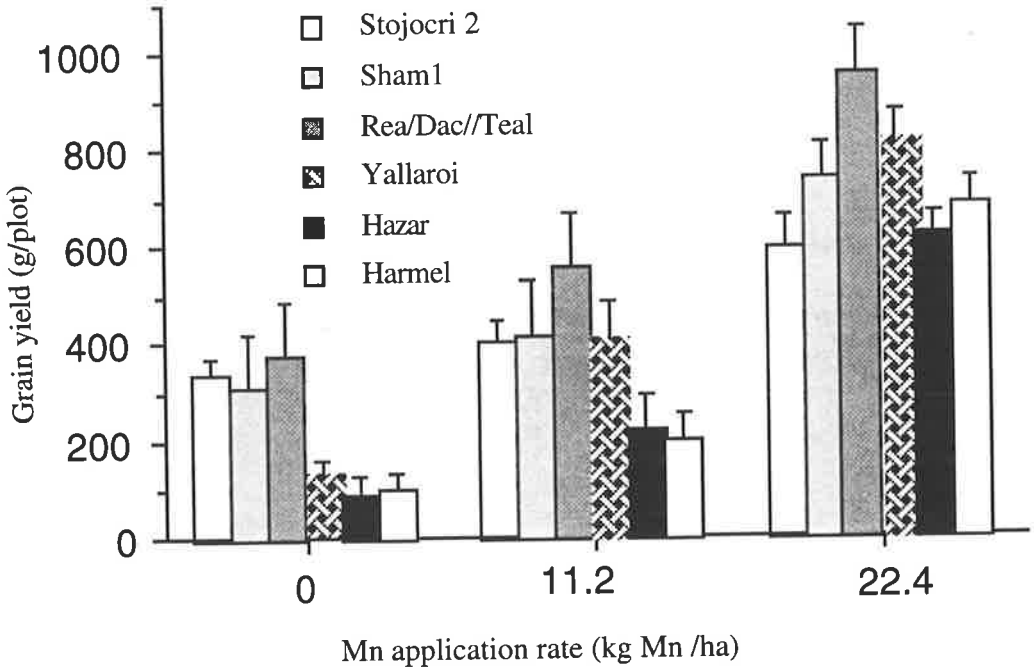

Fig 3.1 The grain yield (g/plot) of six durum wheats differing in Mn efficiency at three levels of Mn supply (kg/ha). Error bars represent standard error of means. Marion Bay, South Australia, field experiment, 1996.

Yallaroi 0.0 kg Mn ha $^{-1}$ Yallaroi 22.4 kg Mn ha $^{-1}$ Yallaroi 11.2 kg Mn ha $^{-1}$

Stojocri2 11.2 kg Mn ha $^{-1}$ Stojocri2 22.4 kg Mn ha $^{-1}$ Stojocri2 0.0 kg Mn ha $^{-1}$

Hazar 0.0 kg Mn ha $^{-1}$ Hazar 11.2 kg Mn ha $^{-1}$ Hazar 22.4 kg Mn ha $^{-1}$

Fig 3.2 The response of three durum wheat genotypes (Stojocri 2, Hazar and Yallaroi) to applied soil manganese (0.0, 11.2 and 22.4 kg Mn/ha) in terms of early growth, and development of deficiency symptoms 107 days after sowing, Marion Bay, South Australia, 1996.

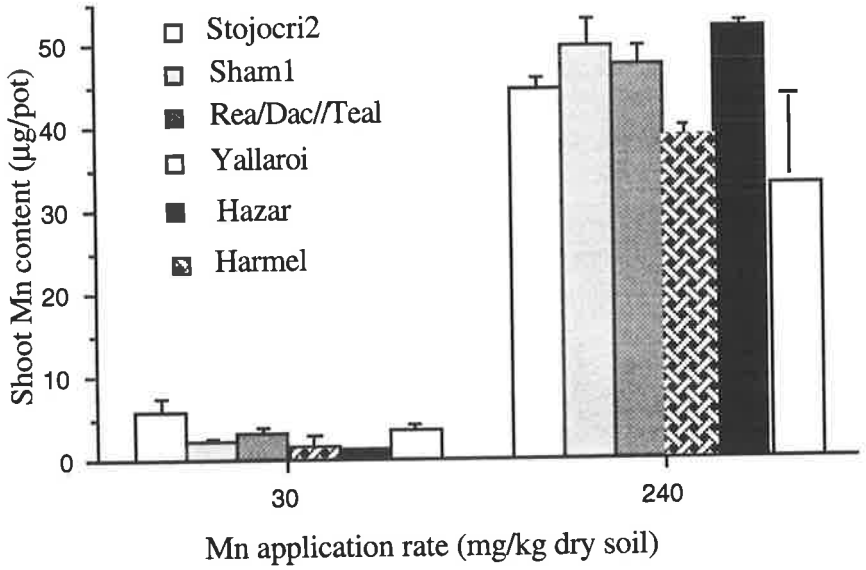

Fig 3.3 Seedling shoot Mn content (µg/pot) of six durum wheat genotypes differing in Mn efficiency 35 DAS at two levels of Mn application (30 and 240 mg Mn/kg dry soil). Error bars represent standard error of means.

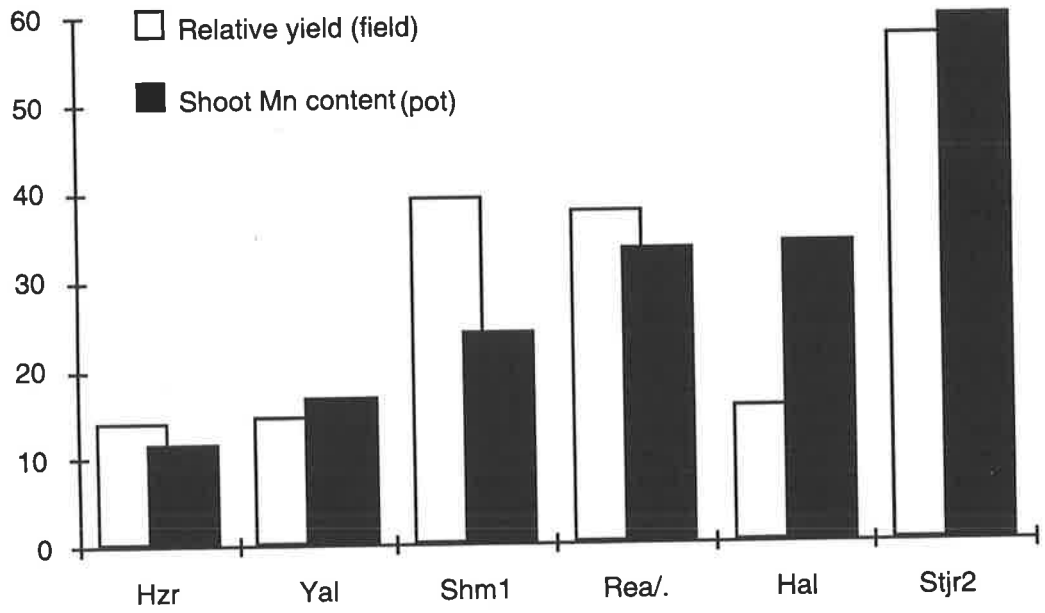

Fig 3.4 Ranking of six durum wheat genotypes on the basis of relative grain yield (grain yield at 0.0 kg/ha Mn/plot *100/grain yield at 22.4 kg Mn/ha) from the field experiment compared with shoot Mn content (mgx10^{-2}/pot) from the pot bioassay.

Table 3.2 Growth measurements from mid-season and maturity harvests of six durum wheat genotypes evaluated at three levels of soil Mn supply (0, 11.2 and 22.4 kg Mn/ha) at a Mn deficient site (Marion Bay, South Australia, 1996). The values are means of four replications.

Parameters are: chlorosis score (**CS**, 1-5 scale), Mn concentration (mg/kg) of youngest emerged leaf blade (**YEB**) at tillering, above ground vegetative growth (g/plot, **BioT**) at tillering, straw dry weight (**Stwyld**, above ground biomass - grain yield, g/plot) at maturity, grain yield (**Gyld**, g/plot), relative grain yield (**Ryld**, %), grain Mn concentration (mg/kg, **GMnc**), grain Mn content (mg/plot, **GMnct**), straw Mn concentration at maturity (mg/kg, **Stwc**), straw Mn content (mg/plot, **Stwct**), shoot Mn content (mg/plot, **Shtct**), above ground biomass at maturity (g/plot, **Bio**) and Mn uptake (Mn content of straw + Mn content of grain, mg/plot, **Mnup**).

Genotype	**CS** (1-5)			**YEB**(mg/kg)			**BioT** (g/plot)			**Stwyld** (g/plot)		
	Mn level (kg/ha)			Mn level (kg/ha)			Mn level (kg/ha)			Mn level (kg/ha)		
	0.0	11.2	22.4	0.0	11.2	22.4	0.0	11.20	22.4	0.0	11.2	22.4
Stojocri 2	3.3	2.7	1.2	7.0	7.2	7.9	119	137	182	529	535	749
Sham 1	3.6	3.4	1.4	6.7	7.9	8.1	86	117	117	457	497	769
Rea/Dac//Teal	4.0	3.6	2.0	6.7	7.5	8.7	39	83	104	269	464	1011
Yallaroi	5.0	4.0	1.5	6.9	7.6	8.2	11	88	98	125	659	986
Hazar	4.9	3.9	2.0	6.6	6.5	6.9	24	89	124	146	300	720
Harmel	4.4	3.6	2.0	6.3	6.7	7.1	30	91	133	216	333	514
LSD 5% Genotype		0.2			0.7			17			141	
LSD 5% Mn level		0.1			0.5			12			99	
LSD 5% Interaction		0.3			ns			31			250	

Table 3.2 (continued)

Genotype	Gyld (g/plot)			Ryld (%)			GMnc (mg/kg)			GMnct (mg/plot)		
	Mn level (kg/ha)			Mn level (kg/ha)			Mn level (kg/ha)			Mn level (kg/ha)		
	0.0	11.2	22.4	0.0	11.2	22.4	0.0	11.2	22.4	0.0	11.2	22.4
Stojocri 2	341	402	596	58	68	100	4.0	4.1	6.7	1.3	1.7	3.9
Sham 1	313	413	739	40	54	100	4.7	3.3	6.2	1.8	1.5	4.8
Rea/Dac//Teal	375	559	955	38	59	100	2.8	2.8	4.9	1.2	1.7	4.6
Yallaroi	126	407	821	15	49	100	2.2	4.7	4.0	0.3	2.3	3.4
Hazar	89	223	628	14	36	100	3.0	2.5	4.1	0.3	0.6	2.6
Harmel	105	201	684	16	31	100	2.1	2.7	3.0	0.2	0.7	2.1
LSD 5% Genotype		65			8			1.3			0.8	
LSD 5% Mn level		49			6			0.9			0.6	
LSD 5% Interaction		115			14			2.3			1.5	

Table 3.2 (continued)

Genotype	Stwc (g/plot)			Stwct (mg/plot)			Bio (mg/plot)			Mnup (mg/plot)		
	Mn level (kg/ha)			Mn level (kg/ha)			Mn level (kg/ha)			Mn level (kg/ha)		
	0.0	11.2	22.4	0.0	11.2	22.4	0.0	11.2	22.4	0.0	11.2	22.4
Stojocri 2	2.5	3.0	19.1	1.4	1.6	12.4	870	938	1344	2.6	3.2	16.4
Sham 1	2.6	2.6	16.9	1.1	1.3	12.7	769	920	1508	2.9	2.8	17.5
Rea/Dac//Teal	2.1	2.2	7.2	0.6	1.0	7.3	644	1023	1966	1.8	2.7	11.9
Yallaroi	2.5	3.4	16.8	0.4	2.4	15.9	251	1067	1808	0.6	4.7	19.3
Hazar	2.8	3.0	4.1	0.5	0.9	3.0	235	523	1349	0.8	1.5	5.6
Harmel	2.3	1.9	4.2	0.4	0.6	2.2	322	534	1198	0.6	1.3	4.3
LSD 5 Genotype		3.5			2.3			181			2.7	
LSD 5% Mn level		2.5			1.6			128			1.9	
LSD 5% Interaction		6.2			4.1			321			4.7	

Table 3.3 Growth measurements of six durum wheat genotypes evaluated at two levels of soil Mn supply (30 and 240 mg Mn/kg dry soil) 35 DAS in a controlled environment chamber. The values are means of three replications. Parameters are: chlorosis score (**CS**, 1-5 scale), Mn concentration (mg/kg) of youngest emerged leaf blade (**YEB**), shoot dry weight (**Shtwt**, g/pot), shoot Mn concentration (mg/kg, **Shtc**), shoot Mn content (µg/pot, **Shtct**), seedling Mn uptake (Mn content of shoot + Mn content of root, µg/pot, **Sdup**), relative shoot dry weight (**Rshtwt**, %).

Genotype	**CS** (1-5)		**YEB** (mg/kg)		**Shtc** (mg/kg)		**Shtwt** (g/pot)	
	Mn level (mg/pot)		Mn level (mg/pot)		Mn level (mg/pot)		Mn level (mg/pot)	
	30	240	30	240	30	240	30	240
Stojocri 2	1.0	1.0	9.7	56.2	10.7	73.1	0.5	0.64
Sham 1	2.5	1.0	8.5	49.0	8.2	66.2	0.3	0.79
Rea/Dac//Teal	1.7	1.0	12.4	90.3	11.1	90.2	0.3	0.53
Yallaroi	2.5	1.0	8.4	67.0	7.5	68.3	0.2	0.57
Hazar	3.3	1.0	6.2	61.1	6.6	70.7	0.2	0.76
Harmel	1.8	1.0	9.3	74.5	8.8	58.8	0.4	0.54
LSD 5% Genotype	0.3		13.3		7.3		0.1	
LSD 5% Mn level	0.2		7.7		4.2		0.1	
LSD 5% Interaction	0.4		ns		ns		0.2	

Table 3.3 (continued)

Genotype	Shtct (µg/pot)		Rshtwt (%)		Sdup(µg/pot)	
	Mn Level (mg/pot)		Mn Level (mg/pot)		Mn Level (mg/pot)	
	6.0	240	30	240	30	240
Stojocri 2	2.4	44.8	83	100	14.4	77.1
Sham 1	3.4	49.8	37	100	3.8	120.1
Rea/Dac//Teal	1.7	47.5	57	100	6.3	94.6
Yallaroi	1.2	38.6	34	100	2.8	76.7
Hazar	3.4	52.0	23	100	1.7	111.1
Harmel	4.0	32.9	70	100	6.7	60.0
LSD 5% Genotype	2.3		14		4.0	
LSD 5% Mn level	5.7		8		2.0	
LSD 5% Interaction	ns		20		ns	

Table 3.4 Correlation coefficients between measured parameters from six durum wheat genotypes evaluated at two levels of soil Mn supply (0.0 and 11.2kg Mn/ha) respectively at Mn deficient site (Marion Bay, South Australia, 1995) and at 35 DAS in controlled enviroment chamber (30 mg/kg dry soil). Parameters scored in the controlled enviroment experiment were: Chlorosis score (**CS**, 1-5 scale), Mn concentration of youngest emerged blade (**YEB**, mg/kg dry weight), shoot Mn concentration (**Shtc**, mg/kg dry weight), shoot Mn content (**Shtct**, µg/pot), seedling Mn uptake (**Sdup**, µg/pot) and relative shoot dry weight (%). Parameters from the field experiment were: chlorosis score (**CS**, 1-5), Mn concentration of youngest emerged blade (**YEB**, mg/kg dry weight), above ground biomass at tillering (**BioT**, g/plot), straw Mn concentration at maturity (**Stwc**, mg/plot), sraw Mn content at maturity (**Stwct**, mg/plot), grain yield (**Gyld**, g/plot), grain Mn concentration (**GMnc**, µg/g), grain Mn content (**GMnct**), straw yield at maturity (**stwyld**, g/plot), above ground biomass at maturity (grain + straw, g/plot, **Bio**), relative grain yield (**Ryld**, %) and Mn uptake (Mn content of straw + Mn content of grain, **Mnup**, mg/plot).

		POT 30 mg Mn/kg dry soil							FIELD 0.0 kg Mn/ha										
		YEB	**CS**	**Shtc**	**Shtct**	**Sdup**	**Shtwt**	**Rshwt**	**CS**	**YEB**	**Gyld**	**GMnc**	**GMnct**	**Mnup**	**Bio**	**Ryld**	**Stwc**	**Stwct**	**BioT**
POT	**CS**	-0.76*																	
	Shtc	0.92*	-0.90*																
	Shtct	0.56	-0.95**	0.82*															
	Sdup	0.48	-0.92*	0.77*	0.99**														
	Shtwt	0.44	-0.89*	0.72	0.98**	0.97**													
	Rshwt	0.62	-0.96**	0.81*	0.95**	0.92**	0.94**												
FIELD	**CS**	-0.53	0.76*	-0.77*	-0.85*	-0.82*	-0.95**	-0.71											
	YEB	0.26	-0.34	0.32	0.32	0.34	0.22	0.10	-0.41										
	Gyld	0.72	-0.70	0.80*	0.81*	0.55	0.51	0.44	-0.85*	0.59									
	GMnc	-0.06	0.05	0.11	0.22	0.20	0.30	0.01	-0.67	0.32	0.60								
	GMnct	0.40	-0.35	0.50	0.40	0.35	0.40	0.20	-0.81*	0.52	0.89*	0.88*							
	Mnup	0.34	-0.45	0.52	0.55	0.51	0.55	0.33	-0.90*	0.56	0.87*	0.89*	0.97**						
	Bio	0.52	-0.65	0.71	0.72	0.69	0.70	0.54	-0.97**	0.56	0.93*	0.76*	0.92*	0.97**					
	Ryld	0.51	0.72	0.75*	0.80*	0.78*	0.76*	0.60	-0.96**	0.62	0.90*	0.66	0.83*	0.91*	0.98**				
	Stwc	- 0.95**	0.59	-0.76*	-0.33	-0.24	-0.22	-0.48	0.27	-0.01	-0.49	0.26	-0.18	-0.07	-0.24	-0.20			
	Stwct	0.20	-0.55	0.49	0.71	0.71	0.73	0.48	-0.91*	0.57	0.74	0.80*	0.81*	0.93*	0.93*	0.93*	0.80*		
	BioT	0.21	-0.60	0.51	0.76	0.76*	0.80*	0.56	-0.93*	0.49	0.70	0.78*	0.78*	0.90*	0.92*	0.92*	0.77*	0.99**	
	Stwyld	0.30	-0.61	0.55	0.74	0.72	0.78*	0.55	-0.96**	0.48	0.77*	0.81*	0.85*	0.94* *	0.95**	0.93*	-0.02	0.98**	0.99**

*, ** significant respectively at P<0.05 and 0.01, data are averages of three replications of six genotypes (n=6) from pot bioassay and four replications of six gentypes from field experiment (n=6).

Table 3.4 (continued)

		YEB		P	O	T	30 mg	Mn/kg	dry	soil								
	CS	-0.76*	CS															
P	Shtc	0.92*	-0.90*	Shtc														
O	Shtct	0.56	-0.95**	0.82*	Shtct													
T	Sdup	0.48	-0.92*	0.77*	0.99**	Sdup												
	Shtwt	0.44	-0.89*	0.72	0.98**	0.97**	Shtwt											
	Rshwt	0.62	-0.96**	0.81*	0.95**	0.92**	0.94**	Rshwt				F	I	E	L	D	11.2 kg	Mn/ha
	CS	-0.36	0.78*	-0.66	-0.90**	-0.90*	-0.92*	-0.96**	CS									
	YEB	0.38	0.18	0.24	0.03	0.03	-0.01	0.65	-0.36	YEB								
	Gyld	0.71	-0.17	0.62	0.25	0.26	0.10	0.12	-0.25	0.77*	Gyld							
F	GMnc	0.06	-0.12	0.07	0.25	0.29	0.20	0.14	-0.25	0.48	0.35	GMnc						
I	GMnct	0.40	-0.03	0.29	0.14	0.14	0.02	0.04	-0.12	0.74	0.77*	0.84*	GMnct					
E	Mnup	0.17	0.07	0.09	0.05	0.05	-0.05	-0.07	-0.06	0.65	0.60	0.92*	0.97*v	Mnup				
L	Bio	0.54	-0.10	0.45	0.23	0.23	0.10	0.10	-0.24	0.83*	0.91**	0.70	0.96**	0.87*	Bio			
D	Ryld	0.54	-0.50	0.68	0.62	0.62	0.51	0.40	-0.71	0.62	0.81*	0.53	0.69	0.59	0.81	Ryld		
	Stwc	-0.49	0.24	-0.39	-0.18	-0.18	-0.22	-0.38	0.01	0.17	0.10	0.71	0.53	0.69	0.37	0.31	Stwc	
	Stwct	-0.08	0.17	-0.14	-0.06	-0.06	-0.12	-0.18	0.01	0.51	0.38	0.94* *	0.88*	0.96**	0.96* *	0.44	0.82*	Stwct
	BioT	0.02	-0.52	0.33	0.69	0.69	0.76	0.49	-0.92*	0.25	0.10	0.39	0.14	0.17	0.20	0.63	0.26	0.18
	Stwyld	0.27	-0.02	0.19	0.19	0.16	0.08	0.05	-0.18	0.74	0.64	0.92*	0.97**	0.98**	0.91	0.65	0.59	0.92*

BioT: 0.27

*, ** significant respectively at P<0.05 and 0.01, data are averages of three replications of six genotypes (n=6) from pot bioassay and four replications of six genotypes from field experiment (n=6).

3.2.3 Discussion

In field screening, better discrimination of genotypes was observed for chlorosis score and grain yield than for YEBs Mn concentration, which is the measure of Mn adequacy or deficiency status of a crop (Table 3.2). Manganese-efficient genotype(s) developed less chlorosis, had higher YEBs Mn concentration, produced more dry matter at tillering and at maturity, maintained higher grain yield and relative grain yield, higher straw yield, higher grain Mn concentration and grain Mn content and finally higher uptake of Mn from the deficient soil compared to inefficient genotypes (Fig 3.1; Table 3.2). Mn-efficient genotype(s) also responded differently to supply of Mn (genotype by Mn), especially in terms of grain yield, relative grain yield and above ground biomass production either at tillering or at maturity compared to inefficient genotype(s) (Fig 3.1; Table 3.2).

In the pot bioassay, efficient genotypes developed a lower chlorosis score, maintained higher YEBs Mn concentration, higher shoot Mn concentration, higher shoot Mn content, higher Mn uptake, higher shoot dry weight and higher relative shoot dry weight compared to inefficient genotypes (Table 3.3). Genotypic discrimination were better in terms of chlorosis score, shoot Mn content, Mn uptake and shoot dry weight than YEBs Mn concentration or shoot Mn concentration (Fig 3.3; Table 3.3).

The higher observed correlation coefficients between the parameters from genotypes at 30 mg Mn/kg dry soil (controlled environment) versus the 0.0 kg Mn/ha (field screening) than at the 11.2 kg Mn/ha rate (Table 3.4) might be explained, in part, by the higher pH (8.5) and 80% $CaCO_3$ content of Wangary soil used in the pot bioassay compared to Marion Bay soil used for the field study (pH=8.1 and 72% $CaCO_3$) and the more severe Mn-deficient nature of the former soil (Huang, 1996).

In an attempt to find a rational basis for choosing the most reliable selection criterion and confirming the result of the previous experiments (Section 2.2), study of the correlation matrix of parameters measured in the field with 0.0 kg Mn/ha compared to the controlled environment chamber at 30 mg Mn/kg dry soil (Table 3.4) revealed that: shoot Mn content, Mn uptake and shoot Mn concentration from the controlled environment chamber correlated highly ($P<0.05$) with grain yield, relative grain yield and development of chlorosis score (Table 3.4; Fig 3.4). Considering the higher correlation for shoot Mn content versus either grain yield or relative grain yield, and the clear discrimination of genotypes on the basis of shoot Mn content compared to shoot Mn concentration shoot Mn content was selected as the selection criterion for further screening (Fig 3.4; Tables 3.3 and 3.4).

The experiments in this and the previous Chapter differed in that the Mn content of the seed used was controlled in the Chapter 3 studies. Comparison of ranking order of genotypes from the controlled environment experiment and field screening (Tables 3.2 and 3.3) in respect to the previous pot and field experiments (Tables 2.4 and 2.5) revealed that:

In the pot bioassay

(i) The ranking order for Sham 1, Rea/Dac//Teal, Yallaroi and Harmel changed, but this remained unchanged for Stojocri 2 and Hazar which had been ranked the highest and the lowest, respectively, for development of Mn deficiency symptoms.

(ii) The ranking order for YEBs Mn concentration changed for all the genotypes, except Harmel which ranked third.

(iii) The ranking order changed for all genotypes in respect of shoot Mn concentration.

(iv) Hazar was ranked last in both experiments while the order changed for the five other genotypes in respect to shoot Mn content.

(v) The ranking order for Hazar and Rea/Dac//Teal remained the same for shoot dry weight and relative shoot dry weight, while that for the other four genotypes changed.

In the field screening

(vi) The ranking order for development of chlorosis score remained unchanged for Stojocri 2, Sham 1 and Rea/Dac//Teal, but changed to some extent for the other genotypes.

(vii) Above ground biomass production at tillering remained constant for Stojocri 2 but changed to some extent for other genotypes.

(iix) Ranking order for Stojocri 2, Harmel and Hazar remained unchanged in respect of grain yield but changed for the other genotypes.

(ix) Relative grain yield changed for all genotypes.

In summary, in controlled environment experiments, the use of seed with uniform Mn content altered, to some extent, the ranking of genotypes with respect to chlorosis score, YEBs Mn concentration, shoot Mn concentration, shoot dry weight, relative shoot dry weight and shoot Mn content, shoot Mn concentration and chlorosis score (Tables 2.4 and 3.3). The same trend, resulting from using seeds with uniform Mn content, was also observed in the field study (Tables 2.5 and 3.2) where ranking of genotypes was changed

for chlorosis score, biomass production at tillering, grain yield and relative grain yield. The fact that the above parameters in the previous study were confounded by variation in seed Mn reserve has been demonstrated (Section 2.3). As a result, for all parameters, the efficient genotype(s) ranked either first or second while one of the inefficient ones (Hazar or Yallaroi) always ranked last, both in pot bioassay and field experiments. This change in ranking order between the two studies has confirmed the significance of seed Mn reserve in early growth and development of durum wheat, and also the importance of using seed with uniform Mn reserves for screening based on yield and yield-dependent characters as addressed by Longnecker *et al.* (1991b) and Marcar and Graham (1986).

3.3 Pedigree analysis of the origin of a manganese inefficient and moderately manganese efficient durum wheat genotype

To determine the source of Mn efficiency for further exploitation of intra-specific genotypic variation, a pot bioassay was undertaken to study performance at two levels of Mn supply of the parents and progenitors of two durum genotypes differing in Mn efficiency. These two genotypes were selected on the basis of their large differences in grain yield (Sections 2.3 and 3.2 2) when evaluated in two consecutive growing seasons (1995-96) at the Mn-deficient site (Marion Bay, South Australia). Plant height and maturity type were the same, and they also came from the same breeding program.

