BREEDING MAIZE FOR STRESS TOLERANCE

by

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CONTENTS

Abstract

A considerable amount of variability in salinity tolerance has been found among 72 accessions of maize based upon the root growth response of 10-day old seedlings grown in salinised solutions. The NOPT models of van Genuchten and Hoffman (1984), were effective for quantifying salinity tolerance by Ct, the concentration at which root growth begins to decrease, the concentration causing a 50% decrease in root length (C50), and the concentration causing zero root growth (C0). From these data selecting individuals with high Ct and C50 would be a useful means of quantifying salinity tolerance. Moderately high to high estimates of heritability indicated that this variability has a strong genetic basis.

North Carolina design II (NCM II) and Triple Test Cross (TTC) analyses were carried out to estimate the components of genetic variation in maize accessions. Additive and non-additive effects were found to be involved in the inheritance of salinity tolerance at the seedling stage. Analysis of the root length data of TTC progenies revealed epistatic effects, with some indication of dominance towards tolerance. This type of gene action may be exploited with advantage in maize, a cross-pollinated crop, for enhancing salinity tolerance.

Tolerance to aluminium and manganese toxicity at the seedling stage for another set of 72 maize accessions was also examined in solution culture. Of the 72 accessions 50 were those assessed for NaCl tolerance. 0.22 mM Al and 2.0 mM Mn gave better separation of aluminium and manganese tolerance and susceptibility assessed on the basis respectively of relative root length, and leaf chlorosis and necrosis. There was considerable variability among accessions in aluminium and manganese tolerance. When grown in Al and Mn containing solution, root growth was greater than when the same accessions were grown in Al alone at the same concentration. Accessions showing tolerance to aluminium, did not necessarily show tolerance to manganese, suggesting that different mechanisms control tolerance to these two metals.

The data, root length at 0.11 mM Al, and leaf chlorosis and necrosis at 1.0 mM Mn, when analysed using NCM II procedure, suggested some degree of cytoplasmic inheritance for aluminium tolerance. Additive and dominance effects were involved in the inheritance of aluminium and manganese tolerance, there being for Al and Mn tolerance a greater proportion of dominance effects.

Both aluminium and/or manganese tolerant and non-tolerant accessions accumulated more organic metabolites, amino acids, proline, and carbohydrates, under aluminium and manganese stress than in control conditions. The tolerant accessions accumulated more solutes than the non-tolerant accessions.

Even using a limited number of primers, RAPD patterns generated through PCR amplifications of an F_2 population of a cross between two salt tolerant accessions revealed considerable polymorphism. RAPD was very sensitive to amplification conditions, and it was very difficult to reproduce the patterns.

The conclusion from the study is that heritable genetic variation for tolerance to salinity, Al, and Mn exists in maize, and the production of plants with increased tolerance to these stresses is feasible through selection and breeding.

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General Introduction

Chapter 1

CHAPTER 1

General Introduction

Stress tolerance is the principal keyword of this thesis. The term stress has been derived from the Latin word *stringere*, which means constraining or impelling force. The term is being used with diverse meanings in physical, medical, and biological sciences. In plant sciences, several definitions of the term stress have been forwarded, but in most, it is considered to mean a significant deviation from the conditions optimal for plant growth. In order to accommodate its diverse forms, Grime, (1979) defined stress as, "the external constraints which limit the rate of dry matter production of all or part of the vegetation below its genetic potential". To conform to the agriculture systems, Jones and Jones (1989) modified this simple concept and used economic yield rather than dry matter production, though the two quantities are often closely related.

Generally, plants are considered to be under stress when they experience a relatively severe shortage of an essential constituent or an excess of potentially toxic or damaging substance. Plants undergoing the latter situation usually face the former situation as well. Studies regarding the responses of plants to an excess of NaCl, Al, and Mn, including some aspects of their genetic, physiological, and molecular basis, forms the subject matter for this thesis.

Only about 10% of the world's arable land may be classified in a non-stress category (Dudal, 1976). According to the more recent estimates, made by United Nation Environment Programme, world wide, 1035×10^6 ha, out of a total of 5169×10^6 ha dry land is affected by chemical degradation (UNEP, 1992).