3.3.1 Materials and methods

Nineteen durum genotypes (cultivated varieties, breeding lines and landraces) including the progenitors of Sham 1 and Hazar (Brajcich *et al.*, 1986), a moderately efficient and

inefficient genotype, were used in this study. Following is a list of the genotypes, along with the abbreviations used (Table 3.5):

Sham 1 = Plc"s"/Ruff"s"//Gta/Rtte"s"

Plc"s"=Gll"s"/Jo"s"

Ruff"s"=Jo"s"/3/Ld357E/2*Tc60//Gll"s"

Gta=Gaviota=Cr"s"/4/T.Pol.185309//T.Gle/2*Tc60/3/Glls

Rtte"s"=Rolette

Plc"s"=Peliccano=Gll"s"/Jo"s"

Gll"s"=Tme/2*Tc60//ZB/Wls

Jo"s"=Bye*2/Tc60//Tac1252/3*Tc60

Gll"s"=Grulla=Tme/2*Tc60//ZB/Wls

Tme=Tremez Molle Enano (Mexico)

Tc60=Cvc/Ld357//Cp

ZB=Zenati Bouteille

WLS=Wells

Tc60=Cvc/Ld357//Cp

Cvc=Carvaca (Spain)

Ld357=Langdon 357

Cp=Cappelli

Jo"s"=Bye*2/Tc60//Tac125Z/3*Tc60

Bye=Barrigon Yaqui (*Triticum turgidum*), Mexico125 (Iberian Durum)

Ruff"s"=Jo"s"/3/Ld357E/2*Tc60//Gl"s"

Gta=Gaviota=Cr"s"/4/T.Pol.185309//T.Gle/2*Tc60/3/Glls

Cr"s"=Crane=Bye*2/Tc60/Stw63/3/ZB/Wls/4/Gll"s"

T.Pol.185309=*Triticum polonicum*

T.Gle=*Triticum glutinosum* Enano (Mexico)

Stw63=Stewart 63

Hazar=T. dur. T. sph-Ram/Gll//M.sadova/3/Aa/Cr/4/21563//61-130/Lds

T. dur. T. sph= (unknown)

Ram=Ramsey

M.sadova=Mariza sadovo (USSR)

Aa=Tnhinga=Tme/2*Tac60//Zb/2*Wls

21563=JO"S"=Bye*2/Tc60//Tac1252/3*Tc60

61-130=Advanced Line from N. Dakota

Lds =Leeds

Sham 1 (named also Waha or Frig"S") and Hazar both originated from CIMMYT/ICARDA, and the former has wide adaptation in the WANA region, having been released as a commercial variety in Algeria, Cyprus, Jordan, Saudi Arabia, Syria and Turkey. Seed of progenitors of Sham 1 and Hazar was provided by the Australian Winter Cereal Collection, and the seed varied in Mn content (0.9-2.7 µg Mn/seed) and also in seed size.

To avoid the confounding effects of seed Mn reserve on the screening process, the selected genotypes were grown under controlled environment conditions (14 hour light/10 hour dark photoperiod at 25°C day/15°C night) under a range of Mn treatments to enhance production of seed with similar Mn content. The genotypes were grown in University of California (UC) potting mix and received either no foliar spray, one foliar spray (at milky dough stage) or two foliar sprays (at milky dough stage and at dough stage) of Mangasol (as described in Section 2.3.2). This allowed selection of seed with reasonably similar Mn content for all genotypes, following the same procedure as explained in Section 3.2. Seed Mn content, genotype abbreviations, geographic source and agronomic data are presented in Table 3.5.

Manganese treatment

The genotypes were evaluated at two levels of Mn supply (25 and 240 mg/kg dry soil). The level of Mn selected (25 mg/kg) in this study was lower than in Section 3.2 to compensate for the higher seed Mn content (Table 3.5) to achieve the same level of Mn stress. Soil preparation, conditions of growth, duration of experiment and harvest practices were the same as described in Section 2.2.

3.3.2 Results

Shoot manganese content

Sham 1, Hazar and their parents differentiated on the basis of shoot Mn content at the low level of Mn supply (25 mg/kg soil) (Fig 3.5). Hazar, the inefficient genotype, showed the lowest ($P<0.05$) Mn content followed by two of the its parents Wells and Leeds (Fig 3.5). In contrast, the other parents Jori and Crane demonstrated higher Mn content than Hazar ($P<0.05$) (Fig 3.5) (see Appendix A, Table C1 for analysis of variance).

Sham 1 and all the immediate individual parents (Plc"s", Ruff"s", Gta and Rtte"S") of CIMMYT origin expressed the same level of Mn efficiency as Sham 1 ($P<0.05$) (Fig 3.5). Among progenitors of the parents were Zenati Bouteille, Cappelli, Caravaca and Tac125Z (Iberian durum) which were dominant ancestors. These generally expressed the same level of efficiency as Sham 1, with the exception of Zenati Bouteille which ranked the highest ($P<0.05$) (Fig 3.5) (see Appendix A, Table C1 for analysis of variance). These early progenitors (Zenati Bouteille, Cappelli, Caravaca and Tac125Z) make up the backbone of the intermediate parents (Tc60, Jo"s", Gll"s" and Cr"s") which were used extensively in crosses and backcrosses with Ld357 (Tc60=Cvc/Ld357//Cp), Barrigon Yaqui (Jo"s"=Bye*2/Tc60//Tac125Z/3*Tc60), Wells (Gll"s"=Grulla=Tme/2*Tc60//ZB/Wls), Stewart 63 (Cr"s"=Crane=Bye*2/Tc60/Stw63/3/ZB/Wls/4/Gll"s"), for incorporation of earliness, short stature, good agronomic type (Table 3.5), adaptation, better quality and disease resistance.

Shoot manganese concentration

The same trend as for Mn content was observed in shoot Mn concentrations for the progenitors of Sham 1 and Hazar: Zenati Bouteille expressed the highest shoot Mn concentration while the lowest shoot Mn concentration was observed in Hazar and its progenitors, Ramsey, Wells and Leeds (Fig 3.6) (see Appendix A, Table C2 for analysis of variance). However, differentiation of genotypes was more distinct on the basis of shoot Mn content than concentration (Fig 3.6).

Deficiency symptoms

Genotypic variation based on deficiency symptoms was not as distinct as that based on shoot content due to the effect of higher seed Mn content (0.47 µg Mn/seed compared to 0.23 in Section 3.2) (Fig 3.6). Nevertheless, the genotypes still expressed a trend similar to that observed for shoot Mn content: Hazar and its progenitors, Ramsey and Wells, ranked as the most sensitive in terms of development of chlorosis symptoms. No deficiency symptoms were observed for Sham 1 and its immediate parent (Plc"s" and Gta) or its efficient progenitors (Zenati Bouteille, Cappelli and Barrigon Yaqui) (Fig 3.7) (see Appendix A, Table C3 for analysis of variance).

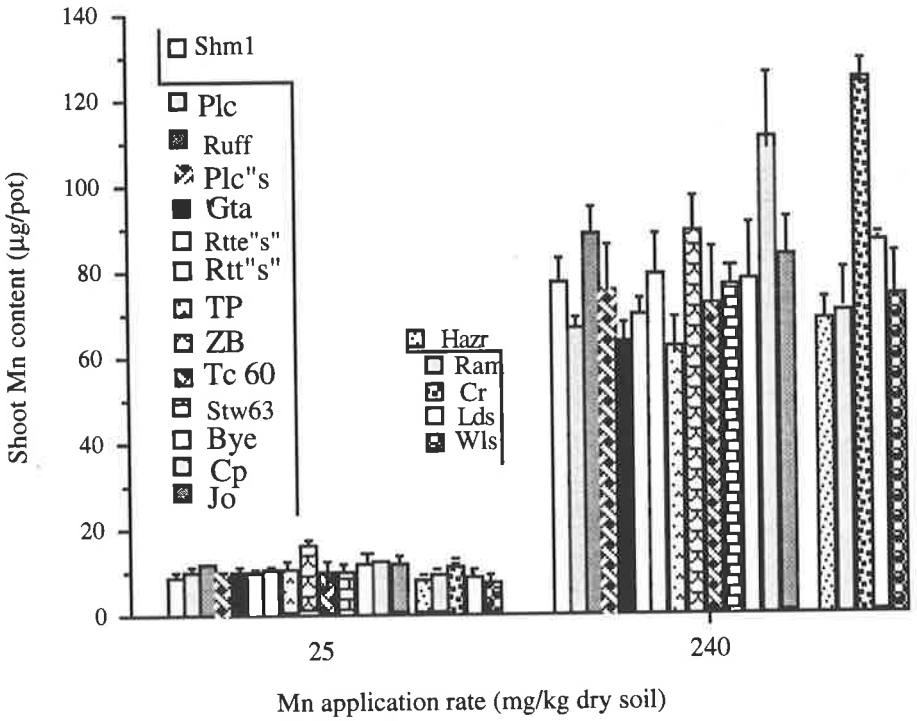

Fig. 3.5 The shoot Mn content (µg/pot) of parents of Mn-efficient (Sham1) and Mn-inefficient (Hazar) durum wheat genotypes (including Sham1 and Hazar) 35 DAS at two levels of Mn supply.

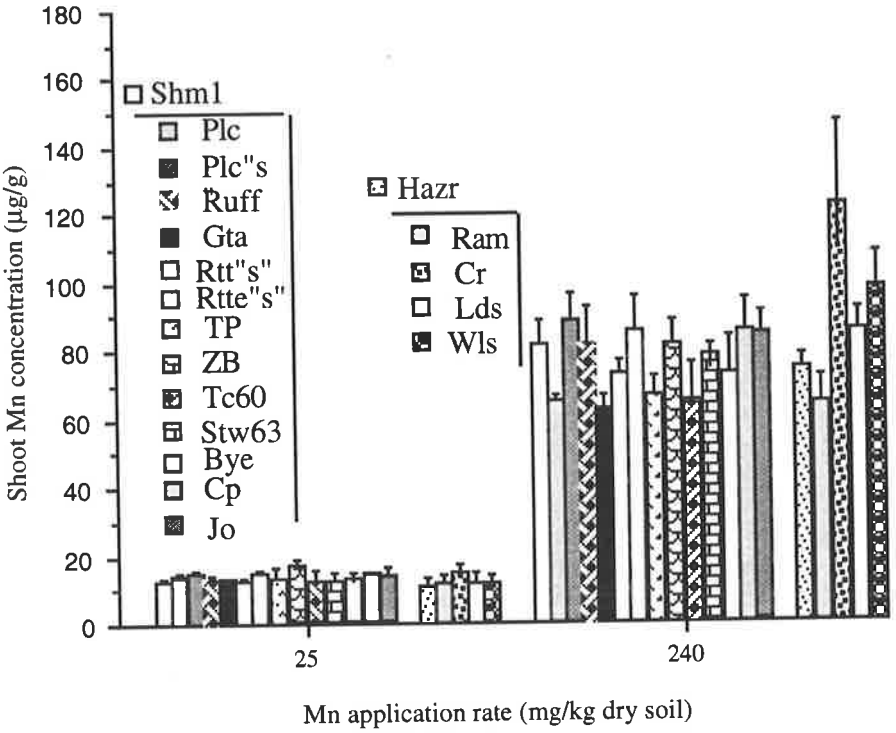

Fig. 3.6 The shoot Mn concentration (mg/kg) of parents of Mn-efficient (Sham1) and Mn-inefficient (Hazar) durum wheat genotypes (including Sham1 and Hazar) 35 DAS at two levels of Mn supply.

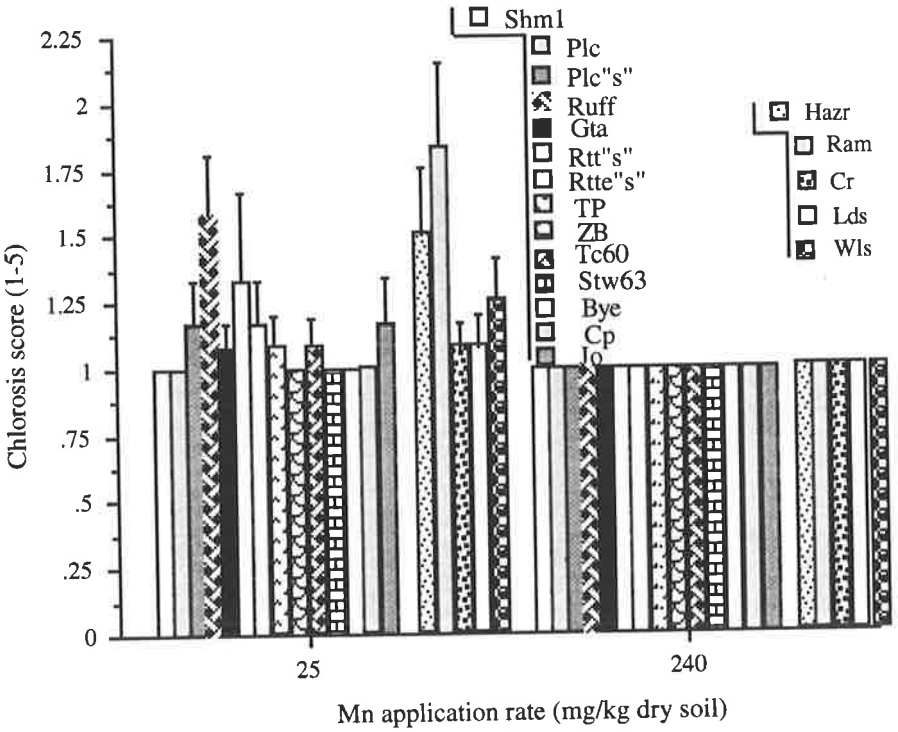

Fig. 3.7 The development of Mn deficiency symptoms and chlorosis score (1-5) in Mn-efficient (Sham1) and Mn-inefficient (Hazar) durum wheat genotypes and their parents 35 DAS at two levels of soil Mn supply.

Table 3.5 Name, abbreviation (Abb), Australian Winter Cereal Collection reference number (AUS), source, seed Mn content, plant stature and maturity data of two durum wheat genotypes differing in Mn efficiency and their 19 parents and progenitors.

name	Abb	AUS reference Number	Source *	Mn content (µg/seed)	Plant stature **	Maturity
Sham 1	Shm1	23839	Mex/Sy	0.46	D	Early
Pelicano	Plc	19444	Mex	0.48	D	Early
Pelicano"s"	Plc"S"	5895	Mex	0.43	D	Medium
Ruff	Ruff	18147	Mex	0.44	Semi D	Early
Gaviota"s"	Gta	20404	Mex	0.42	Semi D	Medium
Rollette1	Rtt"S"	19549	USA	0.47	Semi D	Med-Early
Rollette2	Rtte"S"	15506	USA	0.36	Semi D	Med-Early
Hazar	Haza	24287	Leb/Sy	0.45	D	Med-Early
Ramsey	Ram	999	USA	0.35	Tall	Late
Crane	Cr	15946	Mex	0.43	D	Early
Leeds	Lds	11862	USA	0.48	Semi D	Medium
Wells	Wls	3529	USA	0.41	Tall	Late
Tremez Preto	TP	1414	Portugal	0.49	Tall	Late
Zenati Bouteille	ZB	19534	Algeria	0.46	Med	Med
Tehuancan 60	Tc 60	1377	Spain	0.42	Tall	Late
Stewart 63	Stw 60	12059	USA	0.46	Tall	Late
Bye=BarrigonYaqui *(Triticum turgidum)*	Bye	12284	Mex	0.44	Med	Med-Early
Cappelli	Cp	2068	Italy	0.42	Tall	Late
Jori	Jo	14077	Mex	0.47	D	Medium

*Source; Mex=Mexico, Sy=Syria and Leb=Lebanon

**Plant stature; D=Dwarf and Med=Medium

3.3.3 Discussion

This study was undertaken to understand the origin of Mn efficiency in durum wheat germplasm. The lowest level of efficiency was demonstrated in Hazar (in terms of shoot Mn content, development of chlorosis score and shoot Mn concentration) and its two immediate parents (Leeds and 61-130) and its indirect parent (Wells) (Figs 3.5, 3.6 and 3.7). The use of Mn-inefficient parents originating from the USA and North Dakota in crosses with the other parental lines of Hazar (Jori, Ramsey and Crane), and the lack of selection pressure (for an unfavourable high pH environment) explains very well its poor tolerance to Mn deficiency. The genotype 61-130 from North Dakota demonstrated poor tolerance to deficiency in a preliminary study, so was not included in this experiment. The two other parental genotypes (*T. dur. T. sph* and *M. sadova*) were not available for this study.

Moderate Mn efficiency was observed in Sham 1 and its four immediate parents of CIMMYT origin (Plc"s", Ruff"s", Gta and Rtte"S"). It was also intermediate to the observed variation of its progenitors ($P<0.05$) (Figs 3.5, 3.6 and 3.7) (see Appendix A, Tables C1, C2 and C3 for analysis of variance). The main progenitors of the immediate parents were from Italy (Cappelli), Iberia (Tac 125Z= Iberian durum), Portugal (Tremez Preto) and Algeria (Zenati Bouteille). These expressed the same level of tolerance as Sham 1, or in the case of Zenati Bouteille higher tolerance (Figs 3.5, 3.6 and 3.7). Included in the parentage of Sham 1 are genotypes including Ld 357, Bye, Wells and Stewart 63 (developed in CIMMYT and North America, probably for the incorporation of shorter crop stature and superior agronomic type) and these gave rise to the production of lines of intermediate Mn efficiency (Tehuancan 60 and Grulla). These intermediate lines, in further crosses with each other or with early progenitor lines, formed the immediate parental lines and varieties (Peliccano, Ruff, Gaviota"s' and Rollette) of Sham 1. It is

interesting that among progenitor lines and parental varieties of Sham 1, none had lower Mn efficiency than Sham 1, with one land race from Algeria (Zenati Bouteille in Figs. 3.5, 3.6 and 3.7) which was more efficient. Two other parental genotypes (T.Pol.185309 and Caravaca) were not available for the study.

Generally, the agronomic type and tall stature of the progenitors which evolved mainly in dry climates, mostly under supplementary irrigation, were unsuited to and consequently performed poorly under favourable conditions. This explains the necessity of incorporation of short stature, better agronomic types and probably disease tolerance and quality traits. Sham 1 was selected on the results of a preliminary screening (Section 2.3); however, the same pattern as Sham 1 for tolerance to Mn deficiency exists among the parents and progenitors of Stojocri 2 (Stk/4/Jo/3/Jo/Cr//Cit 71), another durum genotype with relatively high Mn efficiency.

Tracing back the pedigree of Sham 1 revealed that this cultivar, in contrast to Hazar, was intermediate in Mn efficiency compared to parental lines, which indicated inheritance of the traits from parents without selection pressure. There was no evidence that dominance or transgressive segregation had affected transmission of the trait. Moderate Mn efficiency expressed by several progenitors made determination of the origin of Mn efficiency difficult. Nevertheless, the highest Mn efficiency observed in the trial ($P<0.05$) is that Zenati Bouteille, and this focuses attention on Algeria as a possible source of Mn efficiency in durum wheat. Further study of accessions derived from that region is warranted, while the results of this study justify studying entries from other centres of origin.

The study of origin of tolerance to either deficiency or toxicity of micronutrients has provided valuable information which has application for the further exploitation of intra-

specific variation in crop species. Graham *et al.* (1983) noticed a common parent in the pedigree of Mn-inefficient barley genotypes originating from Alexandria. It has been proposed that Mn efficiency in barley is controlled by a single dominant gene and simply inherited (Sparrow *et al.*, 1983). In contrast, tolerance to Mn toxicity in bread wheat appeared to be a quantitative trait and pedigree analysis of the origin of the trait revealed that tolerance had been introduced from Brazilian cultivars into Canadian germplasm (Moroni *et al.*, 1991).

Moderate tolerance to boron toxicity has been observed in genotypes from the centre of origin of durum wheats (Yau *et al.*, 1995; Jamjod, 1996). For both Mn and B, the tolerance of durum genotypes originating from different sources in the region dominated by calcareous, high pH soil in the dryland cereal growing areas of WANA, strengthened the idea that durum wheat originally possessed moderate tolerance to Mn deficiency. The moderate tolerance to Mn deficiency was brought about during a long process of evolution and domestication in the centres of origin, whereas the introduction of durum into North America was accompanied by loss of this tolerance due to continuing improvement of the crop on non-calcareous soils of high rainfall and adequate availability of Mn.

Chapter 4

Inheritance of manganese efficiency in durum wheat (*Triticum turgidum* L. var *durum*)

4.1 Introduction

The advantages of a genetic approach, compared to the alternative agronomic methods, to tackle the problem of Mn deficiency as a widespread constraint for durum wheat in alkaline soils, have been discussed in previous chapters. Considerable genotypic variation in durum germplasm, measured either as relative grain yield at Mn-deficient field site or as a higher shoot Mn content in the pot bioassay, has been demonstrated (Section 3.2). Inheritance of Mn efficiency in barley appears to be simply inherited and controlled by a single, dominant major gene in the cross of Weeah (Mn-efficient) and Galleon (Mn-inefficient) (Longnecker *et al.*, 1988), and observed to be heritable in Mn-efficient durum, being transmitted from progenitors of moderately Mn-efficient genotypes to their offspring (Section 3.3).

The existence of considerable genetic variation for Mn efficiency and reliable selection criterion has made the breeding for Mn-efficient durum wheat varieties feasible; however, a breeding effort would be facilitated if the mode of inheritance was well understood.

This is the subject of the series of experiments presented in this chapter. The response of parents, (Stojocri 2 and Hazar as Mn-efficient and Mn-inefficient genotypes, respectively) and F_1, F_2 and F_3 generations, was examined using the pot bioassay developed in Chapter 2 to determine the mode of inheritance and the number of genes involved in expression of Mn efficiency. An investigation of the dominance relationship and presence of maternal effect was also carried out by studying response of the F_1 hybrid and its reciprocal at different levels of soil Mn supply.

4.2 Materials and methods

The mode of inheritance of Mn efficiency in durum wheat was studied in a cross of Stojocri 2 (Mn-efficient) and Hazar (Mn-inefficient) genotypes, including the reciprocal F_1 hybrids and the F_2 and $F_{2:3}$ generations, under controlled environment conditions.

Genotypes and seed production

The genotypes, Stojocri 2 (Stk/4/Jo/3/Jo/Cr//Cit 71) and Hazar (T. dur. T. sph-Ram/Gll//M.sadova/3/Aa/Cr/4/21563//61-130/Lds) were selected from the previous study on the basis of relative grain yield (% of -Mn/+Mn from field plots) being 58% and 15%, respectively, and shoot Mn content 35 days after sowing in the controlled environment chamber as described in Section 3.2. The pedigrees and origins of the two genotypes are presented in Section 3.2. Seeds were selected from a single head of each genotype in a field observation nursery at Coonalpyn in 1994, progeny tested and multiplied in the glasshouse for crossing purposes. The two genotypes were reciprocally crossed. The response of F_1 hybrid plants, including the reciprocal cross, were studied at five levels of Mn supply and compared with the parents. Additional F_1 plants were grown in potting

mix (UC soil; 14 hrs light/8 hrs dark photoperiod at 25 °C day/15 °C night) to produce F_2 seeds which were used for the study in the corresponding generation (F_2) of Mn efficiency in a controlled environment chamber. F_2 plants were transplanted to Uc potting mix after testing for response to Mn deficiency, and grown to maturity to produce F_3 seed. Twenty of these were randomly selected for further evaluation.

Screening methods

The parents, F_1, F_2 and F_3 populations were studied for Mn efficiency in a controlled environment chamber (15°C day/10°C night, 10-hrs light/14 hrs dark photoperiod). Soil preparation was as described in Section 2.2. The level of Mn supply was varied for the evaluation of the different generations, and this is described in the materials and methods of the individual sections in this chapter.

Genetic analysis

Response of F_1 hybrids and their reciprocal cross in comparison to parents was analysed by analysis of variance.

The genetic analysis carried out in F_2 and F_3 generations was based on using seedling shoot Mn content. The expression of Mn efficiency was measured as a quantitative trait, and the number of genes controlling the response was estimated on the basis of the variance of the segregating population compared to the expected variance of one and two gene models. The variance components of parents were used in calculation of the expected variance. Using the assumption of no linkage, no epistasis and no dominance, the expected variance of the segregating population was calculated based on a modification of the model of Mather and Jinks (1977) employed by Chantachume (1995) and Jamjod (1996) as:

One gene segregation

$$VF_2 = 1/2d^2 + E$$

$$VF_3 = 3/4d^2 + E$$

where - VF_2 and VF_3 are variances of F_2 and F_2 derived F_3 populations

- d is the departure from midpoint (m) of the mean of the homozygous genotypes (AA and aa).

-E is the environmental variance.

The variance of F_1 was estimated from the average variance of the two parents. Therefore

$$E = 1/2Vp_1 + 1/2Vp_2$$

Two gene segregation

$$VF_2 = 1/4d^2 + E$$

$$VF_3 = 3/8d^2 + E$$

The confidence intervals of observed variances for the F_2 or F_3 populations were calculated as described by (Chantachume, 1995) and (Jamjod, 1996) as:

$$(VO \times df)/2\alpha < \text{Confidence interval} < (VO \times df)/2\beta$$

where - VO is the observed variance of the F_2 or F_3 population

-df is the degrees of freedom of n-1

-n is the number of plants of an F_2 population or number of F_2 derived F_3 families

-2α and 2β are lower and upper level chi-square values at P=0.95, df=n-1.

For both, the F_2 and F_3 generations, populations were identified as deviating significantly from the expected variances for single or two gene models when the expected variance was outside the range of the confidence interval of the observed variance.

F_2-derived F_3 populations

The results from the screening of F_2-derived F_3 were interpreted in two steps.

(i) A comparison was made between the observed variance and the expected variance for the one and two genes models.

(ii) for a cross that did not deviate from the two gene model in the statistical analysis, families were classified into four categories (homozygous efficient, homozygous intermediate, segregating or homozygous inefficient). The observed frequency of each category was compared to the expected frequency for a two gene model by chi-square analysis.

For the classification of families, the means and variances of individual families were compared to the confidence interval of the parental mean and the LSD of the parental variance, which were calculated as described by Chantachume (1995) and Jamjod (1996). The confidence interval of each parental mean was calculated as:

Confidence interval = $\pm$ m * SQRT (Vp x ($1/n_1+1/n_2$)

Where -m is the mean of the parent

-n_1 is the number of plants within the family

-n_2 is the number of plants of the parent which were tested

-t_1 is the t-value at the probability of 0.05/n2 and with degrees of freedom of

(n-1) (n+2)

-Vp is the variance of the parent

- F_1 is the F- value at the probability of $0.05/n_3$ and with degree of freedom of

(n_1-1), $((n_2-1)) + (n_3-1))$, where

n_1 is the number of plants within a family,

n_2 and n_3 are the number of plants for each of the two parents.

When the mean of a family was within the confidence interval of either of the parents and the variance of the family was less than the LSD of the parental variance, the family was assigned as either homozygous inefficient or homozygous efficient. When the mean of a family was higher than the inefficient but lower than the efficient parent and had a variance less than the LSD of the parental variance, the family was classified as homozygous intermediate. Families with a variance greater than the parents were classified as segregating.

Following the classification of the individual families, chi-square analysis was used for testing the goodness of fit of the observed segregation ratio to values expected for several models. F_2-derived F_3 populations were tested for the monogenic segregation ratio of 1 homozygous efficient : 2 segregating : 1 homozygous inefficient and for the digenic segregation ratio of 1 homozygous tolerant : 2 homozygous intermediate : 12 segregating : 1 homozygous inefficient.

Chi-square analysis was not performed at the F_2 generation because the response to Mn application was expressed as a quantitative trait and there was no clear cut point for distinguishing between alternative categories.

Relationship between F_2 and F_3

The analysis of the relationship between F_2 and F_3 families was carried out by regression analysis using the StatView program version 4.1 for Macintosh computers.

4.3 Response of F_1 hybrid to increasing levels of manganese application

A pot bioassay with three replications was carried out to study the response of the F_1 hybrid and its reciprocal of a cross between a moderately Mn-efficient durum wheat genotype, and Mn-inefficient durum wheat genotype at five levels of soil Mn supply. The results were statistically analysed using analysis of variance.

4.3.1 Materials and methods

Soil and pot bioassay

The crosses were carried out in a glasshouse, using potting mixture (UC) for growing the parents. The pot bioassay was conducted in a controlled environment, as described in Chapter 2, using five levels of Mn supply: 0, 10, 30, 60, 120 mg Mn/kg dry soil. Only two levels of Mn (10 and 60 mg Mn/kg dry soil) were employed for the reciprocal cross due to limited available number of seed. The pot bioassay used Mn deficient Wangary soil

and, other than the Mn treatments, preparation, growth conditions, duration of the experiment and harvest procedures were the same as described in Section 2.2.

The response of F_1 hybrid

The F_1 hybrid (Stojocri 2/Hazar) of a cross between Stojocri 2 and Hazar and its reciprocal cross (Hazar/Stojocri 2) were compared to the parents under the conditions described above. Parental seed with similar Mn content (1.5-1.7 µg Mn/seed) was selected for this study, while F_1 hybrids had an unavoidably higher content (2 µg Mn/seed). Three seedlings of each parent, F_1 hybrid and reciprocal F_1 were maintained for each treatment and the experiment replicated three times. Shoot Mn content, shoot dry weight and chlorosis score were subject to analysis of variance using Super Anova program for Macintosh computers.

4.3.2 Results

The F_1 hybrids and the parents responded to increased Mn supply with increased shoot Mn content (Fig 4.1) and reduced chlorosis score (Fig 4.2), but the responses were different (genotype by Mn interaction, $P<0.01$). The response of F_1 hybrid was intermediate to the parents for shoot Mn content, but for chlorosis score efficiency was dominant so that the F_1 was close in response to the efficient parent Stojocri 2 and there was no difference between reciprocal F_1 hybrids (Figs 4.3 and 4.4) in the range of applied Mn supply. There was no significant variation ($P<0.05$) among parents and the F_1 hybrid in terms of chlorosis score at higher Mn application (Fig 4.2). This observation confirms once again that the observed difference at lower Mn supply in terms of shoot Mn content

and chlorosis score was not only due to genotype or Mn, but also their interaction (Figs 4.1, 4.2, 4.3 and 4.4) (see Appendix A, Tables D1-4 for analysis of variance).

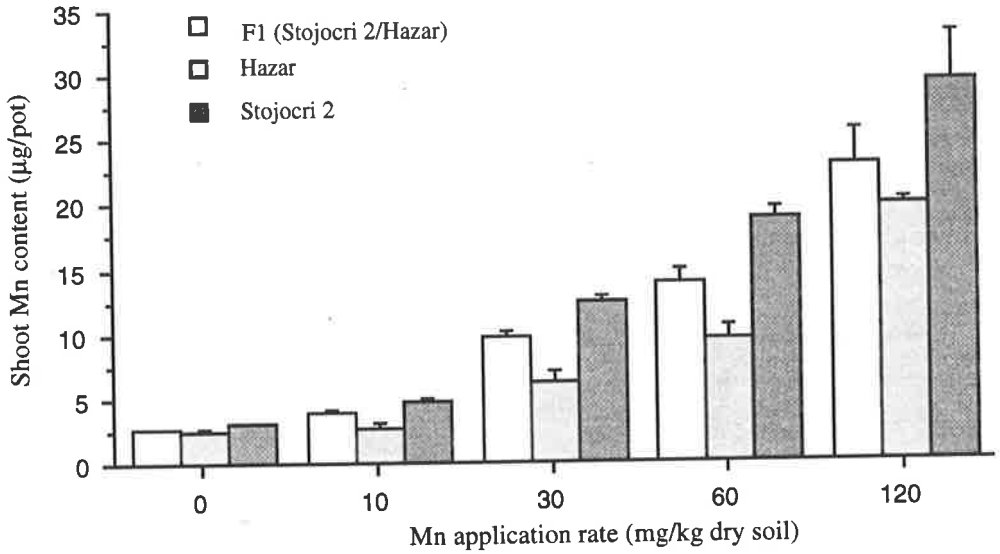

Fig 4.1 Variation in shoot Mn content (µg Mn/pot) 35 DAS of the F_1 hybrid of a cross between Stojocri 2 (Mn-efficient durum wheat) and Hazar (Mn-inefficient genotype) compared to the parents at five levels of soil Mn application. The vertical bars represent the standard error of means.

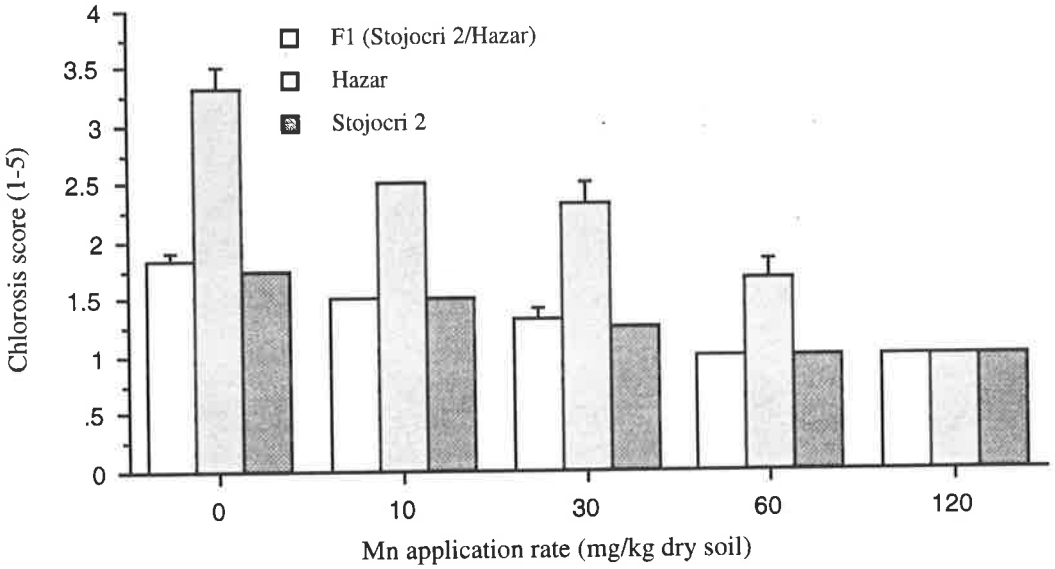

Fig 4.2 Variation in chlorosis score (1-5) 35 DAS of the F_1 hybrid of a cross between Stojocri 2 (moderately Mn-efficient durum wheat) and Hazar (Mn-inefficient genotype) as compared to parents at five levels of soil Mn application. The vertical bars represent the standard error of means.

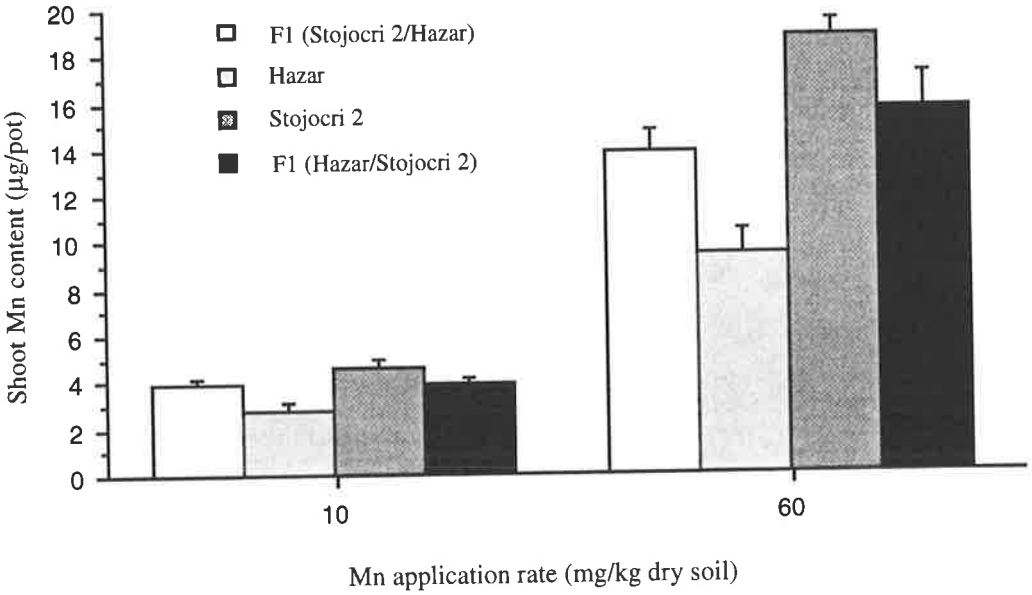

Fig 4.3 Variation in shoot Mn content (µg Mn/pot) 35 DAS of the F_1 hybrid of a cross between Stojocri 2 (moderately Mn-efficient durum wheat) and Hazar (Mn-inefficient genotype) and its reciprocal compared to the parents at two levels of soil Mn application. The vertical bars represent the standard error of means.

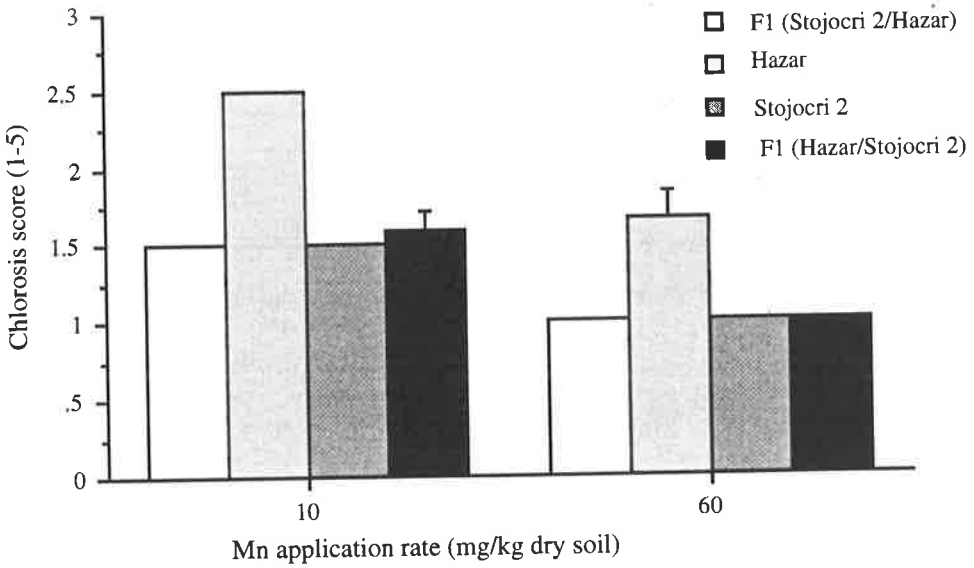

Fig 4.4. Variation of chlorosis score (1-5) 35 DAS of F1 hybrid of a cross between Stojocri 2 (moderately Mn-efficient) and Hazar (Mn-inefficient) durum wheat genotypes and its reciprocal cross as compared to the parents at two levels of soil Mn application. The vertical bars represent the standard error of means.

4.3.3 Discussion

The response of the F1 hybrid in terms of shoot Mn content was generally intermediate (no dominance) between both parents, and seemed fairly complete dominance for the chlorosis score (Figs 4.1 and 4.2). The lower expression of chlorosis in the F_1 compared to the parents made the decision about the nature of expression difficult. The unavoidably higher seed Mn content (2 µg Mn/seed) of F_1 compared to the parents (1.5-1.7 µg/seed), may have caused the expressed lower level of chlorosis development and the higher shoot Mn content in F_1 compared to the parents. So the nature of expression was intermediate (incomplete dominant) gene action, the degree of dominance dependent on availability of Mn. This phenomenon was observed in bread wheat (Paull *et al.*, 1992), peas (Bagheri, 1994) and durum wheat (Jamjod, 1996) in response to toxic concentration of boron.

Such a response seemed consistent with the hypothesis of Knight (1973) that for quantitative traits the response of F1 hybrid relative to the parents will vary according to the intensity of environmental stress. Statistically significant difference was not observed between the F_1 hybrids and the reciprocal (Figs 4.3 and 4.4) (see Appendix A, Tables C3 and C4 for analysis of variance) indicating absence of maternal effects (due to limited number of seeds the comparison was carried out at only two Mn levels). On the basis of these results, 25 mg Mn/kg dry soil was selected as an appropriate level for discrimination of genotypes and F_1 hybrid for shoot Mn content and chlorosis score (Figs 4.1 and 4.2) (see Appendix A, Tables C1-2 for analysis of variance) for further studies of F_2 and F_2-derived F_3.

4.4 Response of F_2 and F_2-derived F_3 families

Two separate experiments under controlled environment chamber conditions were conducted with 134 and 240 pots, respectively, for the study of F_2 and F_2:F_3 populations derived from the cross of Stojocri 2 by Hazar.

4.4.1 Materials and methods

Manganese level

Under controlled environment chamber conditions, F_2 or F_3 were grown with 25 mg Mn/kg dry soil. Soil preparation, conditions of growth and duration of study were the same as described in Section 2.2.

F_2 segregating population

A total of 134 individual seedlings, including twelve seedlings of each parent, were grown with one seedling per pot. Pots were harvested at ground level 35 DAS, the tops were dried and analysed for Mn content. Contents of whole pots (including the crown, roots and soil) of each seedling were transferred to 150 mm diameter pots containing UC potting mix. Plants were transferred to controlled environment conditions (14 hrs light/8 hrs dark photoperiod at 25°C day/15°C night) for a period of 95 days, by which time plants had matured and F_2-derived F_3 seed could be collected.

F_2-derived F_3 population

Two hundred and twenty single seedlings (including ten individuals of each of twenty randomly selected F_2-derived families plus the two parents with ten seedlings of each) were studied in the same way as the F_2 population.

Genetic analysis

The genetic analysis, based on the shoot Mn content of a total of 110 F_2 and 200 F_3 plants, was calculated as described in Section 4.2.

4.4.2 Results

F_2 segregating population

It was not possible to classify the F_2 segregating population into discrete categories, mainly due to the continuous nature of the frequency distribution which resulted in no distinct point for distinguishing between alternative categories (Fig 4.5). The estimation of the number of genes conferring Mn efficiency was based on the comparison of observed variance with expected variance, with the assumption of no dominance, no

linkage and no epistasis. The expected variances of the F_2 population segregating for either one or two genes were both within the confidence interval of the observed variance (Table 4.1).

F_2-derived F_3 population

Expected variance of the F_3 population for both one or two gene models was within the confidence interval of observed variance (Table 4.1). The observed variance (1.5) was closer to the expected variance of segregation at two (1.4) rather than one gene (2.4) (Table 4.1). However, the chi-square analyses failed to support the two gene model (Tables 4.2 and 4.3).

Response of F_2-derived F_3 families

The shoot Mn content of F_2-derived F_3 families was regressed against shoot Mn content of the F_2 individuals, and a coefficient of determination of 0.41 and slope of 0.44 was observed (Fig 4.6) which was indicative of a reasonable relationship of offspring to parent that would enable selection for Mn efficiency in early segregating generations (Fig 4.6).

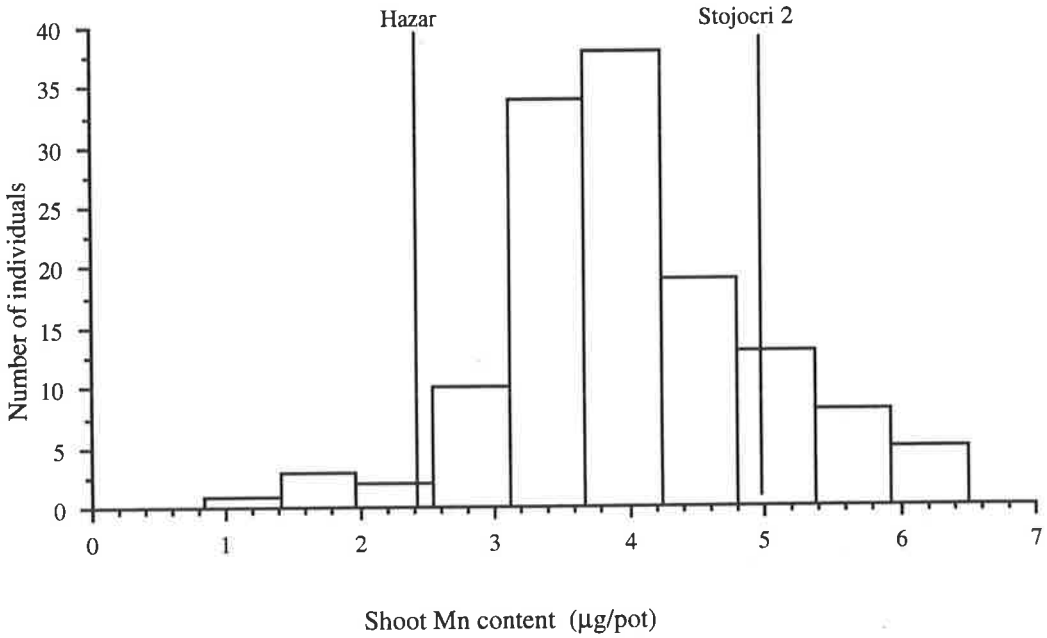

Fig 4.5 Distribution of shoot Mn content (µg Mn/pot) 35 DAS of 110 F_2 plants of a cross between Stojocri 2 (Mn-efficient durum wheat) and Hazar (Mn-inefficient genotype) with mean Mn content of parents of 4.96 and 2.43, respectively.

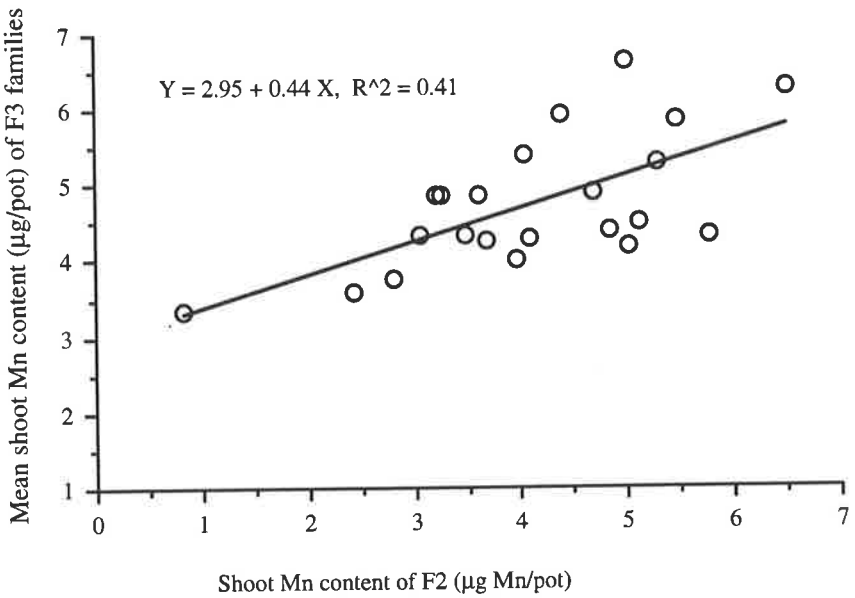

Fig 4.6 The relationship between seedling shoot Mn content of F_2 plants (µg Mn/pot) and mean shoot Mn content of 20 randomly selected F_3 individual families of a cross between Stojocri 2 (moderately Mn-efficient durum wheat) and Hazar (Mn-inefficient durum wheat) grown at 25 mg Mn/kg dry soil, sampled 35 days after sowing.

Table 4.1 Observed variance of F_2 and $F_{2:3}$ population and their parents, estimated parameters and expected variance for one and two gene models for F_2 and $F_{2:3}$, based on shoot Mn content of 35 DAS evaluated at 25 mg Mn/kg dry soil.

Cross		Observed variance			Estimated parameters				Expected variance	
P_1	P_2	VP_1	VP_2	VF_2	CI	E	m	d	1 gene	2 genes
F_2 population										
Stojocri 2	Hazar	0.3	0.2	0.9	0.7-1.1	0.3	3.7	1.3	1.1	0.7
$F_{2:3}$ population										
Stojocri 2	Hazar	0.9	0.4	1.5	1.3-4.8	0.7	5.1	1.5	2.4	1.4

E=Environmental variance, m=mid-point of parent means

d=departure of parents data from mid-point

CI= Confidence interval of the observed variance of the F_2 and F_3 populations

Table 4.2. Mean shoot Mn content (µg/pot) 35 DAS of parents and $F_{2:3}$ population of a cross between moderately Mn-efficient (Stojocri 2) by Mn-inefficient (Hazar) durum wheat genotypes evaluated at 25 mg Mn/kg dry soil under controlled environment conditions.

Cross		P_1		P_2		F_3	
P_1	P_2	Mean	CI	Mean	CI	Mean	LSD *
Stojocri 2	Hazar	6.64	5.7-7.5	3.9	2.9-4.2	4.8	0.99

CI = Confidence interval of parental mean

* = LSD of parental variance

Table 4.3 Chi-square analysis of $F_{2:3}$ families from a cross of moderately Mn-efficient (Stojocri 2) by Mn-inefficient (Hazar) durum wheat genotypes, based on shoot Mn content 35 DAS expected to segregate at two loci.

Cross	Model		Number of F_3 families				Σ	P (df=3)
			Ef	Int	Seg	Inef		
Stojocri 2/Hazar	1:12:12:1	Exp.	1	2	12	1	17.2	0.1%
		Obs.	3	12	2	3		

Model = Expected ratio homozygous-efficient : homozygous intermediate : segregating : homozygous-inefficient = 1:2:12:1

Ef = homozygous-efficient, **Int** = homozygous intermediate, **Seg** = segregating, **Inef** = homozygous-inefficient

4.4.3 Discussion

Variation in Mn efficiency was demonstrated in the F_2 and $F_{2:3}$ populations using shoot Mn content measured quantitatively. Observed variation in the segregating population resulted mostly from inherent genetic variation (0.86 and 1.48, respectively, in F_2 and $F_{2:3}$) since the environmental variance was kept to a minimum (environmental variance of 0.27 and 0.61, respectively in F_2 and $F_{2:3}$) by using seed of uniform size and similar Mn content, and conducting experiments under controlled environmental conditions (Table 4.1). Otherwise the environmental effect would have confounded the expression of genetic variance as observed in the study of Mn deficiency in soybean carried out solely on the evidence of chlorosis symptoms in field trials (Graham *et al.*, 1995).

The comparison of observed variance to expected variance in both the F_2 and $F_{2:3}$ was consistent with the two gene model (Table 4.1). Nevertheless, misclassification was

observed in assigning the $F_{2:3}$ families to either segregating or intermediate categories and as a result the chi-square analysis failed to show the null hypothesis as being acceptable ($P<0.01$) (Tables 4.2 and. 4.3).

The observed linear relationship between the mean of shoot Mn content of $F_{2:3}$ families compared to F_2 population and the regression coefficient ($r=0.64$) provide the evidence of the feasibility of selection for Mn efficiency in early generations in a breeding program.

The most probable model for genetic control of Mn efficiency in the cross Stojocri 2 (Mn-efficient) by Hazar (Mn-inefficient) is two genes with additive effect. Tolerance to Mn deficiency in soybean (Graham *et al.*, 1995), tolerance to Mn toxicity in bread wheat (Moroni *et al.*, 1991) and tolerance to boron toxicity in durum wheat (Jamjod, 1996) were also reported to be quantitative traits. However, to determine the total number of genes involved in expression of Mn efficiency in durum wheat, further study of cross combinations of genotypes with a larger range of Mn efficiency is demanded.

Chapter 5

Use of aneuploidy in the study of manganese efficiency in durum wheat

5.1 Introduction

In the previous chapter the mode of inheritance of Mn efficiency was studied using the F_2 and $F_{2:3}$ of the cross of Mn-inefficient (Hazer) by Mn-efficient (Stojocri 2) durum wheat. It was demonstrated that Mn efficiency in that specific cross was probably under the control of two loci with additive effects. The number of genes conferring a trait determines the size of backcross generations and selection intensity necessary to incorporate the genes into the recurrent parent. An understanding of the location of genes conferring Mn efficiency would increase the chance of identifying markers linked to the trait. Identification of a closely linked marker to the trait has the potential of increasing the precision of selection and making selection in early generations more efficient and, as a result, reducing the size of population and the number of backcrosses and hastening the process of production of Mn-efficient genotypes.

Bread wheat (*T. aestivum* L.) has a close cytogenetic relationship to durum wheat, as both have A and B-genome chromosomes. The chromosomes in three genomes (AABBDD) of bread wheat were found to contain similar genes and hence homoeologous chromosomes were able to compensate for each other (Sears, 1966). The elimination or addition of individual chromosomes, not involving the entire genome (aneuploidy), can be used to determine and study the effect of gene(s) on that chromosome, locate genes to

chromosomes, map genes relative to the centromere, transfer chromosomes from one cultivar or species to another and identify chromosome homologies. Several sets of aneuploids have been produced in hexaploid wheat (*Triticum aestivum* L.) Chinese Spring (CS) by Sears (1954, cited by Joppa, 1987). These aneuploids included nullisomic, monosomic, trisomic, tetrasomic, telosomic, isosomic and nullisomic-tetrasomic, and they have played a pivotal role in elucidating the genetic control of particular characters in Chinese Spring hexaploid wheat (Sears, 1966) and in the study of wheat genetics.

5.2 Aneuploid study in durum wheat

In contrast to bread wheat, aneuploidy has not been extensively used in durum wheat. Unavailability of aneuploids in durum wheat (by the late 1970s) was mainly due to a smaller acreage of durum wheat compared to bread wheat, and its consequently lesser economic importance. The lower tolerance of tetraploids to aneuploidy, in contrast to hexaploid wheat, and the generally harmful effect of a reduction in chromosome number (monosomic and nullisomic) have been advanced as other reasons for the less extensive development and application of aneuploid studies in durum wheat (Joppa, 1987).

The production and use of monosomics in genetic studies of tetraploid wheat has been limited by lower vigor and fertility of monosomic plants and also the low transmission of n-1 gametes through both female and male gametes. Although a set of trisomics has been produced in durum wheat in the cultivar Cappelli by Simone *et al.* (1983, cited by Joppa, 1987), they have not been used in genetic analysis of tetraploid wheat.

Joppa and Williams (1983a) produced a complete set of disomic substitution lines in durum wheat, by substituting a pair of D-genome chromosomes from Chinese Spring into

Langdon durum wheat, where they have replaced the homoeologous pair of A or B-genome chromosomes. The D-genome disomic substitution lines were produced by crossing Chinese Spring nulli-tetras (nulli for a B or A-genome chromosome and tetra for a homoeologous D-genome chromosome) with durum wheat cv Langdon as described by Joppa (1983a). These produced F_1 hybrids with 13"+1"D+1'-A or B+6'D which were selfed and plants with 2n=28 chromosomes selected. In each case, a pair of D-genome chromosomes was substituted for the homoeologous A or B-genome chromosomes. To reduce the genetic contribution from CS to a minimum, the plants were backcrossed at least five times to Langdon. In each backcross, plants with a chromosome constitution 2n=13"+2' (i.e. monosomic for an A or B-genome chromosome and for a homoeologous D-genome chromosome) were selected and backcrossed to Langdon. After the backcrosses, the lines were selfed or crossed with a set of D-genome disomic-addition lines of Langdon followed by further selfings to produce D-genome disomic substitution lines.