The world population of 5,000 million in 1990 is expected to reach 8,000 million by the year 2020. Populations of less-developed countries, presently totalling 3,700 million, will reach 6700 million by 2020. In the coming 30 years the world population is expected to increase by 2.6 billion people, 97 percent of whom will live in the developing world (FAO, 1998). The growth rate of world agriculture production, on the other hand, is decreasing. It was 3% per year in the 1960s, 2.3% in the 1970s, 2% in 1980-1992 and 1.8% in 1996 (FAO, 1998). The required rate of annual agriculture growth is, however, 3% to meet the food demand of the increasing population (IRRI, 1993). It implies that food production will be put under greater pressure in the future, with a resultant need to increase the productivity of both good and marginal arable land, especially in developing countries.

World crop production is limited by environmental stresses, and abiotic soil stresses such as soil salinity, drought, and soil acidity will continue to be problems for plant productivity throughout the world (Clark and Duncan, 1993). Strategies have to be found to produce food, animal feed, and fibre within these many constraints. Salinity and acidity are major factors limiting plant growth on new and existing arable lands of the world.

Salinity as defined by Blum (1985) is the presence of excessive concentrations of soluble salts in soil solutions that suppress plant growth. The salts responsible for the salinity of saline soils occur in varying proportions in these soils. The properties of soil salinity may be quantified from assessment of the total amount of exchangeable cations that a soil can retain, designated the

cation exchange capacity. The soluble cations which give saline soils their properties are Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-, SO_4^{2-}, NO_3^-, and HCO_3^- are the predominant anions (Tanji, 1990). Of these, chlorides, sulphates, and bicarbonates of sodium, calcium, and magnesium are most commonly found in saline soils and irrigation water. Saline soil has an electrical conductivity (EC) of more than 4 dS m^{-1} in at least some part of the soil profile within 25 cm of the surface; exchangeable sodium < 15%, pH < 8.5. Depending upon the presence and concentrations of these cations and anions, EC (electrical conductivity), and ESP (Exchangeable Sodium Percentage) the US Salinity Laboratory classify salt-affected soils as, saline (EC > 4 mmho, ESP < 15), saline sodic (EC > 4 mmho, ESP >15), and non-saline sodic soils (EC < 4 mmho, ESP > 15). This system is being used world wide for the classification salt affected soils.

Plants are subjected to salinity problems in two situations (Blum, 1985; Tanji, 1990; Pessarakli, 1991), i.e., (1) when they are grown on inherently saline soils, the primary source of salinity, and (2) when they are grown on inherently non-saline soils, but when they are irrigated with saline or brackish water, the secondary source of salinity.

Soil salinity can result from natural processes, and/or from crop irrigation with saline irrigation water under poor drainage conditions. Saline soils are found both under humid and arid climatic conditions (Larcher, 1995). In the humid regions it is possible that soil becomes salty due to salts brought in by winds and clouds originating in oceans and sea-spray, on dunes and in marshes. Moreover,

the salt content of soil can be increased by spray, dust, and run-off water along roads that are salted in winter to keep them free of ice.

Salinity is considered the most serious environmental stress that threatens agricultural productivity in arid and semi-arid regions of the world, which accounts for 36% of the earth's surface (Meigs, 1968). Soil salinity in these regions is greatly increased because of the higher evaporation rates from the soil than the amount of precipitation over the course of the year. Especially large amounts of salt accumulate (1) in hollows where the groundwater table is high, (2) in depressions with no drainage, and (3) in intensively irrigated areas where there is appreciable salt content in the irrigation water and insufficient drainage.

Salt-affected soils are not confined to arid and semi-arid regions only, but they also occur in fertile alluvial plains, river valleys, and coastal areas. In fact, no continent on the earth is free from salt-affected soils. Szabolcs (1993), quoted from the estimates of FAO/UNESCO made for the distribution of saline lands, that 357.3 million ha of land in Australia, 211.7 million ha in North and Central Asia, 129.2 million ha in South America, 87.6 million ha in South Asia, 80.5 million ha Africa, 50.8 million ha in Europe, 20.0 million ha in Southeast Asia, 15.7 million ha in North America, and 2.0 million ha in Mexico and Central America is affected by salinity.

A total of about 950 x 10^6 ha of land is salt-affected world wide (Flowers and Yeo, 1995). There is, however, a great deal of variation in the distribution of saline land over the world's surface so that the regional impact of salinity is much more serious than the average value would imply. For example in Pakistan about

26% of the 16 x 10^6 ha of cultivated land is salt-affected (Ahmad, 1990). Wyn Jones (1981) reported that in Pakistan, about 10 out of 15 million hectares of irrigated land were becoming saline or water logged, and one hundred thousand acres are going out of production annually (Qureshi, 1990), resulting in a per annum loss of 20 billion rupees to the national exchequer (Qayyum and Malik, 1988). The total salt-affected land in Australia is also large, with about one third of the total area having salt-affected soils, which are predominantly sodic (Northcote and Skene, 1972; Fitzpatrick *et al*, 1994). In western Canada, soil salinity has reduced the agricultural production of more than 2.2 x 10^6 ha of dry land and 100,000 ha of irrigated land, and the area affected by salinity is increasing about 10% per annum in dry regions (Vander Pluym *et al*, 1981). Al-Khatib (1991) reported that in Syria 30% of the 1.13 x 10^6 ha in the Euphrates valley exists as salt-affected soils and 3,500 – 5,000 ha of arable land is becoming saline every year.

Salinisation of irrigated lands specially threatens current agricultural productivity. About 20% of irrigated land have suffered from secondary salinisation, (Ghassemi *et al*, 1995). Irrigation has a crucial role in agricultural productivity, and the need for more irrigation appears inevitable to increase the productivity of arid and semi-arid areas which constitute about one third of the world's land surface (UNEP, 1992). However, the problem of salinisation increases with the expansion of area under irrigation, because most of the water in the hydrosphere is saline and much of the fresh water is frozen. Thus, non-saline water for irrigation is becoming limited, and water quality continues to decline.

Szabolcs (1987) quotes FAO and UNESCO estimates that 50% of irrigation schemes are salt-affected, and world wide, about 10 million ha of irrigated land, which is equivalent to all the irrigated land in Africa, are thought to be abandoned each year because of secondary salinisation and alkalisation. In India, some 18-53% of the potential irrigated area is water logged, and 11-38% is saline under 18 irrigation projects (Singh, 1992). The total irrigated land in the world is about 263 x 10^6 ha (FAO, 1998). If half of this is salt-affected, then this makes about 16% of the total salt affected land. In the developing countries of Asia, Africa and Latin America all the indications from projection of population growth, increased dependence on irrigation, and failure of irrigation, are that agricultural salinisation will become a life threatening problem (Flowers and Yeo, 1995).

Salinity is a predominantly environmental constraint to crop production in arid and semi-arid areas (Greenway and Munns, 1980; Downton, 1984), while acid soil toxicity, mainly caused by excess aluminium and manganese, is the biggest crop agriculture problem in tropical and sub tropical areas (Zeigler *et al*, 1995).

Acid soils, pH below 5.0, are found throughout the world, with the largest areas in tropical and sub-tropical regions. Except for extreme situations, pH *per se* rarely has a direct effect on plant growth. At very low pH, 4.2, however, the hydrogen ion concentration may hinder, or even reverse cation uptake by plant roots (Jackson, 1967). The poor fertility of acid soils is mainly due to high levels of aluminium and/or manganese toxicity. Problems posed to plants by these toxicities in the soils of the world are basically of two kinds. The first type, which

is of natural origin, arises either as a consequence of the nature of the parental material from which a particular soil is derived, or from the processes of soil formation. The second type of toxicity is anthropogenic in origin, having been imposed on soils by pollution originating from the increasing growth of the industrial and domestic impacts of humans on their environment (McNeilly, 1994).

On a global scale there are two main geographical belts of acid soils, the humid northern temperate zone, and the humid tropics. The largest pool of potentially arable acid soil exists in the humid tropics, which comprise about 60% of the acid soils of the world (von Uexkull and Mutert, 1995). These authors quote FAO estimates that acid soils cover about 30% of the total ice-free land or about 3950 x 10^6 ha of earth's surface. Of the total acid-soil area, 40.9% occurs in the Americas, 26.4% in Asia, 16.7% in Africa, 9.9% in Europe, and 6.1% in Australia and New Zealand. About 67% of the acid-soil area is under forests, 18% under savannas and prairie vegetation, 4.5% under arable crops, and less than 1% under perennial tropical crops. These soils comprise approximately 1.455 billion ha or roughly half of the non-irrigated arable lands in the world, and as such, they potentially pose a major constraint to the world's agriculture production. Acidification of intensively cropped soils due to acid forming fertilisers is a serious problem in many parts of the world, notably in the USA (Jackson and Reisenauer, 1984) and the former USSR (Breburda, 1990).