The D-genome disomic substitution lines can be used for determination of the chromosomal location of genes, producing homologous disomic substitutions of chromosomes from one line into another and for inducing translocation between homoeologous chromosomes. The chromosomal location of genes in the A or B genome can be determined by making the appropriate crosses and studying the segregation by methods similar to those used in a monosomic analysis of hexaploid wheat (Law *et al.*, 1987) and in tetraploid wheat using D-genome disomic substitution lines as described by Joppa (1987). In this case epistatic relationships of homoeologous genes must be taken into consideration, since D-genome disomic substitution lines have D-genome chromosome which may have gene(s) which could have a dominant or epistatic (covering or prohibiting) effect on the gene of interest.

In this chapter a full set of Langdon D-genome substitution lines and Stojocri 2 were employed in two steps:

(i) A preliminary experiment in which the effect of the substitution of the set of individual D genome chromosomes on seed size and seed Mn content and, consequently, on expression of Mn efficiency in terms of shoot Mn content was investigated.

(ii) The set of D-genome disomic substitution lines was grown to give uniform seed Mn content. The effect of Mn deficiency on these stocks with the same seed Mn content was investigated to identify the feasibility of using these to study further the chromosomal location of gene(s) conferring Mn efficiency in durum wheat.

5.2.1 Materials and methods

Genetic material

A full set of D-genome disomic substitution lines in a Langdon background, Chinese Spring wheat, Langdon and Stojocri 2 were used in this study. The Langdon disomic substitution lines were originally provided by Dr L.R. Joppa, North Dakota University, to Dr Sansanee Jamjod, University of Adelaide, in 1992.

The genetic material for the current project was multiplied in a glasshouse using the University of California (UC) potting mix. Due to variation in seed size among the various D-genome substitution lines, selection of seed with uniform Mn content was not feasible (Fig 5.1), as the seed Mn content ranged from 0.9-3.9 μg Mn per seed (Table

5.1). As a result, the preliminary experiment manifested the confounding effect of seed Mn content on the expression of efficiency in terms of shoot Mn content.

To produce seed of each substitution line with uniform Mn content, the whole set of Langdon substitution lines was grown at three levels of soil Mn application (50, 100 and 150 mg Mn/kg soil) and sprayed with Mangazol at heading. This made the selection of seed with similar Mn content feasible (Table 5.2). The stocks were grown in 150 mm diameter polyethylene pots containing 2 kg of Wangary soil. Conditions for growth were as described in Section 3.3.1.

Manganese level

Two levels of soil Mn application (35, and 160 mg/kg soil) were selected for the pot bioassay. Soil preparation, conditions for growth and duration of experiment were the same as described in Chapter 2. The shoot Mn content of seedlings 35 DAS in the pot bioassay was used as the selection criterion. To demonstrate the effect of the substitution on the growth of seedlings, further data on shoot dry weight, shoot Mn concentration and root dry weight have been presented. The presented data are the mean of three replicates subjected to analysis of variance using Super Anova.

In an attempt to check the effect of seed Mn content on the expression of Mn efficiency, either as shoot Mn content or development of chlorosis score, correlation coefficients were determined for the data set using StatView.

5.2.2 Results

Preliminary experiment

The shoot Mn content and development of Mn deficiency chlorosis score of the D-genome disomic substitution lines, Chinese Spring and Langdon, at a deficient Mn level are shown in Figs 5.2 and 5.3. Significant variation ($P<0.05$) was observed between D-genome disomic substitution lines and the parents (Chinese Spring and Langdon) in terms of shoot Mn content (Fig 5.2) (see Appendix A, Table E1 for analysis of variance) or chlorosis score (Fig 5.3) (see Appendix A, Table E2 for analysis of variance). The expression of Mn efficiency in D-genome disomic substitution lines in terms of either shoot Mn content or chlorosis score were highly in agreement ($r=0.52^{**}$ and $r=-65^{**}$, respectively) with seed Mn content (Figs 5.2 and 5.3). Confounding effect of seed Mn content on expression of Mn efficiency measured as shoot Mn content was observed in all entries except Lnd7D(7B), Lnd5D(5B), Lnd6D(6A), Lnd5D(5A) and Lnd1D(1B) which did not respond to seed Mn reserve (Fig 5.2).

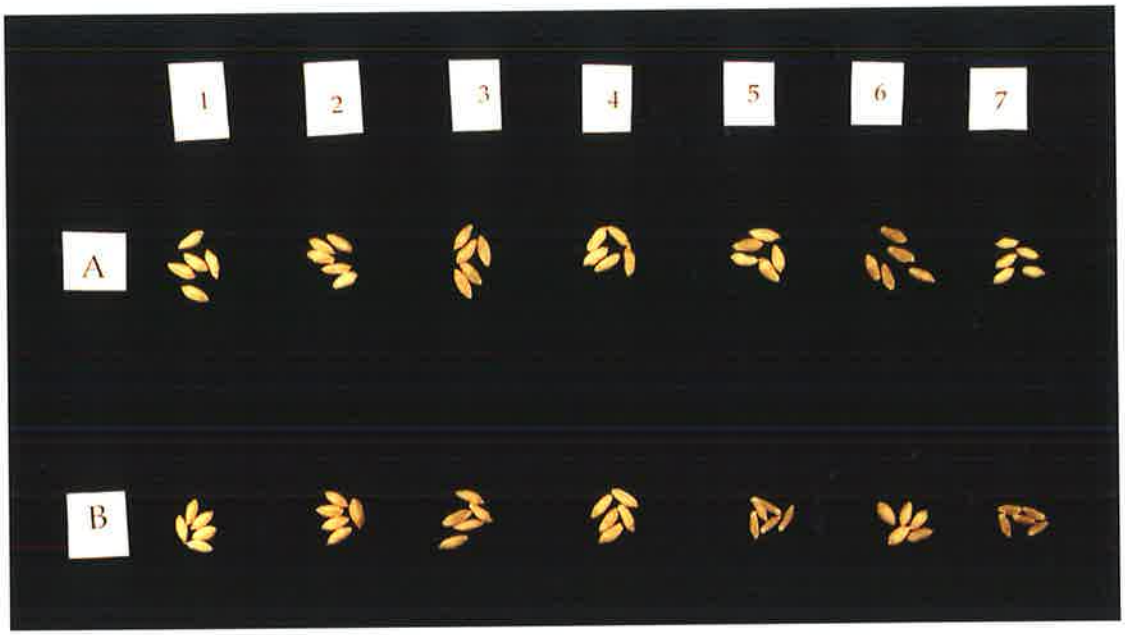

Fig 5.1 Variation of seed size among the Langdon D-genome disomic substitution lines.

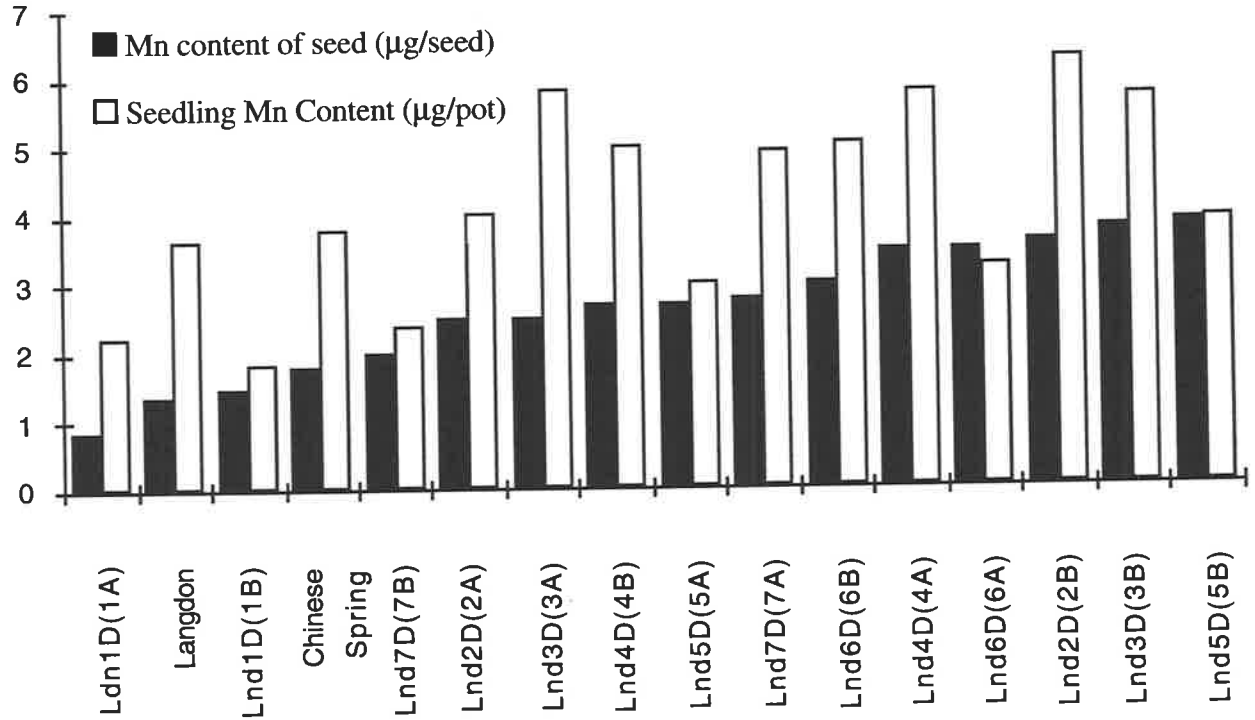

Fig 5.2 Ranking order (r=0.52**) of shoot Mn content (µg/pot) vs seedling Mn content (µg/seed) of Langdon D-genome disomic substitution lines, Chinese Spring and Langdon, 35 DAS in -Mn pot bioassay in a controlled environment chamber.

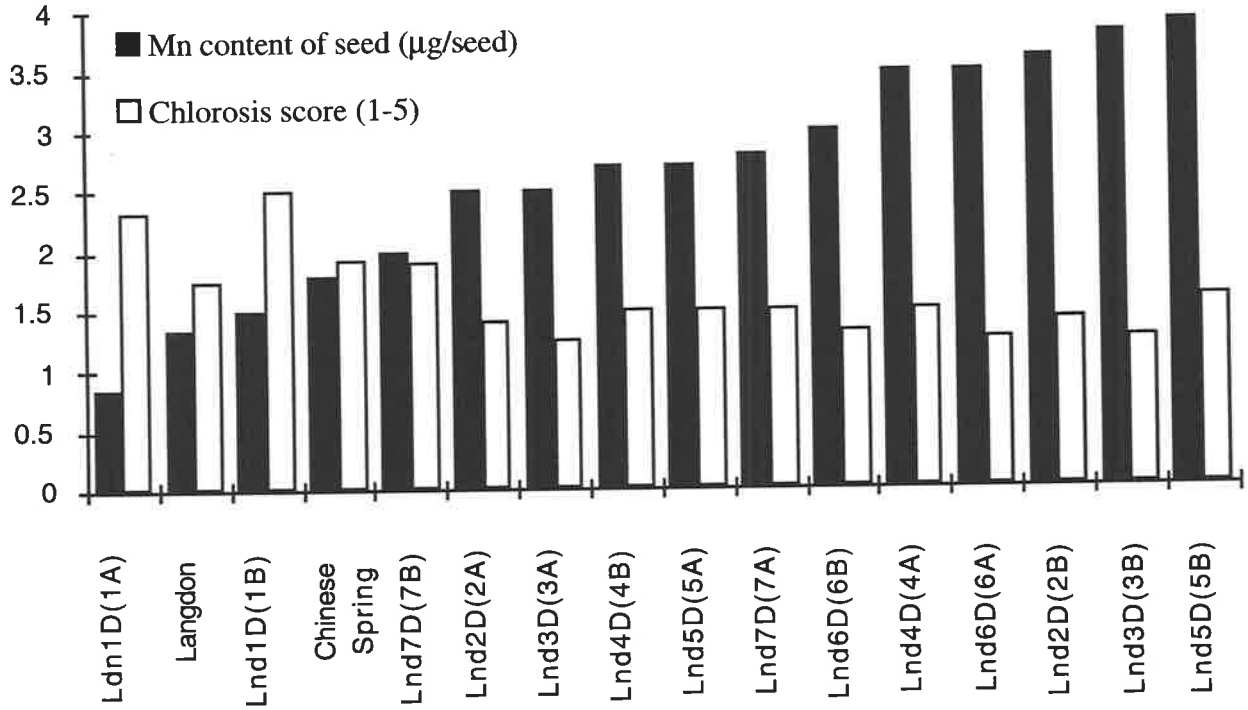

Fig 5.3 Ranking order (r=-0.65**) of chlorosis score (1–5) vs seed Mn content (µg/seed) of Langdon D-genome disomic substitution lines, Chinese Spring and Langdon, 35 DAS in -Mn pot bioassay in a controlled environment chamber.

Table 5.1 Seed Mn concentration (mg/kg), Mn content (µg/seed), seed weight (g/10 seeds), relative vigour and fertility of the full set of D-genome disomic substitution lines, Chinese Spring and Langdon.

Lines	Mn concentration (mg/kg)	Mn content (µg/seed)	Seed wt (g/10 seed)	Vigour**	Fertility**
Lnd1D(1A)	85	0.9	0.1*	Good	Good
Lnd1D(1B)	60	1.5	0.3	Good	Good
Lnd2D(2A)	75	2.5	0.3	Poor	Fair
Lnd2D(2B)	80	3.6	0.5	Fair	Good
Lnd3D(3A)	50	2.5	0.5	Fair	Fair
Lnd3D(3B)	95	3.8	0.4	Fair	Fair
Lnd4D(4A)	92	3.5	0.4	Fair	Fair
Lnd4D(4B)	72	2.7	0.4	Poor	Poor
Lnd5D(5A)	70	2.7	0.4	Good	Poor
Lnd5D(5B)	144	3.9	0.3	Good	Good
Lnd6D(6A)	93	3.5	0.4	Fair	Fair
Lnd6D(6B)	67	3.0	0.4	Fair	Poor
Lnd7D(7A)	74	2.8	0.4	Good	Good
Lnd7D(7B)	90	2.0	0.2*	Poor	Fair
Chinese Spring	73	1.8	0.2	Good	Good
Langdon	45	1.4	0.3	Good	Good

* Small seed, ** observed vigour and fertility in agreement with data presented by (Joppa, 1987)

5.2.3 Discussion

Significant correlation of both shoot Mn content or development of chlorosis score with sown seed Mn content of Langdon D-genome disomic substitution lines was observed (Figs 5.2 and 5.3), which is an indication of the confounding effect of seed Mn content on the expression of Mn efficiency measured by the abovementioned criteria. Due to relatively high seed Mn content (Table 5.1) of the Langdon D-genome disomic substitution lines, and its direct effect on lower expression of chlorosis symptoms (in a range of 1.3-1.9, Fig 5.3), the emphasis below has been given to shoot Mn content rather than chlorosis score.

The confounding effect of seed Mn content was expected to be observed in Lnd7D(7B), Lnd5D(5B), Lnd6D(6A), Lnd5D(5A) or Lnd1D(1B) as shoot Mn content, considering their high seed Mn reserve, but this was not the case. This phenomenon might be explained by:

(i) Fair to poor vigour of seedlings, which was also observed under normal growing conditions in Lnd7D(7B) and Lnd6D(6A) by (Joppa, 1987), but this does not explain those (Lnd5D(5B), Lnd5D(5A) and Lnd1D(1B)) which had good vigour under normal fertility using UC soil (Table 5.1).

(ii) Interaction of respective gene(s) on chromosomes from the D-genome with gene(s) on the homoeologous chromosomes from either A or B genomes as an outcome of substitution might be another possible explanation of the phenomenon.

The observed very low seed Mn reserve in Lnd1D(1A) and Chinese Spring was due to the small seed size, whereas in Langdon it was mainly due to thc lower seed Mn concentration, and in Lnd1D(1B) a combination of both seed size and seed Mn concentration (Fig 5.1; Table 5.1). The substitution of D-genome chromosomes from Chinese Spring into Langdon and its consequences on vigour, fertility, plant stature, maturity and seed size of disomic substitution lines has already been determined (Joppa, 1987); however, the effect on seed Mn concentration and content has not been reported. Variation in seed Mn content and, consequently, on expression of Mn efficiency as pointed out already, are explained to some extent by change in seed size, seed Mn concentration or both, but lower expression of efficiency in entries with higher Mn content probably could be explained just by interaction of gene(s) on chromosomes from the D-genome with the gene(s) on homoeologous chromosomes from either the A or B genome.

The confounding effect of seed Mn reserve on screening for Mn efficiency has been discussed and the necessity of using seeds with similar Mn content in screening has been mentioned earlier (Section 3.2). Results of this preliminary study could be considered as a manifestation of the substitution of D-genome chromosomes in the Langdon background and their effects on seed size and seed Mn reserve and Mn efficiency, leading to a confounding effect an the screening process. However, in the next experiment, the feasibility of using these stocks in genetic analysis with even seed Mn content is described.

5.3 Response of Langdon D-genome disomic substitution lines to manganese-deficiency

To minimise the confounding effect of seed Mn content and to select seed of each substitution line with similar Mn content, the whole set of Langdon D-genome disomic substitution lines, Chinese Spring, Langdon and Stojocri 2 as a check genotype (to monitor the level of soil Mn application and the extent of Mn efficiency) were grown at a range of soil Mn supply (50, 110, 150 mg/kg dry soil) coupled with foliar Mn sprayed at heading. This approach made the selection of seed with uniform Mn content feasible (Table 5.2).

5.3.1 Results

Shoot manganese content

The comparison of shoot Mn content of D-genome disomic substitution lines with Chinese Spring, Langdon and Stojocri 2 (Fig 5.3) revealed that:

(i) The shoot Mn content of Langdon was significantly less than that of Chinese Spring, and the Mn-efficient genotype (Stojocri 2) was intermediate to Chinese Spring and Langdon.

(ii) Generally the shoot Mn content of the substitution lines was not different from the mean of the parents, except Lnd 4D(4B), Lnd 7D(7A) and Lnd 7D(7B), which were significantly less ($P<0.05$) than Langdon.

(iii) Lnd 1D(1B) and Lnd 4D(4B) showed a significant depression in shoot Mn content compared to Lnd 1D(1A) and Lnd 4D(4A), respectively.

(iv) Lnd 5D(5A) and Lnd 5D(5B) performed the same as Langdon and significantly more poorly than Chinese Spring.

(v) Lnd 6D(6A) had a lower shoot Mn content than Langdon, while Lnd 6D(6B) was intermediate to Langdon and Chinese Spring.

(vi) Lnd 7D(7A) and Lnd 7D(7B) were significantly lower than Langdon.

(vii) Comparisons of shoot Mn content (Fig 5.4) (see Appendix A, Table E3 for analysis of variance) were in agreement with data on shoot Mn concentration (Fig 5.5) (see Appendix A, Table E4 for analysis of variance), shoot dry weight (Fig 5.6) (see Appendix A, Table E5 for analysis of variance), and root dry weight (Fig 5.7) (see Appendix A, Table E6 for analysis of variance), except for Lnd 2D(2A), Lnd 2D(2B), Lnd3D(3A) and Lnd 3D(3B), which had relatively higher root and, to a lesser extent, shoot growth compared to Lnd1D(1A) and Lnd 1D(1B).

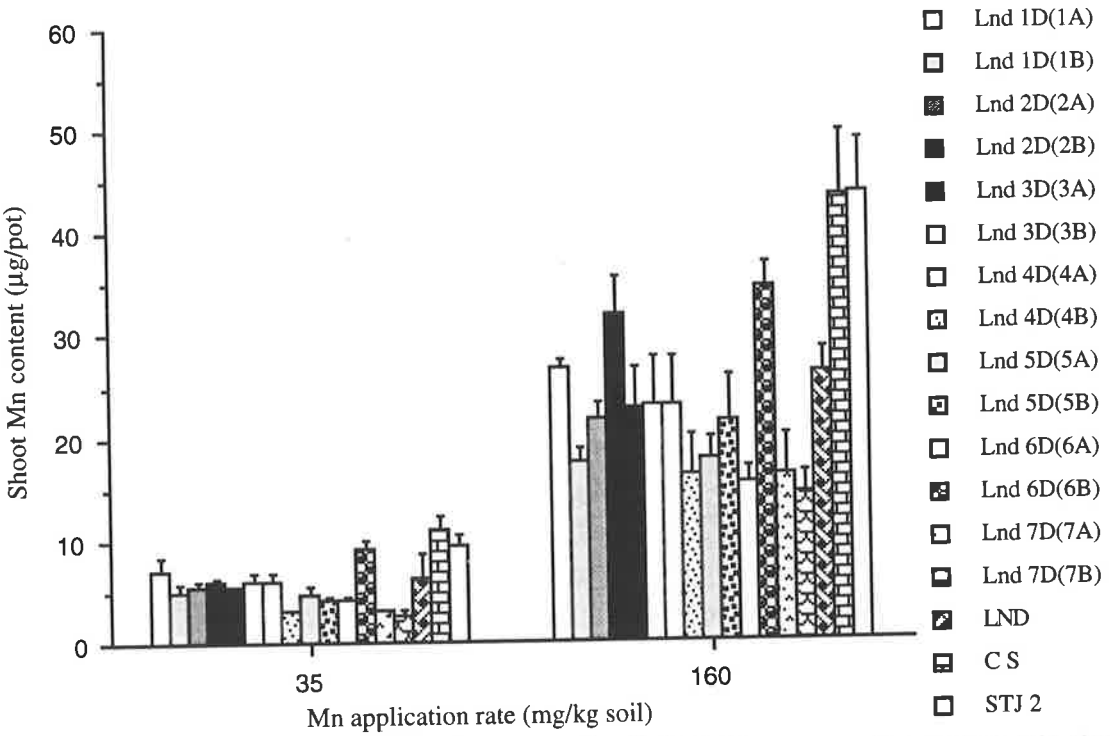

Fig 5.4 Shoot Mn content (µg/pot) of Langdon (LND) D-genome disomic substitutior lines 35 DAS compared with Chinese Spring (CS), Langdon and Stojocri 2 (STJ 2) evaluated at two levels of soil Mn supply (mg/kg dry soil) grown under controlled environment conditions. The error bars represent the standard error of means (n=3).

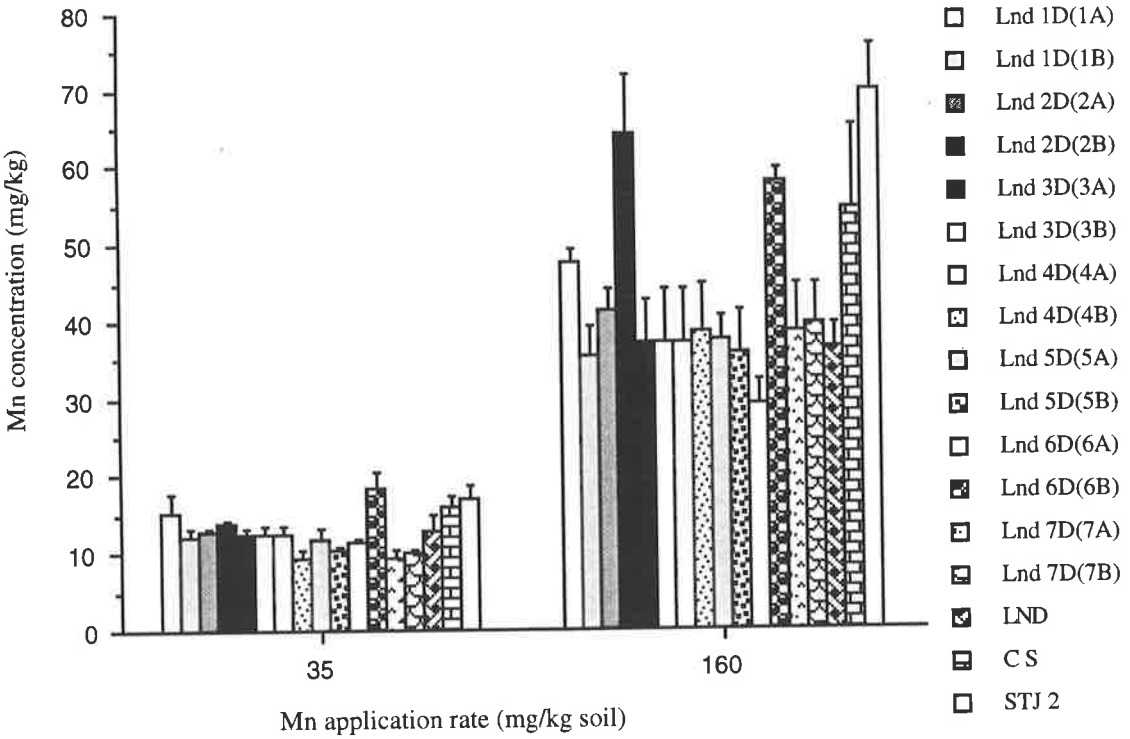

Fig 5.5 Shoot Mn concentration (mg/kg) of Langdon (LND) D-genome disomic substitution lines 35 DAS compared with Chinese Spring (CS), Langdon and Stojocri 2 (STJ 2) evaluated at two levels of soil Mn supply (mg/kg dry soil) grown under controlled environment conditions. The error bars represent the standard error of means (n=3).

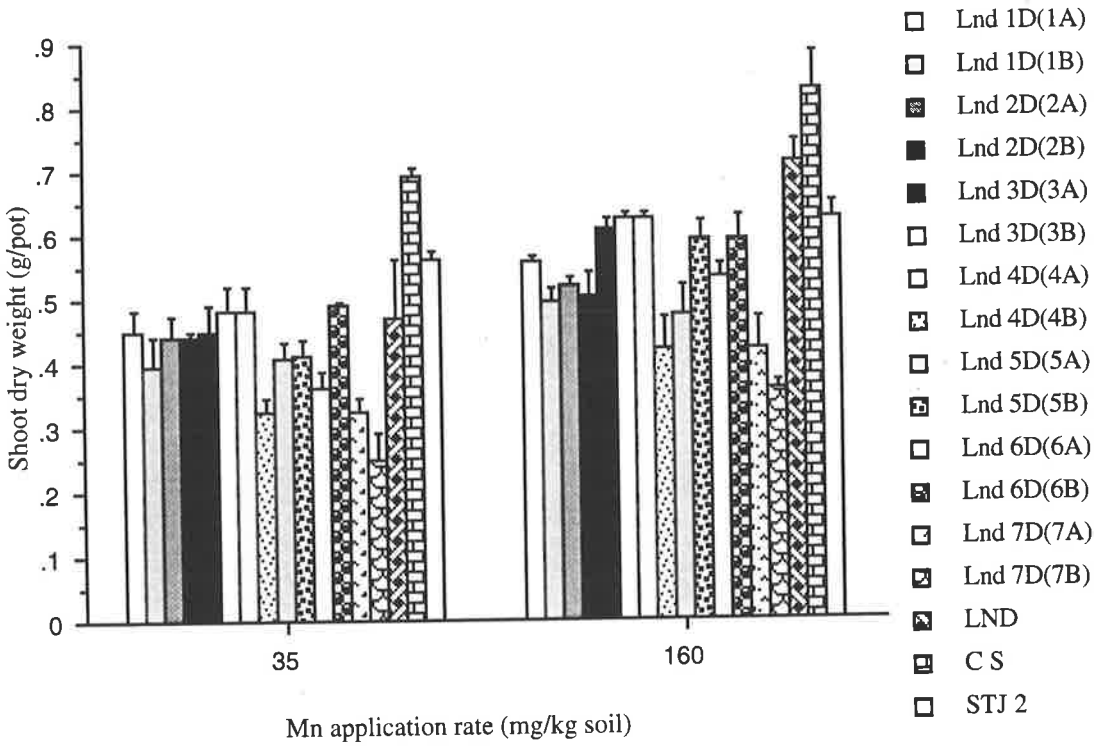

Fig 5.6 Shoot dry weight (g/pot) of Langdon (LND) D-genome disomic substitution lines 35 DAS comparedwith Chinese Spring (CS), Langdon and Stojocri 2 (STJ 2) evaluated at two levels of soil Mn supply (mg/kg dry soil) grown under controlled environment conditions. The error bars represent the standard error of means (n=3).

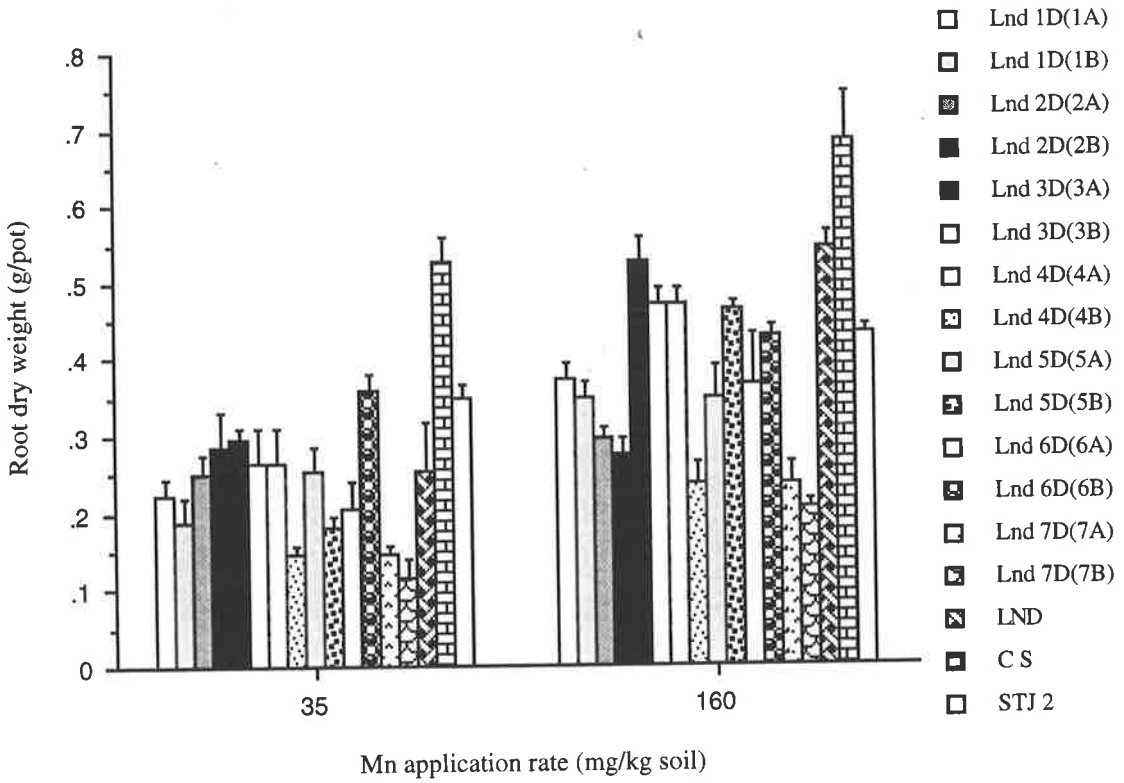

Fig 5.7 Root dry weight (g/pot) of Langdon (LND) D-genome disomic substitution lines 35 DAS compared with Chinese Spring (CS), Langdon and Stojocri 2 (STJ 2) evaluated at two levels of soil Mn supply (mg/kg dry soil) grown under controlled environment condition. The error bars represent the standard error of means (n=3).

Table 5.2 Seed manganese content (μg/seed), seed Mn concentration (mg/kg), seed weight (g/10 seeds) and the level of soil Mn supply required to produce seed with similar Mn contents for D-genome disomic substitution lines, Chinese Spring, Langdon and Stojocri 2.

Lines	Soil Mn concentration (mg/kg)	Mn content (μg/seed)	Mn concentration (mg/kg)	Seed weight (g/10 seed)
Lnd1D(1A)	100	0.79	40	0.2
Lnd1D(1B)	100	0.72	18	0.4
Lnd2D(2A)	100	0.67	22	0.3
Lnd2D(2B)	50	0.69	14	0.5
Lnd3D(3A)	50	0.64	16	0.4
Lnd3D(3B)	50	0.68	23	0.3
Lnd4D(4A)	100	0.65	22	0.3
Lnd4D(4B)	100	0.66	17	0.4
Lnd5D(5A)	50	0.66	17	0.4
Lnd5D(5B)	50	0.76	25	0.3
Lnd6D(6A)	50	0.82	27	0.3
Lnd6D(6B)	100	0.73	18	0.4
Lnd7D(7A)	50	0.76	25	0.3
Lnd7D(7B)	100	0.79	26	0.3
Langdon	50	0.75	25	0.3
Chinese Spring	50	0.65	16	0.4
Stojocri 2	50	0.69	17	0.4

5.3.2 Discussion

Shoot Mn content of the whole set of Langdon D-genome disomic substitution lines, except Lnd 4D(4B), Lnd 7D(7A) and Lnd 7D(7B), was similar to the parents (CS and Langdon) both at deficient and sufficient Mn supply ($P<0.05$) (Fig 5.4). Shoot Mn content of the moderately Mn-efficient genotype Stojocri 2, while not statistically superior to Langdon, was 12% higher at lower Mn supply (Fig. 5.4).

The significantly lower expression of Mn efficiency in 7D(7A) and 7D(7B) compared to Langdon explained the lack of response to seed Mn content in the previous study (Section 5.2.1). The relatively small difference between 7D(7A) and 7D(7B) could further be a result of the substitution on decline of relative vigour in 7D(7B).

The significantly lower expression of Mn efficiency in 4D(4B) in comparison to 4D(4A) could be explained by its lower relative vigour, which was also observed under normal growing conditions.

Substitution of D-genome chromosomes from Chinese Spring with the homoeologous chromosomes from either A or B genome in the Langdon background led to changes in seed size, plant vigour and Mn content, as discussed in Section 5.3. Murata (1991) also has pointed out that root growth of aneuploids might be affected by the aneuploid condition of chromosomes, restricting the application of these stocks in genetic analysis. In contrast, Jamjod (1996) did not report the effect of high concentrations of boron on root length of D-genome disomic substitution lines in comparison to the parents as a barrier to her genetic study of boron tolerance in durum wheat.

Numerous applications of these stocks in genetic analysis of durum wheat have also been reviewed by Joppa (1987). Polyacrylamide gel electrophoresis was performed on

endosperm of the D-genome substitution lines and the locations of gliadin structural genes was detected (Joppa, 1987). Jamjod (1996) also employed these in determining the location of genes (BoT1 and BoT2) conferring tolerance to high concentrations of boron in durum wheat. BoT1 and BoT2 were found, respectively, on chromosome 7B of AUS 10344 (moderately boron tolerant) and AUS 14010 (moderately boron tolerant). Both genes were also found to be on same chromosome of AUS 10110 (boron tolerant durum genotype).

The observed lower expression of Mn efficiency in 7D(7A), 7D(7B) and 4D(4B) could be attributed to:

(i) The negative effect of gene(s) of 7D on expression of gene(s) on homoeologous chromosomes from either the A or B genome.

(ii) The same negative effect caused by substitution of 4B by 4D in 4D(4B) and their influence on relative vigour was one of the barriers in employing these stocks in further genetic analysis of Mn efficiency in durum wheat.

(iii) Another constraint in using these stocks in genetic analysis of Mn efficiency in durum wheat was the expressed level of Mn efficiency in Chinese Spring. It was not efficient enough (not significantly different from Stojocri 2) and had narrow separation from Langdon which is a major barrier in further use in appropriate crosses and study of segregation (Law *et al.*, 1987).

The expression of Mn efficiency in other entries fell between parental genotypes (Chinese Spring and Langdon); however, relatively higher Mn efficiency was observed in 6D(6B) and 1D(1A), and lower efficiency was also demonstrated in 5D(5A) and 5D(5B). This could be explained by:

(i) 6D(6A), 1D(1B) and, to lesser extent, 5D(5B) in the previous study (Section 5.3) were under confounding effect of seed Mn content compared to 6D(6B), 1D(1A) and 5D(5A) (Table 5.1 and Fig 5.2), which confounded their real differences demonstrated in the recent study either as shoot Mn content (Fig 5.4) or shoot Mn concentration (Fig 5.5).

(ii) The study of 6D(6A), 6D(6B), 1D(1A), 1D(1B), 5D(5A) and 5D(5B) under normal growing conditions did not show any differences in relative vigour, so the expressed difference in Mn efficiency could be attributed neither to differences in crop vigour nor in seed Mn content.

(iii) The study of 6D(6A) and also rye (6R)-wheat addition lines for Mn efficiency confirmed the lower Mn efficiency of 6D(6A) (Schlegel *et al.*, 1998), and also demonstrated the positive significant effect of 6R on expression of Mn efficiency (Graham, 1987).

In summary, the greater expression of Mn efficiency seemed to be most probably due to presence of gene(s) for Mn efficiency on 6D and 1D interacting with respective gene(s) from homoeologous chromosomes (1B and 6A) rather than their effect on relative plant vigour or seed size and seed Mn reserve. Considering the additive nature of Mn efficiency genes, it should not be surprising to observe higher efficiency in Lnd 1D(1A) or 6D(6B).

Chapter 6

Use of Amplified Fragment Length Polymorphism (AFLP) in the study of manganese efficiency in durum wheat

6.1 Introduction

In Chapter 3 a precise and accurate screening technique to facilitate breeding and genetic study of Mn efficiency was described. An improved technique should be accurate, fast, less tedious and supplement the current pot bioassay which is subject to environmental variation, limiting its accuracy. The confounding effect of seed Mn content on expression of Mn efficiency, variation arising from using different soil batches and duration of the assay are major limitations of the current Mn pot bioassay. These constraints decrease the accuracy of selection in early generations and make identification of a molecular marker linked to Mn efficiency desirable.

Marker assisted selection (MAS) is the most commonly claimed application for molecular markers. The manipulation of specific traits, in our case the introgression of Mn efficiency from a Mn-efficient parent to high yielding breeding lines, is one of many important objectives in durum breeding programs. Markers have the potential to increase efficiency of the breeding program by enabling breeders to discard unwanted genotypes early in the program, and accelerate introgression of the desired genes into breeding lines

by reducing the number of required backcrosses by selecting for a high proportion of recurrent parent markers.

The development of AFLP (Vos *et al.*, 1995) as a novel method for fingerprinting and its major features giving rise to a preference for this approach over the other marker systems were discussed in Section 1.6.3. The combination of the different marker systems (AFLP and RFLP) and their implementation leading to generation of high density linkage maps in rice, soybean and barley were also discussed (Section 1.6.3). The fact that these maps have the potential of being used in localisation of genes of interest, along with the characterisation and isolation of specific genes, has also been discussed (Section 1.6.3).

The mapping of polyploid species (e.g. durum and bread wheat) has progressed more slowly than that for diploids, as it is difficult to generate a map due to the complexity of the genome, low levels of intra-specific polymorphism and the large number of linkage groups (Marino *et al.*, 1996). These are the major barriers in construction of high density linkage maps and, consequently, detection of the loci of interest.

Bulk segregant analysis (BSA) (Michelmore *et al.*, 1991) is another option to compensate for low map density and to increase the probability of identification of loci of interest. It includes preparation of two bulk DNA samples from extreme segregants in the mapping population, and screening these with an array of markers, with the objective of finding polymorphic markers between the two contrasting bulks. The DNA samples of individuals from two tails of the distribution with similar traits are collected, pooled for each end separately and used as the bulks. The bulks are distinguished by alleles in the region of interest, while the rest of the genome will have a random, but more or less equal, contribution of alleles from the parents distinct from the trait of interest. Screening the mapping population will reveal the linkage between the locus of interest and the

polymorphic markers. Combining these two approaches (AFLP and BSA) has led successfully to identification of markers closely linked to powdery mildew resistance genes in lettuce (Michelmore *et al.*, 1991), and the *Melampsora larici populina* locus (Mer) in *Populus* spp (Cervera *et al.*, 1996).

To identify markers linked to a locus conferring Mn efficiency in durum wheat, AFLP analysis (a novel fingerprinting technique which enhances analysis of a large number of markers) was combined with bulked segregant analysis (BSA) (Michelmore *et al.*, 1991).

6.2 Materials and methods

In order to identify markers linked to a locus conferring Mn efficiency in durum wheat, the F_2 segregating population derived from a cross between Stojocri 2 (a Mn-efficient durum) and Hazar (Mn-inefficient genotype) developed for genetic studies (Chapter 4) was employed and tested with 64 primer combinations.

6.2.1 Plant material

Following harvest of the shoots for determination of Mn concentration, seedlings (including crown, roots and soil) were transplanted into pots (150 mm diameter) with UC potting mix (Section 4.4). Plants were transferred to controlled environment conditions, 14 hrs light/8 hrs dark, photoperiod at 25°C day/15°C night. Twenty days later, leaf samples were harvested from the regrowth for DNA sample preparation from the F_2 population of Stojocri 2/Hazar and also from the parents (Section 4.4).

6.2.2 DNA preparation

The mini prep DNA preparation procedure followed as described by Langridge *et al.* (1996). About 10 cm of the fresh and healthy youngest emerged leaf was collected, placed in a 2.0 ml Eppendorf tube and frozen in liquid nitrogen. The sample was ground with a knitting needle to a fine powder. DNA extraction buffer (750 µl; 1% sarkosyl, 100 mM Tris-HCl, 100 mm NaCl, 10 mM EDTA, pH 8.5) was added and homogenised with leaf powder by vortexing. The same volume (750 µl) of cold phenol/chloroform/iso-amyl-alcohol (25:24:1) was added for extraction, followed by mixing on an orbital rotor for 30 minutes. Phase separation was carried out by centrifuging at 5000 rpm for five minutes and transferring 730 µl of the upper aqueous phase into a fresh Eppendorf tube. The phenol extraction step was repeated using the 730 µl transferred supernatant and adding 730 µl phenol/chloroform/iso-amyl-alcohol. 730 µl of the upper aqueous phase was collected and transferred to a 1.5 ml tube to which 73 µl of 3M Na-acetate (pH 4.8) and 730 µl of isopropanol were added, mixed by inversion and the DNA allowed to precipitate at room temperature for one minute. The tube was centrifuged (15000 rpm) for fifteen minutes in an Eppendorf centrifuge to pellet the DNA. The supernatant was poured off without dislodging the pellet. The pellet was washed by adding 1 ml 70% ethanol followed by five minutes centrifuging (10000 rpm). The ethanol was then discarded by first pouring off the bulk of it and then the remainder was removed by centrifugation and then pipetting out the last drop. The DNA pellet was air dried and resuspended overnight at 4°C in 50 µl R40 [40 µg DNase-free RNAse A/ml 1x TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM Na_2EDTA)].

6.2.3 AFLP marker and bulk segregant analysis

6.2.3.1 Bulk segregant analysis

Bulk segregant analysis (Michelmore *et al.*, 1991) was performed to identify AFLP markers linked to the Mn efficiency loci. Two bulks were made by mixing aliquots of DNA (2 µg) from two sets of five F_2 plants of Stojocri 2/Hazar representing the two extremes in Mn efficiency. The bulks (Mn-efficient bulk and Mn-inefficient bulk and the parents, Stojocri 2 and Hazar) were screened with 64 primer combinations (Table 6.1) to identify polymorphism.

6.2.3.2 AFLP analysis

The AFLP method developed by Vos *et al.* (1995), with some modification was followed and carried out in three steps: (1) preparation of template DNA, (2) selective amplification of template DNA, (3) gel analysis of amplified fragments and autoradiography.

6.2.3.2.1 Preparation of template DNA

The template DNA was produced in the four steps described below:

(i) Restriction digest

One microgram genomic DNA for each of Mn-efficient bulk, Mn-inefficient bulk, Mn-efficient parent and Mn-inefficient parent was digested using two restriction endonucleases, *Mse* I and *Pst* I (Promega, Madison, USA). Five units of each of the endonucleases were used in a total reaction volume of 50 µl containing 5 µl

reaction buffer, 10xRL buffer (100 mM trisHAc, 100 mM MgAc, 500 mM KAc and 50 mM DDT, pH 8.5) and made to the final volume with sterile water. The reactions were incubated at 37°C for three hrs.

(ii) Annealing of adaptors

A stock solution containing both *Mse* I adaptors (*Mse* I and *Mse* II) and *Pst* I adaptors (*Pst* I and *Pst* II) was prepared at 50 µM and 5 µM respectively. The prepared stock solution of adaptors was heated to 90°C for three min and then left to anneal (double stranded) at room temperature for 30 min.

(iii) Ligation of adaptors

The double stranded DNA adaptors were ligated to the end of restriction fragments followed by ethanol precipitation and re-suspension in 60 µl 0.1 M TE (10 mM Tris-HCl, pH 8.0, 1 mM Na_2EDTA). One microlitre of each adaptor (*Mse* I and *Pst* I) was used in a final reaction volume of 10 µl, containing 1µl of reaction buffer (10xRL buffer), 1.2 µl of 10 mM ATP (adenosine 5'-triphosphate) and 1 µl of T4 DNA ligase (1 µ/µl) (Boehringer, Mannheim, Germany) and made to the final volume with sterile water. Ten microlitres of this solution was added to each restriction digest for ligation of adaptors. The digest was incubated at 37°C for three hrs and left at 4°C overnight. The DNA was precipitated by adding 129 µl ethanol and 6 µl 4.8 M sodium acetate (pH 3.2) and placing in liquid nitrogen for five min, followed by centrifuging for five min (10000 rpm). The precipitated DNA pellet was washed with 1 ml 70% ethanol and dried in a speed-vac for ten min followed by re-suspension in 60 µl 0.1 M TE (Tris-EDTA buffer, 10 mM Tris-HCl pH 8.0, 1 mM Na_2EDTA).

(iv) Pre-amplification of DNA

Pre-amplification was performed using primers specific for *Pst* I and *Mse* I adaptors including one selective nucleotide. One microlitre (75 ng/µl) of each of *Mse* C primer and *Pst* A primer were used in a total reaction volume of 21 µl of buffer mix containing 4 µl (1.25 mM) dNTPs (2'-deoxy ribonucleoside 5'-triphosphate), 2.5 µl 10x Tag buffer (50 mM KCl, 20 mM Tris-HCl), 1.5 µl (25 mM) $MgCl_2$, 0.2 µl (5 µ/µl) Tag DNA Polymerase (Promega, Madison, USA) and made to total volume with sterile water. Four microlitres of template DNA (R-L DNA) was added to the buffer, mixed and subjected to PCR. The pre-amplification PCR conditions consisted of twenty cycles of 94°C for 30 seconds, 56°C for one minute and 72°C for one minute. Following PCR, the DNA sample (Template DNA) was diluted 1: 5 in sterilised water before being used in selective amplification.

6.2.3.2.2 Selective amplification of template DNA

In selective amplification, similar primers with three selective bases at the 3' end were used. The *Pst* I primers were end-labelled followed by selective PCR.

(i) End-labelling of primer

A total of eight *Pst* I and eight *Mse* I primers were used giving 64 (8 *Pst* I x 8 *Mse* I) primer combinations (Table 6.1). One microlitre of *Pst* I primers (50 ng/µl) was used for end-labelling in a total reaction volume of 10 µl, including 1.5 µl [γ–^{32}P] dATP (10µCi/ml, Amersham, Braunschweig, Germany), 1µl 10x PNK buffer (10 mM trisHAc, 10 mM MgAc, 50 mM KAc and 5 mM DDT, 0.5

mM spermidine), 0.2 µl T4 PNK (polynucleotide kinase, 10µ/ml) and made to the total volume with sterile water followed by incubation at 37°C for 30-60 min.

(ii) Selective PCR

Selective PCR was carried out using primers containing three selective bases at 3' end. The amplification mix contained 1 µl of each end-labelled *Pst* I primers in a total reaction volume of 18 µl, including 2 µl 10x Tag buffer (50 mM KCl, 20 mM Tris-HCl), 1.2 µl (25 mM) $MgCl_2$, 3.3 µl (1.25 mM) dNTPs (Deoxynucleotide triphosphate, Promega, Madison, USA), 0.5 µl (50 ng/µl) Pst1-1 , 0.6 µl (50 ng/µl) Mse1-1, 0.2 µl (5 µ/µl) Tag Polymerase (Promega, Madison, USA) and made to total volume with sterile water. Two microlitres of template DNA was added to the 18 µl of amplification mix before this was subjected to selective PCR. The PCR reaction conditions for selective amplification consisted of one cycle at 94°C for 30 seconds, 65°C for 30 seconds and 72°C for one minute, followed by nine cycles over which the annealing temperature was decreased by 1°C per cycle with a final step of 25 cycles of 94°C for 30 seconds, 56°C for 30 seconds and 72°C for one minute.

6.2.3.2.3 Gel analysis of amplified fragments

After selective PCR, the amplified fragments were mixed with 20 µl formamide dye (98% formamide, 10 mM EDTA, 0.005% each of xylene cyanol and bromophenol blue) denatured by heating for five minutes at 90°C and chilled on ice prior to loading on the gel. Two microlitres of each sample were loaded on 6% denaturing polyacrylamide gel (10 ml SequaGel buffer, 40 ml Monomer Solution, 400 µl ammonia persulphate 10%

(w/v), National Diagnostics) for separation of amplified fragments. PUC 19 DNA restricted with *Msp* I was used as a molecular weight standard. The gels were transferred to 3 MM paper for drying, and auto-radiography was carried out using Fuji RX medical X-ray film at room temperature for 24-48 hours.

6.3 Results

Genetics of manganese efficiency in durum wheat

In a cross of Stojocri 2 by Hazar, the number of genes and their mode of action was discussed in Chapter 4. That analysis was carried out by the comparison of observed variance to expected variance of the F_2 and $F_{2:3}$ populations based on one and two gene models. The Mn efficiency seemed to be controlled by the additive action of two genes.

A total of 64 primer combinations was used to compare the two bulked samples with the two parents. Approximately 60-70 distinguishable bands were observed for each primer combination and, on average, 50-60 of these bands were polymorphic between the parents. Primers (*Pst* I+ACA/*Mst* I+CAA) and (*Pst* I+ACC/*Mse* I+CAG) each revealed one AFLP, where the band was present in the efficient bulk and efficient parent but absent in inefficient bulk and inefficient parent (Fig 6.1).

6.4 Discussion

The results obtained from the previous study (Chapter 4) suggested that Mn efficiency in the specific cross of Stojocri 2/ Hazar was determined by two genes with additive effect. In the work reported in this chapter, two AFLP markers potentially linked to Mn

efficiency loci were identified using an approach combining BSA and AFLP techniques to screen the segregating population.

Application of AFLP is probably the most powerful DNA fingerprinting method to detect polymorphisms due to the presence or absence of restriction enzyme sites. The most important feature of the AFLP technique is the number of markers that can be screened in each experiment (ten to twenty primer combinations). The use of two restriction enzymes (*Pst* I and *Mse* I) and 64 primer combinations provided a high number of selectively amplified DNA fragments. The high rate of polymorphism obtained between parents creates a greater chance of identifying polymorphic loci for each parental genome.

The reproducibility of AFLP is another positive feature of this technique, as compared to other PCR based techniques such as RAPDs, which are based on use of random primers. Two important features are combined in primers used to obtain AFLP markers:

(i) Complementary characteristic of primer to adaptors which creates a higher specific primer annealing,

(ii) Their selectivity: changing the 3' nucleotides allows amplification of a different set of DNA fragments from the preamplifier fragment. Combining the power of BSA, as a useful tool to screen for the marker tightly linked to the character of interest, and AFLP analysis will provide an efficient approach to identify the markers close to the desired locus.

The two AFLP markers identified in the Mn-efficient parent and Mn-efficient bulk have the potential of being used to screen the segregating population which will lead to a better understanding of the genetics of Mn efficiency in the cross of Stjocri 2/Hazar. If these markers are linked to one or two Mn efficiency genes, they have the potential of being

used to select the efficient individuals in further segregating populations bred from the same efficient progenitor. Combined AFLP and BSA has also been applied in the analysis of disease resistance in *Populus* spp (Cervera *et al.*, 1996). Three AFLP markers closely linked to *Melampsora larici- populina* locus (Mer) were identified to screen a segregating family. One of the identified markers is currently used in the *Populus* spp breeding program (Cervera *et al.*, 1996).

To facilitate screening of progeny derived from the cross of a Mn-efficient progenitor by a simple PCR based assay rather than employing the whole AFLP analysis, cloning and sequencing of AFLP markers could be the next approach. Cloning and sequencing of the AFLP makers has the potential of converting them into a Sequence Characterised Amplified Region (SCAR) for further PCR screening of progeny derived from the same efficient progenitor or those sharing the same Mn efficiency gene.

The use of AFLP markers in other cross combinations of genotypes with different levels of Mn efficiency could lead to identification of other loci conferring Mn efficiency in durum wheat, and will help better understand the genetic basis of Mn efficiency in durum wheat. Cho *et al.* (1996) described the technique employed in cloning the gene of interest (from rice) derived from Selective Restriction Fragment Amplification (SRFA) developed by Zabeau and Vos (1993), or AFLP amplification from either ^{32}P-labelled or silver stained polyacrylamide gels by one round of PCR amplification. The identities of cloned bands were confirmed by further sequence analysis. The inheritance of two cloned AFLP bands were studied by converting them to RFLP clones and mapping them to independent positions on a saturated genetic map of rice as a prelude to their use in a breeding program.

Table 6.1 Selective *Pst* I and *Mse* I primers and adaptors used in screening the Mn-efficient F_2 bulk, Mn-inefficient F_2 bulk and the parents (Stojocri 2 and Hazar) for polymorphism associated with Mn-efficeincy expressed as shoot Mn content (µg/pot).