Aluminium toxicity is the most important factor limiting plant growth in acidic soils throughout the world (Foy, 1984; Wright *et al*, 1989), occurring

almost exclusively on acidic soils below pH 5.0, (Woolhouse, 1983), but it has been reported to occur as high as pH 5.5 in some soils (Foy, 1974). It is the most abundant metal, comprising approximately 7.5% by weight of the Earth's crust (Haug, 1984). Acid soil development, leading to aluminium toxicity as a result of sustained use of acid forming fertilisers, has occurred mainly through the nitrification of ammonium ions (McNeilly, 1994). Aluminium in non-acidic soils having pH > 5 is predominantly bound as insoluble oxide and complex aluminosilicates. However, as soil pH decreases there is a release of ionic Al^{3+} and a reduction in the availability of exchangeable cations such as Ca^{2+}, Mg^{2+}, and K^{+}, and ionic aluminium can be toxic to plants at micromolar concentrations (Parker *et al*, 1989).

The second most important toxicity causing element after aluminium in many acidic soils is manganese (Foy *et al*, 1988). Whilst aluminium toxicity is more important in the soils of Latin America, manganese toxicity is very important in Africa and parts of Asia (S. Pandey, pers com). Manganese in divalent form (Mn^{2+}) is toxic to plant growth, and at low soil pH, MnO_2 is reduced to this toxic species of manganese. Manganese toxicity generally occurs in soil with pH of 5.5 or below, provided the soil contains sufficient total manganese. However, it can also occur at higher pH values in poorly drained or compacted soils where reducing conditions favour the production of divalent manganese which plants can absorb.

Much of crop science is aimed at finding economic optimum management methods for obtaining maximum yields from crop growing. The best way of

avoiding the effects of stress due to salinity is to prevent the stress from occurring. Engineering technology, such as draining saline water and supplying high quality water from remote sources exists, and is essential to combat salinity problems. However it is almost always impractical because it is very expensive and enormous quantities of water are needed, and therefore it is unlikely to be used extensively in the near future if ever. Furthermore, engineering and cultural practices often provide only a temporary solution, and complete eradication of the salinity problem is not possible through these measures. Soil toxicity from aluminium and manganese cannot be avoided in soils, as salinity can be avoided, apart from in extremely small areas (Foy, 1974), and cultural practices are necessary. Whilst application of lime can reduce the toxic effects of aluminium and manganese in the acid soils by increasing the soil pH, it is not universally possible because of the large areas affected and the non-availability of abundant supplies of lime. Moreover, liming the soil surface does little to correct chemical impediments to sub-soil root penetration, and has to be repeated.

The need is, therefore, to find/breed plant material, which not only survives, but also grows and yields well on these affected soils. The plant genetic approach, to encounter the toxic effects of these stresses on crop production, is ecologically clean, energy conserving, and usually cheaper than amending the soil. Hence, it is compatible with national and international goals of economic food production, conservation of soils, water and energy, and control of pollution.

Normal, classical, evolutionary changes in plant populations are to be expected, rather inevitably, in any situation where stress is occurring consistently

(Bradshaw and Hardwick, 1989). Stress as a constraint and stimulant, apart from affecting the individual, also promotes the development of better adapted genotypes, as is very clearly seen along a stress gradient. It is well established now that tolerance to salinity and heavy metals in soil has evolved in the wild, and in some cases, such soils support endemic and rare taxa (Wild and Bradshaw, 1977). Several plant species are known to have metal tolerant populations, the products of natural selection, growing successfully on metal contaminated soils, despite those soils causing severe damage and usually complete death to non-tolerant individuals of the same species (Bradshaw and McNeilly, 1981). This tolerance is specific to the individual metal, and is highly heritable (Gartside and McNeilly, 1974a,b). In contrast, many species of higher plants are found only in coastal areas and deserts, which provide evidence that salinity and drought tolerance have evolved and that those plant species growing in these habitats are obviously the product of natural selection for survival and growth in saline environments. The evidence that tolerance can evolve in response to such environmental stresses suggests there being potential for selection and breeding of crop cultivars having improved stress tolerance. The approach of tailoring the plants through selection and genetic means, to fit the problem soils instead of changing the soil to fit the plant has received considerable attention, and a few varieties with improved tolerance to saline, and acid soil conditions have been developed in different crops.