Enzyme	Selective nucleotide	Sequence	Primer designation
Pst I	**AAC**	GACTGCGTACATGCAG**AAC**	P1
Pst I	**AAG**	GACTGCGTACATGCAG**AAG**	P2
Pst I	**ACA**	GACTGCGTACATGCAG**ACA**	P3
Pst I	**ACC**	GACTGCGTACATGCAG**ACC**	P4
Pst I	**ACG**	GACTGCGTACATGCAG**ACG**	P5
Pst I	**ACT**	GACTGCGTACATGCAG**ACT**	P6
Pst I	**AGC**	GACTGCGTACATGCAG**AGC**	P7
Pst I	**AGG**	GACTGCGTACATGCAG**AGG**	P8
Mse I	**CAA**	GATGAGTCCTGAGTAA**CAA**	M1
Mse I	**CAG**	GATGAGTCCTGAGTAA**CAG**	M2
Mse I	**CAT**	GATGAGTCCTGAGTAA**CAT**	M3
Mse I	**CCA**	GATGAGTCCTGAGTAA**CCA**	M4
Mse I	**CCT**	GATGAGTCCTGAGTAA**CCT**	M5
Mse I	**CGA**	GATGAGTCCTGAGTAA**CGA**	M6
Mse I	**CTA**	GATGAGTCCTGAGTAA**CTA**	M7
Mse I	**CTG**	GATGAGTCCTGAGTAA**CTG**	M8
Pst I	*Pst* I adaptor 1	CTCGTAGACTGCGTACATGCA	
Pst I	*Pst* I adaptor 2	TGTACGCAGTCTAC	
Mse I	*Mse* I adaptor 1	GACGATGAGTCCTGAG	
Mse I	*Mse* I adaptor 2	TACTCAGGACTCAT	

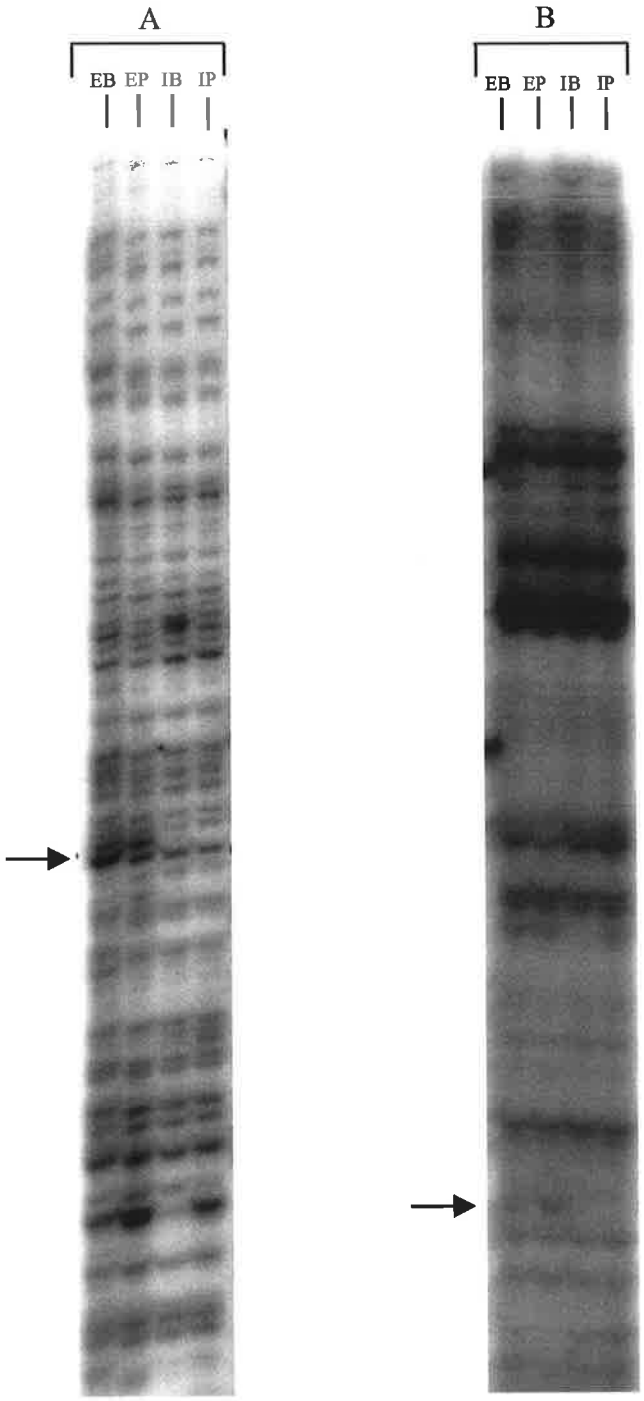

Fig 6.1 AFLP markers potentially linked to the Mn efficiency locus. Bulked segregant analysis (BSA) is presented as a set of four lanes: Mn-efficient bulk (E_B), Mn-efficient parent (E_P), Mn-inefficient bulk (I_B) and Mn-inefficient parent (I_P). The AFLP markers (Pst I+ACA/Mse I+CAA) (A), and (Pst I+ACC/Mse I+CAG) (B), identified by BSA and indicated by an arrow, are present in E_B and E_P but absent in I_B and I_P.

Chapter 7

General Discussion

No variation for Mn efficiency has been reported for durum wheat prior to the work beginning of this thesis in South Australia. Considering the importance of durum as a promising crop, and also the preference of a genetic approach as compared to agronomic solutions in tackling this particular problem, the first milestone of this study was devoted (Chapter 2) to investigating the extent of tolerance of durum wheat (*Triticum turgidum* L. var *durum*) to Mn deficiency. This study identified durum genotypes, notably Stojocri which has higher tolerance than the commercial durum varieties.

The preliminary study of 69 genotypes (including accessions and advanced lines from exotic sources) at Marion Bay (lower Yorke Peninsula) and Coonalpyn (145 km south east of Adelaide) of South Australia, Mn-deficient and micronutrient multiple deficient sites respectively, revealed:

(1) Prevalence of Mn deficiency, expressed as deficiency symptoms, not only in a calcareous Mn-deficient sand field site (Marion Bay) but also at a site representative of extensive area of SA cereal growing zone, Coonalpyn as a multiple-micronutrient deficient site.

(2) Presence of a relatively wide range of variation in severity of deficiency symptoms among genotypes at both sites.

A manganese pot bioassay was employed to study the reason for the observed variation (different expression of Mn deficiency symptoms in the field) among genotypes, using three selected genotypes and a range of applied Mn (Chapter 2).

Higher critical Mn concentration in youngest emerged leaf blades was observed in durum wheat (18-19 mg/kg dry weight) 35 DAS, which was more than the reported 10-12 mg/kg dry weight for either field grown bread wheat (Graham *et al.*, 1985) or barley (11 mg/kg dry weight) (Hannam *et al.*, 1987). The higher critical Mn concentration in YEBs of durum wheat exacerbates its lower tolerance to Mn deficiency as a result of the higher internal Mn requirement than bread wheat and barley, or the differences may be due to change in the experimental conditions. Higher critical level of Mn has also been reported (Graham *et al.*, 1985) in leaf blades of young seedlings grown in solution culture compared to field grown wheat.

Statistically significant response of genotypes to applied Mn was observed for a variety of measured parameters, such as: extent of development of chlorosis, Mn uptake, shoot Mn concentration, shoot Mn content and dry matter production. In other words, the observed difference of these genotypes under field conditions in terms of expression of Mn deficiency symptoms was due to genotypic variation in tolerance to Mn deficiency or genotypic variation in the efficiency of Mn uptake. This was the first reported evidence of genotypic variation of Mn efficiency in durum wheat. However, determination of the extent of genotypic variation (to allow further screening) demanded development of an efficient selection criterion. This was accomplished by concurrent study of several durum genotypes in the field and in controlled environment conditions for two growing seasons (Chapters 2 and 3).

The two years of concurrent field and pot studies on genotypes revealed that genotypes under controlled environment conditions differentiated better on the basis of chlorosis symptoms, shoot Mn contents, Mn uptake and shoot dry weights than did YEBs and Mn concentrations. The Mn-efficient genotypes produced higher seedling dry weight by absorbing more Mn from the deficient soil, thus maintained higher Mn tissue contents and concentration and developed less chlorosis symptoms compared to inefficient genotypes. The same trend was also observed in the field, where Mn-efficient genotypes developed less chlorosis symptoms, produced more biomass at tillering and at maturity, maintained higher grain yield and Mn uptake in comparison to Mn-inefficient genotypes. The measured parameters in the controlled environment (shoot Mn content, shoot Mn concentration and Mn uptake) were highly correlated with those measured in the field experiment (grain yield and relative grain yield). As shoot Mn content gave better discrimination of the genotypes and this showed higher correlation with both grain yield and relative grain yield, this was chosen as the selection criterion for further studies and screening.

The use of genotypes with variable seed Mn content changed, to some extent, the ranking of genotypes (within a narrow range of efficiency) for the measured parameters, both in the field and controlled environment. This phenomenon once again placed emphasis on the importance of using seed with similar Mn reserves in screening genotypes based on yield and yield dependent characters, as addressed by Longnecker *et al.* (1991a) and Marcar and Graham (1986) (Chapters 2 and 3).

The development of selection criterion under controlled environment conditions facilitated screening of a large number of genotypes in a short period of time. However, the growth habit and maturity status of genotypes should be taken into account when screening, considering the fact that Mn deficiency is accentuated in late maturing genotypes and under

long growing seasons. This explains the good level of tolerance of Senatore Cappelli (late maturing) under controlled environment conditions versus its relatively low grain yield in the field.

A relatively wide range of genotypic variation was observed in terms of relative grain yield (15% to 58%) among the genotypes from different sources. The genotypes with higher relative yield (e.g Stojocri 2 and Sham 1) are being used in the breeding program to incorporate Mn efficiency in current Mn-inefficient advanced lines or cultivars with a lower relative yield (e.g Yallaroi with 15% relative yield). The observed extent of variation for the Mn efficiency in the current germplasm (15%-58%), although not very wide, seems to be useful enough to be incorporated into current Mn-inefficient advanced lines through plant breeding.

Study of the progenitors of Sham 1 and Hazar, respectively, as Mn-efficient and Mn-inefficient, showed that genotypes originating from sources of origin of durum wheat often possessed moderate tolerance to Mn efficiency, which had evolved in the wild species and had been retained during a long process of domestication. The introduction of these genotypes and their continuous improvement on non-calcareous soils (USA and Canada) was accompanied by the loss of the genes for tolerance to Mn deficiency.

The expressed level of Mn efficiency in Sham 1 was intermediate to its parental lines which indicates inheritance of the trait from the parents with no selection pressure. There was no evidence of dominance or heterosis in transmission of the trait. This study has provided valuable information on possible sources of Mn efficiency, and drawn attention to Algeria as the country of origin of the efficient progenitors (Zenati Bouteille). This genotype has contributed 3.5% of the ancestors in the pedigree of both Sham 1 and Stojocri 2 as efficient genotypes (Fox *et al.*, 1998). To exploit further intra-specific

variation for Mn efficiency, extensive study of accessions derived from Algeria and other sources (Iraq, Italy, Tunisia, Iran and Greece) is warranted.

To enhance breeding, information on mode of action and the number of genes is necessary to determine the backcrossing procedure. Analysis of response of F_1 hybrid and the study of segregating populations F_2 and F_3 (Chapter 4) led to the conclusion that in the specified cross possibly two genes with an additive effect were involved. However, determination of the total number of genes involved in the full expression of Mn efficiency in durum wheat necessitates cross combinations of genotypes expressing the full range of Mn efficiency which in turn demands further screening (for highly Mn efficient genotypes). Currently the efficient genotype (Stojocri 2) is used in the breeding program. As this genotype has a good agronomic type and is fairly closely related to the recurrent parents, possibly a minimum of only two backcrosses will be required. This should give approximately 88% recovery of recurrent parent.

A better alternative and a complementary tool to the current Mn pot bioassay, possibly with greater accuracy and reduced time, would be the development of the molecular markers and their application in breeding.

Identification of the chromosomal location of genes would facilitate determination of markers linked to the trait. Feasibility of determination of the chromosomal location of genes conferring Mn efficiency in durum wheat using D-genome disomic substitution lines was the subject of the study presented in Chapter 5. There are some barriers in the successful application of these stocks in genetic analysis of Mn efficiency in durum wheat.

(i) The substitution of the D-genome chromosomes from Chinese Spring in Langdon background resulted in changes in seed size, seed Mn concentration and

consequently seed Mn content. This confounding effects of the seed Mn reserves in screening was minimised by production of seed with similar Mn content.

(ii) Lower expression of Mn efficiency was observed in 7D(7A), 7D(7B) and 4D(4B) compared to the parents (Chinese Spring and Langdon). This could be attributed to the negative effect of gene(s) of 7D on the expression of gene(s) on homoeologous chromosomes from either the A or B genomes. The same negative effect was observed in substitution of 4A and 4D for 4B.

(iii) Lower level of Mn efficiency in Chinese Spring makes it an inappropriate genotype in such studies (Law *et al.*, 1987).

The expression of Mn efficiency in all other genotypes (except 6D(6B), 1D(1A)) fell between parental genotypes (Chinese Spring and Langdon). The higher Mn efficiency in 6D(6B) and 1D(1A) could probably be due to presence of gene(s) for Mn efficiency on 6D and 1D interacting with respective gene(s) from homoeologous chromosomes (1B and 6A genome) rather than their having an effect on plant vigor or seed size.

The application of the D-genome disomic substitution lines for the determination of the chromosomal location of genes in the A or B genome, by methods similar to those used in a monosomic analysis in hexaploid (Law *et al.*, 1987) was described by Joppa (1987). Jamjod (1996) successfully employed these in determining the location of genes (BoT1 and BoT2) conferring tolerance to high concentrations of boron in durum wheat. BoT1 and BoT2 were found, respectively, on chromosome 7B of AUS 10344 (moderately boron tolerant) and AUS 14010 (moderately boron tolerant). Both genes were also found to be on the same chromosome of AUS 10110 (boron tolerant durum genotype). However, their application in the genetic analysis of Mn efficiency, and probably other micronutrient efficiencies can be limited by the factors discussed above.

It would be advantageous to develop a more precise and accurate screening technique to compliment the current pot bioassay. Hence the identification of the molecular markers linked to Mn efficiency loci is desirable. The markers have the potential for increasing the efficiency of breeding programs by enabling breeders to discard the unwanted genotypes early in the program thus accelerating the introgression of the desired genes into breeding lines and by reducing the size of the segregating population.

The difficulty of generating a high density map in polyploid species (bread and durum wheat) and, consequently, detection of the loci of interest, has made BSA (Michelmore *et al.*, 1991) an alternative option. This thesis describes an attempt to identify polymorphic markers between two contrasting bulks selected at the two extreme in a mapping population developed from a cross of Stojocri 2 with Hazar (Chapter 6). To identify linked polymorphic markers, AFLP analysis technique, which allows the analysis of large number of markers, was combined with BSA (Michelmore *et al.*, 1991). Primers (*Pst* I+ACA/*Mse* I+CAA) and (*Pst* I+ACC/*Mse* I+CAG) each revealed one AFLP, where the bands were present in the efficient bulk and efficient parent, but absent in the inefficient bulk and inefficient parent.

Screening of the mapping population will reveal the linkage between the locus of interest and the polymorphic markers, which is the next approach and is highly warranted. If these markers are linked to one or two Mn efficiency genes, they have the potential of being used to select efficient individuals in further segregating populations bred from the same efficient progenitor. Combining these two approaches (AFLP and BSA) has successfully led to identification of markers closely linked to powdery mildew resistance genes in lettuce (Michelmore *et al.*, 1991) and *Melampsora larici populina* Locus (Mer) in Populus spp (Cervera *et al.*, 1996). Three AFLP markers closely linked to *Melampsora*

larici- populina Locus (Mer) were identified to screen segregating families. The identified marker is currently used in a *Populus* spp breeding program (Cervera *et al.*, 1996).

To facilitate screening of progeny derived from the cross of a Mn-efficient progenitor, a simple PCR based assay, rather than employing the whole AFLP analysis, cloning and sequencing of AFLP markers, could be the next approach. Cloning and sequencing of the AFLP makers enables them to be converted into a Sequence Characterised Amplified Region (SCAR) for further PCR screening.

Cho *et al.* (1996) described the technique employed in cloning the gene of interest (from rice) derived by Selective Restriction Fragment Amplification (SRFA) developed by Zabeau and Vos (1993) or AFLP amplification from either ^{32}P-labelled or silver stained polyacrylamide gels by one round of PCR amplification. The identities of cloned bands were confirmed by sequence analysis. The inheritance of the two cloned AFLP bands was studied by converting them to RFLP clones and mapping them to independent positions on a saturated genetic map of rice as prelude to their use in a breeding program.

The outcome of studies conducted in the context of this thesis are currently being employed in the durum breeding program by crossing the identified Mn-efficient genotypes (Stojocri 2 and Zenati Bouteille) with advanced lines, followed by two consecutive backcrosses to the recurrent parent. After each backcross, the progeny are screened using the selection criterion developed (shoot Mn content), which will lead to selection of efficient progeny at an early stage.

The search for further intra-specific variation in durum from a collection from the probable geographic sources of Mn efficiency, a better understanding of the genetics of Mn efficiency and the validation of molecular markers linked to Mn efficiency loci would certainly enhance the production of Mn efficient genotypes. This would lead to a

broadening of the adaptation of the crop from its current cultivation in deep fertile soils to micronutrient deficient and marginal soils in lower rainfall areas.

Appendix A

Analysis of variance tables (ANOVA)

Analysis of variance was perfomed using Super Anova package.

Table A1. ANOVA table for YEBs Mn concentration (mg/kg) from Experiment 2.1 (Fig. 2.1)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	121.08	60.54	.33	.7172
Genotype	2	3115.93	1557.97	8.61	.0006
Mn Level	8	52801.33	6600.17	36.47	.0001
Genotype * Mn Level	16	2942.04	183.88	1.02	.4560
Residual	52	9411.65	180.99		

Table A2. ANOVA table for shoot Mn concentration (mg/kg) from Experiment 2.2 (Fig. 2.2)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	528.23	264.11	1.93	.1556
Genotype	2	3293.63	1646.81	12.02	.0001
Mn Level	8	86158.17	10769.77	78.64	.0001
Genotype * Mn Level	16	4373.66	273.35	2.00	.0314
Residual	52	7121.50	136.95		

Table A3. ANOVA table for root Mn concentration (mg/kg) from Experiment 2.1 (Fig. 2.3)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	574.96	287.48	1.73	.1867
Genotype	2	3257.31	1628.65	9.82	.0002
Mn Level	8	150682.17	18835.27	113.59	.0001
Genotype * Mn Level	16	8865.64	554.10	3.34	.0005
Residual	52	8622.55	165.82		

Table A4. ANOVA table for shoot dry weight (g/pot) from Experiment 2.1 (Fig. 2.4)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	.01	2.52E-3	.85	.4341
Genotype	2	.86	.43	144.97	.0001
Mn Level	8	3.09	.39	129.66	.0001
Genotype * Mn Level	16	.30	.02	6.27	.0001
Residual	52	.15	2.98E-3		

Table A5. ANOVA table for root dry weight (g/pot) from Experiment 2.1 (Fig. 2.5)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	8.96E-4	4.48E-4	.21	.8110
Genotype	2	.29	.15	68.98	.0001
Mn Level	8	.98	.12	57.73	.0001
Genotype * Mn Level	16	.17	.01	5.07	.0001
Residual	52	.11	2.13E-3		

Table A6. ANOVA table for plant dry weight (g/pot) from Experiment 2.1 (Fig. 2.6)

Source	df	Sum of Squares	Mean Squares	F-Value	P-Value
Replication	2	.01	4.70E-3	.53	.5929
Genotype	2	2.16	1.08	121.22	.0001
Mn Level	8	7.52	.94	105.44	.0001
Genotype * Mn Level	16	.88	.05	6.15	.0001
Residual	52	.46	.01		

Table A7. ANOVA table for shoot Mn content (μg/pot) from Experiment 2.1, (Fig. 2.7)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	184.00	92.00	1.82	.1717
Genotype	2	2453.88	1226.94	24.31	.0001
Mn Level	8	26531.70	3316.46	65.72	.0001
Genotype * Mn Level	16	5133.82	320.86	6.36	.0001
Residual	52	2624.28	50.47		

Table A8. ANOVA table for root Mn content (μg/pot) from Experiment 2.1 (Fig. 2.8)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	13.23	6.61	.17	.8483
Genotype	2	2701.98	1350.99	33.72	.0001
Mn Level	8	16758.28	2094.78	52.29	.0001
Genotype * Mn Level	16	5597.62	349.85	8.73	.0001
Residual	52	2083.28	40.06		

Table A9. ANOVA table for seedling uptake (μg/pot) from Experiment 2.1 (Fig. 2.9)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	106.11	53.05	.44	.6481
Genotype	2	10148.66	5074.33	41.83	.0001
Mn Level	8	85037.55	10629.69	87.63	.0001
Genotype * Mn Level	16	21218.54	1326.16	10.93	.0001
Residual	52	6307.51	121.30		

Table A10. ANOVA table for chlorosis score (1-5) from Experiment 2.1 (Fig. 2.10)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	.01	3.09E-3	.19	.8242
Genotype	2	3.34	1.67	104.97	.0001
Mn Level	8	87.99	11.00	691.43	.0001
Genotype * Mn Level	16	4.88	.31	19.18	.0001
Residual	52	.83	.02		

Table A11. ANOVA table for relative shoot dry weight (%) from Experiment 2.1 (Fig. 2.11)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	253.01	126.50	1.63	.2057
Genotype	2	828.88	414.44	5.34	.0078
Mn Level	8	80018.30	10002.29	128.93	.0001
Genotype * Mn Level	16	2137.28	133.58	1.72	.0316
Residual	52	4034.26	77.58		

Table A12. ANOVA table for chlorosis score (1-5) from Experiment 3.2 (Table 2.4)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	.10	.05	2.91	.0593
Genotype	23	11.41	.50	29.73	.0001
Mn Level	1	95.88	95.88	5742.42	.0001
Genotype * Mn Level	23	11.41	.50	29.73	.0001
Residual	94	1.57	.02		

Table A13. ANOVA table for YEBs Mn concentration (mg/kg) from Experiment 3.2 (Table 2.4)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	45.85	22.93	1.48	.2322
Genotype	23	2584.64	112.38	7.27	.0001
Mn Level	1	33641.37	33641.37	2176.19	.0001
Genotype * Mn Level	23	959.68	41.73	2.70	.0700
Residual	94	1453.13	15.46		

Table A14. ANOVA table for shoot Mn concentration (mg/kg) from Experiment 3.2 (Table 2.4)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Rep	2	83.79	41.89	7.42	.2100
Var	23	2070.15	90.01	15.94	.0001
Mn	1	31022.95	31022.95	5495.47	.0001
Var * Mn	23	867.65	37.72	6.68	.1001
Residual	94	530.65	5.65		

Table A15. ANOVA table for shoot dry weight (g/pot) from Experiment 3.2 (Table 2.4)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replicatio	2	.44	.22	7.73	.0900
genotype	23	2.46	.11	3.80	.0001
Mn Level	1	14.89	14.89	527.53	.0001
Genotype* Mn Level	23	1.64	.07	2.52	.0009
Residual	94	2.65	.03		

Table A16. ANOVA table for relative shoot dry weight (%) from Experiment 3.2 (Table 2.4)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	485.26	242.63	20.11	.1100
Genoype	23	10176.56	442.46	36.67	.0001
Mn Level	1	135178.78	135178.78	11204.61	.0001
Genotype * Mn Level	23	10176.56	442.46	36.67	.1100
Residual	94	1134.07	12.06		

Table A17. ANOVA table for shoot Mn content (mg/kg) from Experiment 3.2 (Table 2.4)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	628.08	314.04	7.59	.2120
Genotype	23	3717.23	161.62	3.90	.0001
Mn Level	1	44834.53	44834.53	1083.28	.0001
Genotype * Mn Level	23	2108.30	91.67	2.21	.1040
Residual	94	3890.47	41.39		

Table A18. ANOVA table for chlorosis score (1-5) from Experiment 3.2 (Table 2.5)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Replication	2	.10	.05	.47	.6272	Rep * Gen
Genotype	23	36.79	1.60	14.65	.0001	Rep * Gen
Replication * Genotype	46	5.02	.11	3.37	.0001	Residual
Mn Level	1	34.71	34.71	1.1E3	.0001	Residual
Genotype * Mn Level	23	2.67	.12	3.59	.0001	Residual
Residual	48	1.55	.03			

Table A19. ANOVA table for above-ground biomass at tillering (g/plot) from Experiment 3.2 (Table 2.5)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Replication	2	3250.31	1625.16	1.32	.2780	Rep * Gen
Genotype	23	165122.32	7179.23	5.82	.0001	Rep * Gen
Replication * Genotype	46	56782.88	1234.41	2.23	.0033	Residual
Mn Level	1	113811.77	113811.77	205.86	.0001	Residual
Genotype * Mn Level	23	29494.35	1282.36	2.32	.0070	Residual
Residual	48	26537.75	552.87			

Table A20. ANOVA table for grain yield (g/plot) from Experiment 3.2 (Table 2.5)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Replication	2	10384.29	5192.15	.76	.4741	Rep * Gen
Genotype	23	3002552.94	130545.78	19.07	.0001	Rep * Gen
Replication * Genotype	46	314877.71	6845.17	1.38	.1347	Residual
Mn Level	1	3732946.01	3732946.01	753.81	.0001	Residual
Gen otype* Mn Level	23	702977.49	30564.24	6.17	.0001	Residual
Residual	48	237700.00	4952.08			

Table A21. ANOVA table for relative grain yield (%) from Experiment 3.2 (Table 2.5)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Rep	2	401.93	200.97	2.36	.1054	Rep * Gen
Gen	23	21024.66	914.12	10.75	.0001	Rep * Gen
Rep * Gen	46	3910.40	85.01	.95	.5739	Residual
Mn Lev	1	118049.51	118049.51	1.3E3	.0001	Residual
Gen * Mn Lev	23	21024.66	914.12	10.17	.0001	Residual
Residual	48	4312.33	89.84			

Dependent: RLYLD

Table B1. ANOVA table for chlorosis score (1-5) from Experiment 3.2, Table. 3.2 (field)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Replication	3	2.85	.95	6.33	.1955	Rep * Genotype
Genotype	5	14.09	2.82	18.79	.0001	Rep * Genotype
Rep * Genotype	15	2.25	.15	3.68	.0007	Residual
Mn Level	2	18.29	9.14	66.29	.0001	Genotype * Mn
Genotype * Mn lev	10	1.38	.14	3.39	.0033	Residual
Residual	36	1.47	.04			

Dependent: CS

Table B2. ANOVA table for YEBs Mn concentration (mg/kg) from Experiment 3.2, Table. 3.2 (field)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Replication	3	51.22	17.07	33.48	.1001	Rep * Genotype
Genotype	5	11.20	2.24	4.39	.0116	Rep * Genotype
Replication * Genotype	15	7.65	.51	.63	.8276	Residual
Mn Level	2	15.16	7.58	18.68	.0004	Genotype * Mn
Genotype * Mn Level	10	4.06	.41	.50	.8758	Residual
Residual	36	28.99	.81			

Table B3. ANOVA table for above-ground biomass at tillering from Experiment 3.2, Table. 3.2 (field)

Source	df	Sum of Sq...	Mean Sq...	F-Value	P-V...	Error Term
Replication	3	7410.02	2470.01	1.93	.1685	Rep * Genotype
Genotype	5	52140.38	10428.08	8.14	.0007	Rep * Genotype
Replication * Genotype	15	19219.29	1281.29	2.97	.0038	Residual
Mn Level	2	66902.58	33451.29	33.80	.0001	Genotype * Mn
Genotype * Mn level	10	9898.20	989.82	2.29	.0136	Residual
Residual	36	15554.41	432.07			

Table B4. ANOVA table for straw yield at maturity (g/plot) from Experiment 3.2, Table. 3.2 (field)