The plant genetic approach has great potential for solving the problem of crop production under environmental stresses, particularly in developing countries

which must use a 'low input' system of agriculture (Foy, 1997). The approach of tailoring the plants through selection and genetic means depends upon two basic components. Firstly the existence of variability within the crop under improvement, in response to a particular stress, and secondly this variability must have a significant genetic component.

Analyses have shown that variability in salinity tolerance, and aluminium and manganese tolerance does exist both within and between plant species. Previous studies have demonstrated the occurrence of variation in salt tolerance in a number of crop species (Maas and Hoffman, 1977), for instance, in wheat and barley (Epstein *et al*, 1980; Ashraf and McNeilly, 1988), lucerne (Al-Khatib *et al*, 1994a), rice (Chowdhry *et al*, 1995), millets (Kebebew and McNeilly, 1995), and others. Reviewing plant adaptation to acid soils, Foy (1988) listed 23 species for which there is evidence of intra-specific variation in tolerance to aluminium and manganese toxicity. It is well recognised now that variation for tolerance to environmental stresses exists, and this may be used to breed crop varieties, which could guarantee a stable production under particular stress conditions.

A thorough knowledge about the genetic basis of variation in tolerance to a given stress is absolutely necessary to plan an effective breeding programme. In many crop species, considerable progress has been made in exploring the genetic basis of aluminium tolerance. Some of these crops include rice (Howeler and Cadavid, 1976), wheat (Camargo, 1981; Aniol, 1990), sorghum (Gourley *et al*, 1990) and soybean (Bianchi-Hall *et al*, 1998). The evidence indicates that tolerance to aluminium is genetically controlled, and that it is inherited as a

dominant trait, involving both additive and non additive action of three or more genes. The amount of evidence about the genetic basis of salinity tolerance and manganese tolerance is not great. Such evidence as does exist for salinity tolerance, e.g. for sorghum (Azhar and McNeilly, 1988), rice (Gregoria and Senadhira, 1993), pearl millet (Kebebew and McNeilly, 1996), and tomato (Foolad, 1996b), suggests that the character is under QTL control and is highly influenced by environmental variation, and both additive and non additive genetic effects are involved. The breeding procedure depends upon the pattern of inheritance, qualitative or quantitative, the number of genes with major effects, and the nature of the action of those genes. Such information about the genetic basis and components of variation can help in devising a selection strategy for tolerance, and predicting progress through selection.

To facilitate breeding for salt tolerance, adoption of new selection criteria based on knowledge of the physiological mechanisms or characters contributing to salt tolerance has been proposed (Noble and Shannon, 1988; Yeo and Flowers, 1990). Partial exclusion of ions and synthesis of organic solutes are the broad physiological mechanisms by which cultivated crops respond to salt stress. However, in spite of the large amount of research on aluminium toxicity, there is no consensus on the physiological mechanism(s) of aluminium toxicity or tolerance in plants (Taylor, 1991). Understanding about the physiology of manganese tolerance even lags behind that for aluminium tolerance (Foy *et al*, 1988). Reviewing the physiology of stress tolerance, Larcher (1995) has proposed some non-specific physiological mechanisms that follow a stereotypic pattern,

whatever the nature of stress factor. Accumulation of organic solutes such as free amino acids, proline, and carbohydrates, under stress conditions are among those non-specific mechanisms. Some information about the physiological mechanism of tolerance may be obtained by estimating the accumulation of these organic solutes from aluminium and/or manganese tolerant and non-tolerant accessions when grown under these toxicities.