Source	df	Sum of Sq...	Mean Sq...	F-Value	P-V...	Error Term
Replication	3	645056.41	215018.8	2.87	.0716	Rep * Genotype
Genotype	5	716906.73	1.43E5	1.91	.1521	Rep * Genotype
Replication * Genotype	15	1.13E6	75034.18	2.60	.0095	Residual
Mn Level	2	3.1E6	1.55E6	18.81	.0004	Genotype * Mn
Genotype * Mn level	10	823129.09	82312.91	2.85	.0101	Residual
Residual	36	1.04E6	28845.96			

Table B5. ANOVA table for Grain yield (g/plot) from Experiment 3.2, Table. 3.2 (field)

Source	df	Sum of Sq...	Mean Sq...	F-Value	P-V...	Error Term
Replication	3	524056.46	1.75E5	5.41	.1100	Rep * Genotype
Genotype	5	760095.04	1.52E5	4.71	.0087	Rep * Genotype
Replication * Genotype	15	484163.49	32277.57	5.27	.0001	Residual
Mn level	2	3.31E6	1.66E6	60.92	.0001	Genotype * Mn
Genotype * Mn level	10	271933.28	27193.33	4.44	.0004	Residual
Residual	36	220515.29	6125.42			

Table B6. ANOVA table for relative grain yield (%) from Experiment 3.2, Table. 3.2 (field)

Source	df	Sum of Sq...	Mean Sq...	F-Value	P-V...	Error Term
Replication	3	1971.05	657.02	4.11	.1258	Rep * Genotype
Genotype	5	6201.27	1240.25	7.77	.0009	Rep * Genotype
Replication * Genotype	15	2395.52	159.70	1.69	.0980	Residual
Mn Level	2	62819.75	31409.88	71.50	.0001	Genotype * Mn
Genotype * Mn Level	10	4392.92	439.29	4.65	.0003	Residual
Residual	36	3400.99	94.47			

Table B7. ANOVA table for grain Mn concentration (mg/kg) from Experiment 3.2, Table. 3.2 (field)

Source	df	Sum of Sq...	Mean Sq...	F-Value	P-V...	Error Term
Replication	3	79.61	26.54	11.85	.1003	Rep * Genotype
Genotype	5	39.54	7.91	3.53	.0261	Rep * Genotype
Replication * Genotype	15	33.59	2.24	.93	.5456	Residual
Mn level	2	45.97	22.98	8.73	.0064	Genotype * Mn
Genotype * Mn Level	10	26.32	2.63	1.09	.3965	Residual
Residual	36	87.07	2.42			

Table B8. ANOVA table for grain Mn content (mg/plot) from Experiment 3.2, Table. 3.2 (field)

Source	df	Sum of Sq...	Mean Sq...	F-Value	P-V...	Error Term
Replication	3	6.43E7	2.14E7	12.88	.0002	Rep * Genotype
Genotype	5	2.56E7	5.11E6	3.07	.0418	Rep * Genotype
Replication * Genotype	15	2.5E7	1.7E6	1.68	.1000	Residual
Mn Level	2	1.01E8	5.06E7	48.57	.0001	Genotype * Mn
Genotype * Mn Level	10	1.04E7	1.04E6	1.05	.4209	Residual
Residual	36	3.56E7	9.89E5			

Table B9. ANOVA table for straw Mn concentration at maturity (mg/kg) from Experiment 3.2, Table. 3.2 (field)

Source	df	Sum of Sq...	Mean Sq...	F-Value	P-V...	Error Term
Replication	3	54.49	18.16	.80	.5122	Rep * Genotype
Genotype	5	351.84	70.37	3.11	.0403	Rep * Genotype
Replication * Genotype	15	339.87	22.66	1.30	.2534	Residual
Mn Level	2	1269.07	634.54	10.73	.0032	Genotype * Mn
Genotype * Mn Level	10	591.41	59.14	3.39	.0033	Residual
Residual	36	628.44	17.46			

Table B10. ANOVA table for straw Mn content at maturity (μg/plot) from Experiment 3.2, Table. 3.2 (field)

Source	df	Sum of Sq...	Mean Sq...	F-Value	P-V...	Error Term
Replication	3	47817669	1.6E7	1.47	.2629	Rep * Genotype
Genotype	5	2.6E8	5.2E7	4.80	.0081	Rep * Genotype
Replication * Genotype	15	1.63E8	1.08E7	1.38	.2110	Residual
Mn Level	2	1.02E9	5.09E8	14.23	.0012	Genotype * Mn
Genotype * Mn Level	10	3.58E8	3.58E7	4.54	.0004	Residual
Residual	36	2.84E8	7.88E6			

Table B11. ANOVA table for overground biomass (g/plot) from Experiment 3.2, Table. 3.2 (field)

Source	df	Sum of ...	Mean Sq...	F-Value	P-V...	Error Term
Replication	3	2.23E6	7.43E5	4.42	.1205	REP * Genotype
Genotype	5	2.6E6	5.2E5	3.09	.0408	REP * Genotype
Replication * Genotype	15	2.5E6	1.68E5	3.52	.0010	Residual
Mn Level	2	1.28E7	6.4E6	36.72	.0001	Genotype * Mn
Genotype * Mn level	10	1.7E6	1.74E5	3.65	.0020	Residual
Residual	36	1.72E6	47716.29			

Table B12. ANOVA table for Mn uptake (mg/plot) from Experiment 3.2, Table. 3.2 (field)

Source	df	Sum of Sq...	Mean Sq...	F-Value	P-V...	Error Term
Replication	3	2.15E8	7.2E7	3.94	.1295	Rep * Genotype
Genotype	5	4.02E8	8.03E7	4.42	.0113	Rep * Genotype
Replication * Genotype	15	2.72E8	1.82E7	1.77	.0798	Residual
Mn Level	2	1.76E9	8.78E8	21.74	.0002	Genotype * Mn
Genotype * Mn Level	10	4.04E8	4.04E7	3.94	.0011	Residual
Residual	36	3.69E8	1.03E7			

Table B13. ANOVA table for development of chlorosis score (1-5) from Experiment 3.2, Table. 3.3 (controlled environment chamber)

Source	df	Sum of Squares	Mean Square	F-Value	P-V...
Replication	3	.22	.07	1.91	.1467
Genotype	5	6.60	1.32	34.08	.0001
Mn level	1	15.56	15.56	401.87	.0001
Genotype * Mn level	5	6.60	1.32	34.08	.0001
Residual	33	1.28	.04		

Table B14. ANOVA table for YEBs Mn concentration (mg/kg) from Experiment 3.2, Table. 3.3 (controlled environment chamber)

Source	df	Sum of Squares	Mean Square	F-Value	P-Val...
Replication	3	450.38	150.13	1.82	.1622
Genotype	5	2573.78	514.76	6.25	.0004
Mn level	1	39334.76	39334.76	477.55	.0001
Genotype * Mn level	5	1788.93	357.79	4.34	.0038
Residual	33	2718.13	82.37		

Table B15. ANOVA table for shoot Mn concentration (mg/kg) from Experiment 3.2, Table. 3.3 (controlled environment chamber)

Source	df	Sum of Squares	Mean Square	F-Value	P-V...
Replication	3	36.91	12.30	.49	.6908
Genotype	5	1363.47	272.69	10.89	.0001
Mn level	1	46743.84	46743.84	1866.31	.0001
Genotype * Mn level	5	916.84	183.37	7.32	.0001
Residual	33	826.52	25.05		

Table B16. ANOVA table for shoot dry weight (mg/pot) from Experiment 3.2, Table. 3.3 (controlled environment chamber)

Source	df	Sum of Squares	Mean Square	F-Value	P-V...
Replication	3	.01	3.10E-3	.55	.6507
Genotype	5	.24	.05	8.41	.0001
Mn level	1	1.25	1.25	223.01	.0001
Genotype * Mn level	5	.36	.07	12.89	.0001
Residual	33	.19	.01		

Table B17. ANOVA table for shoot Mn content (μg/pot) from Experiment 3.2, Table. 3.3 (controlled environment chamber)

Source	df	Sum of Squares	Mean Square	F-Value	P-V...
Replication	3	19.80	6.60	.89	.4581
Genotype	5	507.67	101.53	13.64	.0001
Mn level	1	20422.88	20422.88	2743.30	.0001
Genotype * Mn level	5	605.05	121.01	16.25	.0001
Residual	33	245.67	7.44		

Table B18. ANOVA table for relative shoot dry weight (%) from Experiment 3.2 (controlled environment chamber)

Source	df	Sum of Squares	Mean Square	F-Value	P-V...
Replication	3	149.07	49.69	.52	.6704
Genotype	5	5397.31	1079.46	11.33	.0001
Mn level	1	29192.01	29192.01	306.46	.0001
Gentype * Mn level	5	5397.31	1079.46	11.33	.0001
Residual	33	3143.38	95.25		

Table B19. ANOVA table for seedling Mn uptake (µg/pot) from Experiment 3.2, Table. 3.3 (controlled environment chamber)

Source	df	Sum of Squares	Mean Square	F-Value	P-V...
Replication	3	81.52	27.17	.38	.7715
Genotype	5	4486.00	897.20	12.38	.0001
Mn level	1	84599.78	84599.78	1167.76	.0001
Genotype * Mn level	5	6408.48	1281.70	17.69	.0001
Residual	33	2390.72	72.45		

Table C1. ANOVA table for shoot Mn content (μg/pot) from Experiment 3.3, Figure. 3.5 (controlled environment chamber)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	283.16	141.58	1.41	.2504
Genotype	18	7385.52	410.31	4.09	.0001
Mn Level	1	138728.92	138728.92	1382.57	.0001
Genotype * Mn Level	18	5931.31	329.52	3.28	.0002
Residual	74	7425.25	100.34		

Table C2. ANOVA table for shoot Mn concentration (mg/kg) from Experiment 3.3, Figure. 3.6 (controlled environment chamber)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	561.15	280.58	1.51	.2270
Genotype	18	5825.00	323.61	1.75	.0500
Mn Level	1	126992.88	126992.88	684.86	.0001
Genotype * Mn Level	18	5085.82	282.55	1.52	.1060
Residual	74	13721.73	185.43		

Table C3. ANOVA table for chlorosis score (1-5) from Experiment 3.3, Figure. 3.7 (controlled environment chamber)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	.02	.01	.29	.7515
Genotype	18	1.44	.08	2.21	.0093
Mn Level	1	.92	.92	25.38	.0001
Genotype * Mn Level	18	1.44	.08	2.21	.0093
Residual	74	2.69	.04		

Table D1. ANOVA table for shoot Mn content (μg/pot) from Experiment 4.3 (controlled environment chamber) and Fig 4.1

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	17.02	8.51	1.71	.1998
Genotype	2	227.60	113.80	22.82	.0001
Mn level	4	2725.24	681.31	136.60	.0001
Genotype * Mn level	8	110.44	13.80	2.77	.0218
Residual	28	139.66	4.99		

Table D2. ANOVA table for chlorosis score (1-5) from Experiment 4.3 (controlled environment chamber) and Fig 4.2

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	.03	.02	.85	.4388
Genotype	2	7.23	3.62	184.12	.0001
Mn level	4	9.51	2.38	121.02	.0001
Genotype * Mn level	8	2.60	.33	16.55	.0001
Residual	28	.55	.02		

Table D3. ANOVA table for shoot Mn content (μg/pot) from Experiment 4.3 (controlled environment chamber) and Fig 4.3

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	.10	.05	.02	.9774
Genotype	3	98.63	32.88	14.94	.0001
Mn level	1	677.81	677.81	308.08	.0001
Genotype * Mn level	3	44.29	14.76	6.71	.0049
Residual	14	30.80	2.20		

Table D4. ANOVA table for chlorosis score (1-5) from Experiment 4.3 (controlled environment chamber) and Fig 4.4

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	.03	.02	1.00	.3927
Genotype	3	3.01	1.00	65.11	.0001
Mn level	1	2.22	2.22	144.03	.0001
Genotype * Mn level	3	.11	.04	2.41	.1109
Residual	14	.22	.02		

Table E1. ANOVA table for shoot Mn content (μg/pot) from Experiment 5.2.2 (controlled environment chamber) and Fig 5.3

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Genotype	16	760.60	47.54	6.84	.0001
Mn level	1	4301.70	4301.70	618.97	.0001
Mn level*Genotype	15	491.40	30.71	4.42	.0001
Replication	2	5.64	2.82	.41	.6680
Residual	66	458.68	6.95		

Table E2. ANOVA table for chlorosis score (1-5) from Experiment 5.2.2 (controlled environment chamber) and Fig 5.3

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Genotype	15	3.58	.22	5.56	.0001
Mn level	1	10.81	10.81	268.68	.0001
Mn level * genotype	16	3.58	.22	5.56	.0001
Replication	2	.45	.22	5.56	.0059
Residual	66	2.65	.04		

Table E3. ANOVA table for shoot Mn content (μg/pot) from Experiment 5.3 (controlled environment chamber) and Fig 5.4

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	.51	.26	.01	.9875
Genotype	16	3085.72	192.86	9.46	.0001
Mn level	1	8890.75	8890.75	436.17	.0001
Genotype*Mn level	16	1171.60	73.22	3.59	.0001
Residual	66	1345.34	20.38		

Table E4. ANOVA table for shoot Mn concentration (mg/kg) from Experiment 5.3 (controlled environment chamber) and Fig 5.5

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	9.85	4.93	.10	.9078
Genotype	16	4334.70	270.92	5.33	.0001
Mn level	1	23929.84	23929.84	470.58	.0001
Genotype*Mn level	16	2211.14	138.20	2.72	.0023
Residual	66	3356.23	50.85		

Table E5. ANOVA table for shoot dry weight (g/pot) from Experiment 5.3 (controlled environment chamber) and Fig 5.6

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Replication	2	.01	.01	1.73	.1860
Genotype	16	1.03	.06	18.66	.0001
Mn level	1	.37	.37	105.62	.0001
Genotype*Mn level	16	.05	3.42E-3	.99	.4806
Residual	66	.23	3.47E-3		

Table E6. ANOVA table for root dry weight dry weight (g/pot) from Experiment 5.3 (controlled environment chamber) and Fig 5.7

Source	df	Sum of Squares	Me...	F-Value	P-Value
Replication	2	.02	.01	4.28	.1178
Genotype	16	1.06	.07	25.08	.0001
Mn level	1	.51	.51	191.26	.0001
Genotype*Mn level	16	.16	.01	3.85	.0001
Residual	66	.17	3E-3		

Appendix B

Correlation coefficient matrix tables

Correlation coefficient matrix was produced using Statview 4.02 statistical package.

Table 2.2.1D-I Correlation coefficient matrix between characters including chlorosis score (CS), youngest emerged blade Mn concentration (YEB), root dry weight (RDWT), root Mn concentration (RMnCn), root Mn content (RMnCt), shoot dry weight (StDWT), relative shoot dry weight (RSTDW), shoot Mn concentration (StMnCn), shoot Mn content (StMnCt), seedling dry weight (SDDWT) and seedling Mn uptake (SDUP); average of three durum wheat genotypes differing in Mn efficiency at 60, 90, 120, 160, 240 and 360 mg Mn/kg soil dry weight. The critical values of Spearman's rank correlation coefficient for n=9 observation at α=0.05 and α=0.01 are respectively 0.600 and 0.783.

Table 2.2.1D Correlation coefficient matrix table at Mn=60.00 mg/kg dry dry soil

Mn=60 mg/kg dry wt	CS	YEB	RDWT	RMnCn	RMnCt	StDWT	RStDW	StMnCn	StMnCt	SDDWT	SDUP
Chlorosis score (CS)	1	-.555	-.864	-.541	-.794	-.864	-.829	-.505	-.901	-.876	-.882
YEB	-.555	1	.336	.205	.245	.214	.490	.918	.704	.266	.460
Root dry weight (RDWT)	-.864	.336	1	.684	.944	.965	.961	.228	.808	.984	.932
Root Mn conc..(RMnCn)	-.541	.205	.684	1	.870	.728	.654	.275	.684	.721	.833
Root Mn content (RMnCt)	-.794	.245	.944	.870	1	.958	.885	.209	.805	.961	.965
Shoot dry weight (StDWT)	-.864	.214	.965	.728	.958	1	.896	.153	.792	.996	.934
Relative st d wt (RSTDW)	-.829	.490	.961	.654	.885	.896	1	.381	.856	.926	.917
Shoot Mn conc.(StMnCn)	-.505	.918	.228	.275	.209	.153	.381	1	.716	.189	.443
Shoot Mn cont (StMnCt)	-.901	.704	.808	.684	.805	.792	.856	.716	1	.810	.932
Seedling D wt (SDDWT)	-.876	.266	.984	.721	.961	.996	.926	.189	.810	1	.944
SeedlingUptake(SDUP)	-.882	.460	.932	.833	.965	.934	.917	.443	.932	.944	1

9 observations were used in this computation.

Table 2.2.1E Correlation coefficient matrix table at Mn=90.00 mg/kg dry dry soil

Mn=90 mg/kg dry soil

	CS	YEB	RDWT	RMnC	RMnCT	StDWT	RSTDW	StMnC	StMNCt	SDDWT	SDUP
Chlorosis score (CS)	•	•	•	•	•	•	•	•	•	•	•
YEB	•	1	.194	.503	.262	.299	.640	.719	.543	.260	.391
Root dry weight (RDWT)	•	.194	1	.777	.975	.967	.627	.590	.905	.988	.960
Root Mn conc..(RMnCn)	•	.503	.777	1	.890	.841	.602	.787	.899	.823	.908
Root Mn content (RMnCt)	•	.262	.975	.890	1	.969	.628	.665	.937	.979	.988
Shoot dry weight (StDWT)	•	.299	.967	.841	.969	1	.719	.580	.904	.995	.955
Relative st d wt (RSt DWT)	•	.640	.627	.602	.628	.719	1	.580	.722	.689	.679
Shoot Mn conc.(StMnCn)	•	.719	.590	.787	.665	.580	.580	1	.863	.589	.764
Shoot Mn cont (StMnCt)	•	.543	.905	.899	.937	.904	.722	.863	1.000	.911	.980
Seedling D wt (SDDWT)	•	.260	.988	.823	.979	.995	.689	.589	.911	1.000	.965
SeedlingUpta.(SDUP)	•	.391	.960	.908	.988	.955	.679	.764	.980	.965	1.000

9 observations were used in this computation.
A variable had a variance that was zero or missing.

Table 2.2.1F Correlation coefficient matrix table at Mn=120.00 mg/kg dry dry soil

Mn=120 mg/kg dry soil

	CS	YEB	RDWT	RMnC	RMnCt	StDWT	RSTDW	StMnCn	StMnCt	SDDWT	SDUP
Chlorosis score (CS)	•	•	•	•	•	•	•	•	•	•	•
YEB	•	1	-.333	.554	-.193	-.24	-.235	.904	.559	-.276	.144
Root dry weight (RDWT)	•	-.333	1	.183	.970	.951	.394	.038	.575	.985	.877
Root Mn conc..(RMnCn)	•	.554	.183	1	.395	.171	.436	.664	.600	.184	.530
Root Mn content (RMnCt)	•	-.193	.970	.395	1	.895	.418	.183	.657	.942	.935
Shoot dry weight (St DWT)	•	-.240	.951	.171	.895	1	.496	.098	.643	.989	.863
Relative st d wt (RSTDW)	•	-.235	.394	.436	.418	.496	1	-.073	.204	.448	.357
Shoot Mn con.(StMn Cn)	•	.904	.038	.664	.183	.098	-.073	1	.821	.085	.502
Shoot Mn cont (StMn Ct)	•	.559	.575	.600	.657	.643	.204	.821	1	.629	.882
Seedling D wt (SDDWT)	•	-.276	.985	.184	.942	.989	.448	.085	.629	1.000	.885
SeedlingUptake(SDUP)	•	.144	.877	.530	.935	.863	.357	.502	.882	.885	1.000

9 observations were used in this computation.
A variable had a variance that was zero or missing.

Table 2.2.1G Correlation coefficient matrix table at Mn=160.00 mg/kg dry dry soil

Mn=160 mg/kg dry soil

	CS	YEB	RDWT	RMnC	RMnct	StDWT	RSTDW	StMnCn	StMnCt	SDDWT	SDUP
Chlorosis score (CS)	.	.	.	.	.	.	.	.	.	.	.
YEB	.	1	.163	.769	.504	-.036	.136	.874	.561	.042	.560
Root dry weight (RDWT)	.	.163	1	.072	.901	.860	.678	.407	.683	.943	.806
Root Mn conc..(RMnCn)	.	.769	.072	1	.486	.024	.142	.724	.529	.044	.533
Root Mn content (RMnCt)	.	.504	.901	.486	1.000	.745	.675	.699	.839	.831	.944
Shoot dry weight (StDWT)	.	-.036	.860	.024	.745	1	.694	.373	.757	.981	.783
Relative st d wt (RSTDW)	.	.136	.678	.142	.675	.694	1	.407	.613	.716	.666
Shoot Mn conc.(StMnCn)	.	.874	.407	.724	.699	.373	.407	1	.879	.401	.837
Shoot Mn cont (StMn Ct)	.	.561	.683	.529	.839	.757	.613	.879	1.000	.756	.972
Seedling D wt (SDDWT)	.	.042	.943	.044	.831	.981	.716	.401	.756	1	.820
SeedlingUptake(SDUP)	.	.560	.806	.533	.944	.783	.666	.837	.972	.820	1.000

9 observations were used in this computation.
A variable had a variance that was zero or missing.

Table 2.2.1H Correlation coefficient matrix table at Mn=240.00 mg/kg dry dry soil

Mn=240 mg/kg dry soil

	CS	YEB	RDWT	RMnCn	RMnCt	StDWT	RSTDW	StMnCn	StMnCt	SDDWT	SDUP
Chlorosis score (CS)	.	.	.	.	.	.	.	.	.	.	.
YEB	.	1.000	-.598	.156	-.444	-.724	-.412	.370	-.173	-.692	-.324
Root dry weight (RDWT)	.	-.598	1	-.227	.731	.921	.154	.055	.734	.970	.768
Root Mn conc..(RMnCn)	.	.156	-.227	1	.483	-.273	-.493	.509	.182	-.256	.350
Root Mn content (RMnCt)	.	-.444	.731	.483	1	.616	-.198	.463	.816	.675	.954
Shoot dry weight (StDWT)	.	-.724	.921	-.273	.616	1	.176	-.200	.595	.988	.635
Relative st d wt (RSTDW)	.	-.412	.154	-.493	-.198	.176	1	-.636	-.366	.173	-.296
Shoot Mn conc.(StMnCn)	.	.370	.055	.509	.463	-.200	-.636	1	.654	-.107	.585
Shoot Mn cont (St Mn Ct)	.	-.173	.734	.182	.816	.595	-.366	.654	1	.657	.952
Seedling D wt (SDDWT)	.	-.692	.970	-.256	.675	.988	.173	-.107	.657	1	.699
SeedlingUptake(SDUP)	.	-.324	.768	.350	.954	.635	-.296	.585	.952	.699	1

9 observations were used in this computation.
A variable had a variance that was zero or missing.

Table 2.2.1I Correlation coefficient matrix table at Mn=360.00 mg/kg dry dry soil

Mn=360 mg/kg dry soil	CS	YEB	RDWT	RMnCn	RMnCt	StDWT	RSTDW	StMnCn	StMnCt	SDDWT	SDUP
Chlorosis score (CS)	•	•	•	•	•	•	•	•	•	•	•
YEB	•	1.000	-.555	.047	-.4	-.567	-.813	.110	-.332	-.553	-.383
Root dry weight (RDWT)	•	-.555	1	.736	.967	.992	.680	.488	.877	.997	.966
Root Mn conc..(RMnCn)	•	.047	.736	1	.856	.706	.222	.596	.699	.731	.814
Root Mn content (RMnCt)	•	-.400	.967	.856	1	.951	.584	.473	.824	.963	.955
Shoot dry weight (StDWT)	•	-.567	.992	.706	.951	1	.726	.425	.851	.998	.944
Relative st d wt (RSTDW)	•	-.813	.680	.222	.584	.726	1	-.046	.454	.700	.543
Shoot mn conc.(StMnCn)	•	.110	.488	.596	.473	.425	-.046	1	.819	.457	.676
Shoot Mn cont (StMnCt)	•	-.332	.877	.699	.824	.851	.454	.819	1	.864	.955
Seedling D wt (SDDWT)	•	-.553	.997	.731	.963	.998	.700	.457	.864	1	.957
SeedlingUptake(SDUP)	•	-.383	.966	.814	.955	.944	.543	.676	.955	.957	1.000

9 observations were used in this computation.
A variable had a variance that was zero or missing.

Appendix C

1 Material

1.1 Enzymes

1.1.1 Restriction Enzymes

Pst1	Promega, Madison, USA
Mse1	Promega, Madison, USA

1.1.2 Other Enzymes

RNase (ribonuclease)	Boehringer Mannheim
T4 DNA ligase	Boehringer, Mannheim, Promega
T4 DNA polymerase	New England Biolab, Schwalbach, Germany)
Tag DNA polymerase	Promega, Madison, USA

1.2 Nucleotides and radio nucleotides

Deoxynucleotide triphosphate (dNTP)	Promega, Madison, USA
$^{32}P\gamma$ ATP (10 µCi/µl)	Amersham, Braunscweig, Germany

1.3 Buffers and stock solutions

DNA extraction buffer: 1% sarkosyl, 0.1 M Tris-HCl (pH 8.5), 0.1 M NaCl, 0.01 M Na_2EDTA.

10xPCR buffer: 500 mM KCl, 200 mM Tris-HCl, 25 mM $MgCl_2$, 1 mg/ml BSA, pH 8.4.

R40: 40 µg DNase-free RNase A/ml 1x TE buffer.

10x RL buffer: 100 mM trisHAc, 100 mM MgAc, 500 mM KAc and 50 mM DDT (pH 8.5).

1x TE buffer: 10 mM Tris-HCl (pH 8.0), 1 mM Na_2EDTA.

1.4 Abbreviations of chemicals

ASP	ammonium per sulphate
ATP	adenosine 5'-triphosphate
dNTPs	2'-deoxy ribonucleoside 5'-triphosphates
DNA	deoxyribonucleic acid
DTT	dithiothreitol
RNase	ribonuclease
TE	Tris-EDTA buffer
TEMED	N,N,N',N'-tetramethylethylenediamine
Tris	tris[hydroxymethy]amino methane

References

Akerman, S., Tammisola, J., Regina, M., Kauppinen, V., and Lapinjoki, S. (1996) Segregation of AFLP markers in *Betula pendula* (Roth). *Forest. Genet.* **3**, 117-123.

Asher, J. S., and Graham, R. D. (1993) Agronomic value of seed with high nutrient content. In *Wheat in heat-stressed enviroments: Irrigated, dry areas and rice-wheat Farming systems* (Edited by D. A. Saunders, & G. P. Hettel), pp. 297-308. UNDP/ARC/BARI/CIMMYT, Dinajpur, Bangladesh.

Bagheri, A. (1994) Boron tolerance in grain legumes with particular reference to genetics of boron tolerance in peas. PhD Thesis, The University of Adelaide,

Bartlett, R. J. (1988) Manganese redox reactions and organic interactios in soils. In *Manganese in soils and plants* (Edited by R. D. Graham, R. J. Hannam, & N. C. Uren), pp. 59-74. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Becker, J., Vos, P., Kuiper, M., Salamini, F., and Heun, M. (1995) Combined mapping of AFLP and RFLP markers in barley. *Mol. Gen. Genet.* **249**, 65-73.