Substantial progress has been made in crop agriculture using conventional plant breeding methods despite limited basic understanding of the physiological and biochemical mechanisms. However, in some situations, genetic advance through plant breeding has been slow due to the complex and ambiguous natures of the trait(s), such as stress tolerance. A more comprehensive understanding of the physiological and biological mechanisms, possibly, would contribute positively and efficiently to breed crop plants for salinity tolerance. Molecular analysis of the genome at the DNA level can provide a greater advantage because DNA sequences are the same in all of the living cells of a plant, regardless of physiological or developmental state of the tissue. DNA marker technology has provided a new source of information and an impetus for modifying some plant breeding methods. DNA marker technology is now integrated into existing plant breeding programmes all over the world in order to allow researchers to transfer and combine genes at a rate and precision not previously thought of (Mohan *et al*, 1997). Marker-assisted selection (MAS) can be used to pyramid the major genes for salinity tolerance (Bohnert and Jensen, 1996). DNA markers have been utilised

to determine the number of genes controlling trait inheritance and for gene tagging in wheat (Andersson *et al*, 1994; Schachermayr *et al*, 1994).

With the advent of the polymerase chain reaction (PCR) DNA marker technology gained a new impetus. Several techniques have become available to generate genetic markers. The use of these techniques has the potential to facilitate selection for complex traits in early generations. Random amplified DNA polymorphism (RAPD) is one of the PCR techniques that are relevant to the population-level variation (Williams *et al*, 1990). RAPD patterns have been shown useful by Bagheri *et al* (1995) for clarifying phylogenic relationships within a species, and also to provide useful genetic markers for varietal identification in peas (*Pisum sativum* L.) Using the RAPD method, Foolad and Chen (1998) identified 13 RAPD markers at eight genomic regions that were associated with quantitative trait loci (QTL) affecting salt tolerance during germination in tomato. They concluded that simultaneous or sequential transfer of QTL at different development stages would lead to the development of cultivars with salt tolerance throughout the ontogeny of the plant.

Maize (*Zea mays* L.), together with wheat and rice, is one of the most important cereal crops being used both as human food and animal feed. It has a very long and intense evolution. It apparently originated in Mexico, the oldest archaeological record (7000 years) being found in the Tehuacan valley, then spread to Americas, Europe, and Asia (Benson and Pearce, 1987). Now it is grown on over 14 million ha of land all over the world, with an annual production of about 600 million metric tonnes (FAO, 1998). About 300 races of maize have

been recognised, and most of the races and thousands of cultivars within races have been developed in the tropics (Paterniani, 1990). Races of maize are known that grow as far north as Southern Canada while others range to the extremes of the tropical forest and desert oasis. Some grow at sea level and others at almost 3000 metre elevation. It is grown extensively in the temperate, sub-tropical and tropical zones, and is probably the most wide spread crop in the world after barley. Because it is a natural cross-pollinator, it is highly heterogeneous and responsive to selection (Neuffer, 1994). Therefore, it is most likely that maize accessions could be found or developed for cultivation in areas affected by salinity, and aluminium and manganese toxicity.

The main aim of this PhD project was to supply basic information about the existence of genetic variability for tolerance to salinity, and aluminium and manganese stresses, and the potential of exploiting that variability in maize accessions through genetic means. In Chapter 2 variability in salinity tolerance in maize accessions, the majority of them were land races from different ecological areas, was assessed using the non-linear least square inversion method of van Genuchten and Hoffman (1984). The method has been shown useful in identifying salinity tolerant and non-tolerant accessions in barley (Martinez-Cob *et al*, 1987), millets (Kebebew and McNeilly, 1995), and wheat (Steppuhn *et al*, 1996). Two biometrical procedures, Triple Test Cross and North Carolina Model II were followed to gain information about the genetic basis of salinity tolerance and the results are presented in Chapter 3. The work described in Chapter 4 assesses separately the variability in maize accessions in response to aluminium and

manganese toxicities. Individual and combined impacts of these metals on selected maize accessions were also examined in that Chapter. In Chapter 5 estimates of components of genetic variation and heritabilities obtained following the North Carolina Model II method were used to examine the genetic basis of variability for aluminium and manganese stresses. Accumulation of organic solutes, amino acids, proline, and carbohydrates, in response to aluminium and manganese were examined in Chapter 6. Preliminary work was carried out in Chapter 7 to study DNA polymorphism in the F_2 generation of a cross between two salt tolerant accessions.