Bozzini, A. (1988) Origin, distribution and production of durum wheat. In *Durum wheat: chemistry and technology* (Edited by G. Fabriani, & C. Lintas), pp. 1-14. American Association of Cereal Chemist (AACC), St. Paul, Minnesota, USA.

Brajcich, P., Pfeiffer, W., and Autrique, E. (1986). Durum Wheat. Names; Parentage; Pedigrees and Origins . The International Maize and Wheat Improvement Center (CIMMYT).

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

Brooks, B. J. (1993). Durum wheat, Annual Report 1993 . Waite Agricultural Research Institute, Adelaide, South Australia.

Brooks, B. J., Rathjen, A. J., and McDonald, G. K. (1994) Durum wheat and breeding in South Australia. In *7th Australian Wheat Assembly* (Edited by J. G. Paull, I. S. Dundas, K. W. Shepherd and G. J. Hollamby), pp. 107-110. The Unversity of Adelaide, Adelaide.

Burnell, James. N. (1988) The biochemistry of manganese in plants. In *Manganese in soils and plants* (Edited by R. D. Graham, R. J. Hannam, & N. C. Uren), pp. 125-133. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Cakmak, I., Ozturk, L., Karanlik, S., Marschner, H., and Ekiz, H. (1996) Zinc-efficient wild grasses enhance release of phytosiderophores under zinc deficiency. *Plant Nutr.* **19**, 551-563.

Campbell, L. C., and Nable, R. O. (1988) Physiological functions of manganese in plants. In *Manganese in soils and Plants* (Edited by R. D. Graham, R. J. Hannam, & N. C. Uren), pp. 139-149. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Cervera, M. T., Gusmao, J., Steenackers, M., Peleman, J., Storme, V., Vanden Broeck, A., Van Montagu, M., and Boerjan, W. (1996) Identification of AFLP molecular markers for resistance against *Melampsora larici-populina* in Populus. *Theor. Appl. gent.* **93**, 733-737.

Chantachume, Y. (1995) Genetic studies on the tolerance of wheat to high concentration of boron. PhD Thesis, The University of Adelaide,

Cho, Y. G., Blair, M. W., Panaud, O., and McCouch, S. R. (1996) Cloning and mapping of variety-specific rice genomic DNA sequence: amplified fragment lenght polymorphism (AFLP) from silver-stained polyacrylamide gels. *Gen* **39**, 373-378.

Cooper, L. (1997). 1996-97 Annual Report. . Durum wheat Growers Association of South Australia Inc.

Dudal, R. (1976) Inventory of the major soils of the world with special reference to mineral stress hazards. In *Plant adaptation to mineral stress in problem soils* (Edited by M. Wright), Ithaca, The Cornell University.

Elstner, E. F. (1982) Oxygen activation and oxygen toxicity. *Annu. Rev. Plant Physiol.* **33**, 73-96.

Fales, S. L., and Ohki, K. (1982) Manganese deficiency in wheat: influence on growth and forage quality of herbage. *Agron. J.* **74**, 1070-1073.

Fox, P. N., Magana, R. I., Lopez, C., Sanches, H., Herrera, R., Vicarte, V., White, J. W., Skovmand, B., and Makay, M. C. (1998) The International Wheat Information System, IWIS, Version 2 on CD. CIMMYT, Mexico.

Foy, C. D., and Da Silva, A. R. (1991) Tolerance of wheat germplasm to acid subsoil. *J. Plant Nutr.* **14**, 1277-1295.

Gale, M. D., Sharp, P. J., Chao, S., and Law, C. N. (1989) Application of genetic markers in cytogenetic manipulation of wheat genome. *Gen.* **31**, 137-142.

Gilkes, R. J., and McKenzie, R. M. (1988) Geochemistry and mineralogy of manganese in soil. In *Manganese in soils and plants* (Edited by R. D. Graham, R. J. Hannam, & N. C. Uren), pp. 23-35. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Glenn, J. K., Akileswaran, L., and Gold, M. H. (1986) Mn (II) oxidation is the principal function of the extracellular Mn-peroxidase from *phanerochaete chrysosporium. Arch. Bochem. Biophys.* **251**, 688-696.

Graham, M. J., Nickell, C. D., and Hoeft, R. G. (1995) Inheritance of tolerance to manganese deficiency in soybean. *Crop Sci.* **35**, 1007-1010.

Graham, R. D. (1980) Susceptibility to powdery mildew of wheat plants deficient in copper. *Plant Soil* **56**, 181-185.

Graham, R. D. (1984) Breeding for nutritional charactristics in cereals. *Adv. plant nutr.* **1**, 57-102.

Graham, R. D. (1987). Development of wheats with enhanced nutrient efficiency: progress and potential. In (A. Klatt), CIMMYT/UNDP. Chiang Mai, Thailand. 305-320.

Graham, R. D. (1988) Genotypic differences in tolerance to manganese deficiency. In *Manganese in soils and plants* (Edited by R. D. Graham, R. J. Hannam, & N. C. Uren), pp. 261-276. Kluwer Acdemic Publishers, Dordrecht, The Netherlands.

Graham, R. D., Ascher, J. S., and Hynes, S. C. (1992) Selecting zinc-efficient cereal genotypes for soil of low zinc status. *Plant Soil* **146**, 1-2.

Graham, R. D., Davies, W. J., and Asher, J. S. (1985) The critical concentration of manganese in field-grown wheat. *Aust. J. Agric. Res.* **36**, 155-169.

Graham, R. D., Davies, W. J., Sparrow, D. H. B., and Ascher, J. S. (1983) Tolerance of barley and other cereals to manganese-deficient calcareous soils of South Australia. In *Genetic aspects of plant nutrition* (Edited by M. R. Saric, & B. C. Loughman), pp. 339-345. Martinus Nijhoff/Dr W Junk., Hague, The Netherlands.

Guesella, J. F. (1986) DNA polymorphism and human disease. *Ann. Rev. Biochem.* **55**, 831-854.

Hannam, D. P., Riggs, J. L. , and Graham, R. D. (1987) The critical concentration of manganese in barley. *J. Plant Nutr.* **10**, 2039-2048.

Hannam, R. J., Graham, R. D., and Riggs, J. L. (1985) Redistribution of manganesc in maturing *Lupinus angustifolius cv.* Illyarrie in relation to level of previous accumulation. *Ann. Bot.* **56**, 821-834.

Hannam, R. J., and Ohki, K. (1988) Detection of manganese deficiency and toxicity in plants. In *Manganese in soils and plants* (Edited by R. D. Graham, R. J. Hannam, & N. C. Uren), pp. 243-259. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Harbard, J. L. (1992) Mechanism of manganese efficiency in barley (*Horedeum vulgare*). M. Ag. Sc. Thesis, The University of Adelaide, South Australia,

Hart, G. E. (1994) RFLP maps of bread wheat. In *DNA-Based markers in Plant* (Edited by R. L. Phillips, & I. K. Vasil), Kluwer Academic Publishers., The Netherlands.

Heenan, D. P., and Campbell, L. C. (1980a) Growth, yield components and seed composition of two soybean cultivars as affected by manganese supply. *Aust. J. Agric. Res.* **31**, 471-476.

Hill, M., Witsenboer, H., Zabeau, M., and Vos, P. (1996) PCR-based fingerprinting using AFLPs as a tool for studying genetic relationship in *Lactuca spp. Theor. Appl. Genet.* **93**, 1202-1210.

Huang, C. (1996) Mechanism of Mn efficiency in barley. PhD, The University of Adelaide, Adelaide, South Australia,

Huang, C., Webb, M. J., and Graham, R. D. (1994) Manganese efficiency is expressed in barley growing in soil system but not in solution culture. *J. Plant Nutr.* **17**, 83-95.

Huang, C., Webb, M. J., Graham, R. D., and Huang, C. Y. (1996) Pot size affects expression of Mn efficiency in barley. *Plant Soil* **178**, 205-208.

Islam, A., Edwards, G., and Asher, C. J. (1980) pH optima for crop growth, The results of a flowing culture experiment with six species. *Plant. Soil* **54**, 339-357.

Jamjod, S. (1996) Genetic studies on the tolerances of durum wheat to high concentration of boron. PhD Thesis, The university of Adelaide, Adelaide, South Australia.

Jenkin, M. J. (1993) The genetics of boron tolerance in barley. PhD Thesis, The University of Adelaide.

Jenkyn, J. F., and Bainbridge, A. (1978) Biology and pathology of cereal powdery mildews. In *The powdery mildews* (Edited by D. M. Spencer), pp. 283-321. Academic Press, London.

Joppa, L. R. (1987) Aneuploid Analysis in tetraploid wheat. In *Wheat and wheat improvement* (Edited by E. G. Heyne), pp. 255-268. ASA, CSSA and SSSA Publishers, Madison, Wisconsin, USA.

Joppa, L. R., and Williams, N. D. (1983a) The Langdon durum D-genome disomic substitutions: Development characteristics and uses. *Agron. Abstr. American Society of agronomy, Madison, WI, p. 68.*

Joppa, L. R., and Williams, N. D. (1983b) Chromosomal location of genes for gliadin polypeptides in durum wheat (*Triticum Turgidum* L.). *Theor. Appl. Genet.* **64**, 289-293.

Joppa, L. R., and Williams, N. D. (1988) Genetics and breeding of durum wheat in the United States. In *Durum wheat: chemistry and technology* (Edited by G. Fabriani, & C. Lintas), pp. 47-92. American Society of Cereal Chemist (ASCC), St. Paul, Minnesota, USA.

Kaur, N. P., Arora, C. L., Sadana, U. S., and Chark, K. S. (1991). Effect of manganese stress on the reproductive phase of wheat. In (K. Dhir, I. Dua, & K. S. Chark), India.

Kaur, N. P., Takkar, P. N., and Nayyar, V. K. (1989) Some physiological studies on differential susceptibility of wheat and triticale to manganese deficiency. *Ann. Biol. Lud.* **5**, 115-122.

Keim, P., Schupp, JM., Travis, SE., Clayton, K., Zhu, T., Shi, L., Ferreira, A., Webb, DM., Zhu, T., and Shi, LA. (1997) A high-density soybean genetic map based on AFLP markers. *Crop. Sci.* **37**, 537-543.

Kimber, G., and Sears, E. R. (1987) Evolution in the genus *Triticum* and the origin of cultivated wheat. In *Wheat and wheat improvement* (Edited by E. G. Heyne), pp. 154-164. ASA, CSSA, SSSA Publisher, Madison, Wisconsin, USA.

King, P. M., McCloud, P. I., Jacka, J., and Graham, R. D. (1992) Crop and pature ratations at Coonalpyn, South Australia: Effect on nutrients in wheat and barley. *Agron Crop Sci.* **168**, 37-48.

Knight, R. (1973) The relation between hybrid vigour and genotype-enviroment interaction. *Theor. appl. Genet.* **43**, 311-318.

Langridge, P. (1994) Molecular markers in wheat breeding and research. In *7th Assembly Wheat Breeding Society of Australia* (Edited by J. G. Paull, I. S. Dundas, & K. W. Shepherd), pp. 137-140. The University of Adelaide, Adelaide.

Langridge, p., Karakousis, A., and Nield, J. (1996). Course Manual of Practical Workshop in Basic Recombinant DNA Techniques Application to Plant Breeding. Centre for Cereal Biotechnology, Waite Agriculture Research Institute, The University of Adelaide, Adelaide, South Australia.

Large, E. C. (1954) Growth stages in cereals. *Plant path.* **3**, 128-129.

Law, C. N., Snape, J. W., and Worland, A, J. (1987) Aneuploidy in wheat and its uses in genetic analysis. In *Wheat Breeding* (Edited by F. G. H. Lupton), pp. 71-108. Chapman and Hall, London.

Lin, J., Kuo, J., Ma, J., Saunders, JA., Beard, HS., and MacDonald, MH. (1996) Identification of molecular markers in soybean comparing RFLP, RAPD and AFLP DNA mapping techniques. *Plant Mol. Biol. Rept.* **14**, 156-169.

Loneragan, J. F. (1968) Nutrient requirements of plants. *Nature Land* **220**, 1307-1308.

Loneragan, J. F. (1988) Distribution and movement of manganese in plants. In *Manganese in soils and plants* (Edited by R. D. Graham, R. J. Hannam, & N. C. Uren), pp. 113-124. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Longnecker, N. E., Graham, R. D., and Card, G. (1991a) Effects of manganese deficiency on the pattern of tillering and development of barley (*Hordeum vulgare* cv. *Galleon*). *Field Crps. Res.* **28**, 1-2.

Longnecker, N. E., Graham, R. D., McCarthy, K. W., Sparrow, D. H. B., and Egan, J. P. (1988) Screening for manganese efficiency in barley (*Hordeum vulgare* L.). In *3rd Int. Symposium of genetic aspects of plant mineral nutrition* (Edited by N. E. Bassam, M. Dambroth, & B. C. Loughman), pp. 273-280. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Longnecker, N. E., Marcar, N. E., and Graham, R. D. (1991b) Increased manganese content of barley seeds can increase grain yield in manganese-deficient conditions. *Aust. J. Agric. Res.* **42**, 1065-1074.

Mackill, D. J., Zhang, Z., Redona, E. D., and Colowit, P. M. (1996) Level of polymorphism and gentic mapping of AFLP markers in rice. *Genome* **39**, 969-976.

Marcar, N. E., and Graham, R. D. (1986) Effect of seed manganese content on the growth of wheat (*Triticum aestivum*) under manganese deficiency. *Plant Soil* **96**, 165-173.

Marcar, N. E., and Graham, R. D. (1987) Genotypic variation for manganese efficiency in wheat. *J. Plant Nutr.* **10**, 9-16.

Marino, C. L., Nelson, J. C., Lu, Y.H., Sorrells, M. E., Leroy, P., Tuleen, N. A., Lopes, C. R., and Hart, G. E. (1996) Molecular genetic maps of the group 6 chromosomes of hexaploid wheat (*Triticum aestivum* L. em. Thell). *Genome* **39**, 369-366.

Marschener, P., Asher, J. S., and Graham, R. D. (1991) Effect of manganese-reducing rhizosphere bacteria on the growth of *Gaeumannomyces graminis* var. *tritici* and on manganese uptake by wheat (*Triticumaestivum* L.). *Biol. Fert. Soils* **12**, 33-38.

Marschner, H. (1988) Mechanism of manganese acquisition by roots from soils. In *Manganese in plants and soils* (Edited by R. D. Graham, R. J. Hannam, & N. C. Uren), pp. 191-201. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Marschner, H. (1995) Manganese. In *Mineral Nutrition of Higher Plants* (Edited by H. Marschner), pp. 324-333. Academic Press Limited, London.

Mather, K., and Jinks, J. L. (1977) *Introduction to biometrical genetics* London, UK.: Chapman and Hall.

Maughan, P. J., Saghai Maroof, M. A., and Buss, G. R. (1996) Amplified fragment lenght polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. *Theor. Appl. Genet.* **93**, 392-401.

McCarthy, K. W., Longnecker, N., Sparrow, D. H. B., and Graham, R. David. (1988). Inheritance of manganese efficiency in barley (*Horedeum vulgare* L.). In (M. J. Webb, R. O. Nable, R. D. Graham, & R. J. Hannam), Manganese Symposium 1988 INC., Adelaide, South Australia. Adelaide, South Australia. 121-123.

McIntosh, R. A. (1987) Gene location and gene mapping in hexaploid wheat. In *Wheat and Wheat Improvement* (Edited by E. G. Heyne), pp. 269-288. Amer. Soc. Agron. Inc. Publ, Madison, Wiconsin, USA.

Michelmore, R. W., Paran, I., and Kesseli, R. V. (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA.* **88**, 9828-9832.

Milbourne, D., Meyer, R., Bradshaw, J. E., Baird, E., Bonar, N., Provan, J., Powell, W., and Waugh, R. (1997) Comparison of PCR-based marker system for analysis of genetic relationship in cultivated potato. *Mol. Breed.* **3**, 127-136.

Moody, D. B., Rathjen, A. J., Cartwright, B., Paull, J. G., and Lewis, J. (1988). Genetic diversity and geographical distribution of tolerance to high levels of soil boron. In (T. E. Miller, & R. M. D. Koebner), Cambridge Laboratory, IPSR, Cambridge.

Moore, G., Gale, M. D., Kurata, N., and Flavell, R. B. (1993) Molecular analysis of small grain cereal genome: current status and prospects. *Bio. Technol.* **11**, 584-589.

Moroni, J. S., Briggs, K. G., and Taylor, G. J. (1991) Pedigree analysis of the origin of manganese tolerance in Canadian spring wheat (*Triticum aestivum* L.) cultivars. *Euphytica* **56**, 107-120.

Munns, D. N., Johnson, C. M., and Jacobson, L. (1963) Uptake and distribution of manganese in oat plants. II. Kinetic model. *Plant Soil* **19**, 193-204.

Murata, R. (1991) Cytogenetic change during seed storage. In *Chromosome Engineering in Plants: Breeding, Evolution* (Edited by P. K. Gupta, & T. Tsuchiya), pp. 211-228. Elsevier Science Publishers, The Netherland.

Nable, R. O., Bar, Akiva A., and Loneragan, J. F. (1984) Functional manganese requirement and its use as a critical value for diagnosis of manganese deficiency in subterranean clover (*Trifolium subterraneum* L. cv. *Seaton Park*). *Ann. Bot.* **54**, 39-49.

Nable, R. O., and Loneragan, J. F. (1984a) Tranlocation of manganese in subterranean clover (*Trifolium subterraneum* L. cv. *seaton park*). I. Redistribution during vegetative growth. *Aust. J. Plant Physiol.* **11**, 101-111.

Nable, R. O., and Loneragan, J. F. (1984b) Translocation of manganese in subterranean clover (*Trifolium subterraneum* L. cv. *Seaton Park*). II. Effect of leaf senescence and of restricting supply of manganese to part of a split root system. *Aust. J. Plant Physiol.* **11**, 1-2.

Nachit, M. M. (1998) Network for integrated research to improve durum wheat productivity in the mediterranean region. In *Wheat: Prospects for Global Improvement (5th International Wheat Conference)* (Edited by H. J. Braun, F. Altay, W. E. Kronstad, S. P. S. Beniwal, & A. McNab), Kluwer Academic Publishers, Dordrecht, The Netherlands.

Norvell, W. A. (1988) Inorganic reaction of manganese in soils. In *Manganese in plants and soils.* (Edited by R. D. Graham, R. J. Hannam, & N. C. Uren), pp. 37-58. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Pakniyat, H., Powel, W., Baird, E., Handley, LL., Robinson, D., and Scrimgeour, CM. (1997) AFLP variation in wild barely (*Horedeum spontaneum C. Koch*) with reference to salt tolerance and associated ecogeography. *Genome* **40**, 332-341.

Paul, S., Wachira, FN., Powel, W., and Waugh, R. (1997) Diversity and genetic differentiation among populations of Indian and Kenya tea (*Camellia sinesis* (L. *O. Kuntze*). *Theor. Appl. Gent.* **94**, 255-263.

Paull, J. G. (1990) Genetic studies on the tolerance of wheat to high concentration of boron. PhD Thesis, The University of Adelaide, South Australia.

Paull, J. G., Nable, R. O., and Rathjen, A. J. (1992) Physiological and genetic control of the tolerance of wheat to high concentration of boron and the implication for plant breeding. *Plant Soil* **146**, 131-146.

Pearson, J, N., and Rengel, Z. (1995a) Uptake and distribution of ^{65}Zn and ^{54}Mn in wheat grown at sufficient and deficient levels of Zn and Mn
I. During vegetative growth. *J. Exp. Bot.* **46**, 833-839.

Pearson, J. N., Jenner, C. F., Rengel, Z., and Graham, R. D. (1996) Differential transport of Zn, Mn and sucrose along the longitudinal axis of developing wheat grains. *Physiol. Planta.* **97**, 332-338.

Pearson, N. P., and Rengel, Z. (1995b) Uptake and distribution of ^{65}Zn and ^{54} Mn in wheat grown at sufficient and deficient levels of Zn and Mn
II. During grain devlopment. *J. Exp. Bot.* **46**, 841-845.

Powell, W., Thomas, WTB., Baird, E., Lawrence, P., Booth, A., Harrower, B., McNicol, JW., and Waugh, R. (1997) Analysis of quantitative traits in barley by the use of amplified fragment lenght polymorphism. *Hered.* **79**, 48-49.

Prince, R. C. (1986) Manganese at active site of chloroplast oxygen-evolving complex. *Tends. Biochem. Sci.* **132**, 491-492.

Reisenauer, H. M. (1988) Determination of plant-available soil manganese. In *Manganese in soils and plants* (Edited by R. D. Graham, R. J. Hannam, & N. C. Uren), pp. 87-98. Kluwer Academic Publisher, Dordrecht, The Netherlands.

Rengel, Z. (1997) Root exudation and microflora population in rhizosphere of crop genotypes differing in tolerance to micronutrient deficiency. In *Plant Nutrition for sustainable food production and enviroment* (Edited by T. Ando, K. Fujita, T. Mae, H. Matsumoto, S. Mori, & J. Sekiya), pp. 242-248. Kluwer Academic Publishers, Tokyo, Japan.

Reuter, D. J., Alston, A. M., and McFarlane, J. M. (1988b) Occurrance and Correction of Manganese Deficiency in Plants. In *Manganese in Soils and Plants* (Edited by R. D. Graham, N. C. Hannam, & N. C. Uren), pp. 205-224. Kluwer Academic Publisher, Dordrecht, The Netherlands.

Reuter, D. J., Cartwright, B., Judson, G. J., Mcfarlane, J. D. , Maschmedt, D. J., and Robinson, J. B. (1988a). Trace Elements in South Australian Agriculture (139). South Australia. Dep. Agric.

Reuter, D. J., and Robinson, J. B. (1986) *Plant analysis. An intepretation manual* Melbourne: Inkata Press.

Robson, A. D., and Snowball, K. (1986) Nutrient deficiency and toxicity symptoms. In *Plant analysis-An interpretation manual* (Edited by D. J. Reuter, & J. B. Robinson), pp. 13-19. Inkata Press, Melborne.

Samuel, G., and Piper, C. S. (1928). *J. Dep. Agric. S. Aust.* 31, 696, 789.

Schondelmaier, J., Steinrucken, G., and Jung, C. (1996) Integration of AFLP markers into map of sugar beet (*Beta vulgaris* L.). *Plant Breed.* **115**, 231-237.

Sears, E. R. (1966) Nullisomic-tetrasomic combination in heaxploid wheat. In *Chromosome manipulation and plant genetics* (Edited by R. Riley, & K. R. Lewis), Oliver and Boyd, London.

Sensi, E., Vignani, R., Rohde, W., and Biriclti, S. (1996) Characterization of genetic biodiversity with *Vitis vinifera* L. Sangiovese and Colorino genotypes by AFLP and ISTR DNA marker technology. *Vitis.* **35**, 183-188.

Shah, S. H., Gorham, J., Foster, B. P., and Wyn Jones, R. G. (1987) Salt tolerance in triticeae: the contribution of the D genome to cation selectivity in hexaploid wheat. *J. Exp. Bot.* **38**, 254-269.

Sharma, P. N. (1992) Pollen fertility in manganese-deficient wheat. *Trop. Agric.* **69**, 21-24.

Sharma, SK., Knox, MR., and Ellis, THN. (1996) AFLP analysis of diversity and phylogeny of *Lens* and its comparison with RAPD analysis. *Theor. Appl. Genet.* **93**, 751-758.

Sharpe, N. (1993). Durum wheat - A specific market. In Proc: 10th Australian Agronomy Conference pp. 40-42. (Edited by G. K. McDonald and W. D. Belloti.), The University of Adelaide, Adelaide, South Australia.

Simpson, D. J., and Robinson, S. R. (1984) Freeze-fracture ultrastracture of thylakoid membrane in chloroplast from manganese-deficient plants. *Plant. Physiol.* **74**, 735-741.

Singh, D. K., and Bharti, S. (1985) Seed manganese content and its relationship with growth characteristics of wheat cultivars. *New Phytol.* **101**, 387-391.

Sparrow, D. H. B., Graham, R. D., Asher, J. S., and Davies, W. J. (1983) Breeding barley tolerant to manganese deficiency. Proc: Aust Plant Breed Conf., Adelaide, South Australia.

Tohme, J., Gonzalez, DO., Beebe, S., and Duque, Mc. (1996) AFLP analysis of gene pool of a wild bean core collection. *Crop Sci.* **36**, 1375-1384.

Uren, N. C., Asher, C. J., and Longnecker, N. E. (1988) Techniques for research on manganese in soil-plant systems. In *Manganese in soils and plants* (Edited by R. D. Graham, R. J. Hannam, & N. C. Uren), pp. 309-328. Kluwer Academic Publisher, Dordrecht, The Netherlands.

Vogel, JM., Rafalski, A., Powell, W., Morgante, M., Andre, C., Hanafey, M., and Tingey, SV. (1996) Application of genetic diagnostics to plant genome and plant breeding. *Hort. Sci.* **31**, 1107-1108.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T. V. D., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. (1995) AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res.* **23**, 4407-4414.

Vose, P. B. (1990) Screening techniques for plant nutrient efficiency: Philosophy and methods. In *Genetic aspects of plant mineral nutrition* (Edited by N. El Bassam, M. Dambroth, & B. C. Loughman), pp. 283-289. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Walter, K. H. (1988) Manganese fertilizers. In *Manganese in soils and plants* (Edited by R. D. Graham, R. J. Hannam, & N. C. Uren), pp. 225-241. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Ware, G. O., Ohki, K., and Moon, L. C. (1982) The Mitscherlich plant growth model for determining critical nutrient deficiency levels. *Agron. J.* **74**, 88-91.

Webb, A. J., and Dell, B. (1990) Effect of manganese supply on development of wheat (*Triticum aestivum*) roots. In *Plant nutrition - physiology and applications* (Edited by M. L. Van Beusichem), pp. 235-239. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Wilhelm, N. S., Fisher, J. M., and Graham, R. D. (1985) The effect of manganese deficiency and cereal cyst nematode infection on the growth of barley. *Plant Soil* **85**, 23-32.

Wilhelm, N. S., Graham, R. D., and Rovira, A. D. (1988) Application of different sources of manganese sulfate decreases take-all (*Gaeumannomyces graminis* var. *tritici*) of wheat grown in a manganese deficient soil. *Aust. J. Agric. Res.* **39**, 1-10.

Wilhelm, N. S., Graham, R. D., and Rovira, A. D. (1990) Control of Mn status and infection rate by genotype of host and pathogen in wheat take-all interaction. *Plant Soil* **123**, 267-275.

Wilson, D. O., Boswell, F. C., Ohki, K., Parker, M. B., Shurman, L. M., and Jellum, M. D. (1982) Changes in soybean seed oil and protein as influenced by manganese nutrition. *Crop Sci.* **22**, 948-952.

Yau, S. K., Nachit, M. M., Ryan, J., and Hamblin, J. (1995) Phenotypic variation in boron-toxicity tolerance at seedling stage in durum wheat (*Triticum durum*). *Euphytica* **185**, 185-191.

Zabeau, M., and Vos, P. (1993) Selective restriction fragment amplification: a general method for DNA fingerprinting. *European Patent Application 9240629.7; Publication number EP 0534858 Al*

Zarcinas, B. A., Cartwright, B., and Spouncer, L. R. (1987) Nitric acid digestion and multi element analysis of plant material by inductively coupled plasma spectrometry. *Commun. Soil Plant Anal.* **18**, 131-136.