The results from this study are discussed in relation to our current understanding of stress tolerance and the possibility of creating increased tolerance, especially for salinity, and aluminium and manganese stress, in future breeding programmes.

Assessment of salinity tolerance in maize, based upon response function estimates

Chapter 2

CHAPTER 2

Assessment of salinity tolerance in maize, based upon response function estimates

2.1. Introduction.

Environmental stresses come in many forms, yet salinity remains as one of the world's oldest and the most serious environmental problems, especially in arid and semi-arid areas where poor crop establishment is the major limiting factor in crop production. Crop yield losses due to salinisation of soils are considerable and in some cases agriculture on salt affected land has been abandoned. Some civilisations such as Sumerians in Mesopotamia, vanished because they failed to respond to this threat (McWilliams, 1986). It is estimated that 7% of the earth's surface and about 5% of cultivated land in the world is already plagued by excess salinity (Flowers *et al*, 1997), primarily caused by inadequate drainage and/or low quality irrigation water. In addition, the greater pressure on arable land to produce more food for growing populations, especially in the third world, is on marginal lands previously not cropped because of their high degree of natural salinity, are now being brought into cultivation (Flowers and Yeo, 1995). The increase in salinization continues at a frightful rate in some regions. In Pakistan alone about 100,000 acres of land go out of crop production each year due to salinity (Qureshi, 1990). Of all the improved agricultural technologies capable of ameliorating the problem of salt-affected soil, the availability of salt tolerant crop plant material would be most cost effective, as was proposed by Epstein and Rains in 1987, and

without genetically based variability in any crop such development is not possible to date.

The available evidence suggests that substantial differences in salt tolerance do exist within and between crop species. Shannon (1982) pointed out that variation for salt tolerance among cultivars has been observed in 30 agricultural species. Maas (1993) showed that plant growth response to soil salinity varies widely among both agricultural crop species and potential halophytic plant species. He found that barley, cotton, and wheat are capable of growing and producing acceptable crop yields with 50% seawater irrigation, and they are therefore relatively salt tolerant.

Maize is the third most important cereal crop in the world after wheat and rice, and is grown all over the world under a wide range of climatic conditions. It is moderately sensitive to salinity and considered as the most salt-sensitive of the cereals (Maas and Hoffman, 1977). Being highly cross-pollinated, maize has become highly polymorphic through the course of natural and domesticated evolution and thus contains enormous variability (Paterniani, 1990) in which NaCl tolerance may exist. Its variability for salinity tolerance is illustrated by the findings of Ashraf and McNeilly (1990). From 10,000 seedlings of cv. Akbar screened at 180 mM NaCl, 18 seedlings survived, and their progeny showed significant tolerance after 8 weeks growth in saline sand culture conditions. Thus, improvement of salt tolerance in maize is clearly possible through selection and breeding.

Several models have been proposed to fit salinity responses of crop accessions for a better quantification of their salt tolerance. The use of the non-linear least square inversion model developed by van Genuchten and Hoffman (1984) has considerably facilitated the evaluation of salinity tolerance at various growth stages. The model has successfully been used for assessing salt tolerance at germination in 24 cultivars of barley (Martinez-Cob *et al*, 1987), at the seedling stage in three species of millet (Kebebew and McNeilly, 1995), and at maturity in wheat (Steppuhn *et al*, 1996).

Using the non-linear least square inversion model of van Genuchten and Hoffman (1984) the work described in this chapter assesses variability in salinity tolerance in 72 maize (*Zea mays* L.) accessions and its genetic basis, using seedling root growth response to NaCl in solution culture.

2.2. Materials and methods

2.2.1. Plant material.

Seventy-two maize accessions, mostly land races, but also including commercial varieties/breeder lines were assessed. Seed of the accessions was obtained from CIMMYT, IPK Germany, CIFP Bolivia, INIA Chile, Zeneca UK, NCRPIS USA, and AARI Pakistan.

2.2.2. Screening protocols.

The response of ten-day-old seedlings of the 72 accessions were examined using four NaCl concentrations, 0 (control), 60 mM, 80 mM and 150 mM, all prepared in half strength nutrient solution (see Appendix 1.2 for composition of the solution) formulated by Rorison and described by Hewitt (1966).

Thirty seeds of each accession were sown on rafts of black alkathene beads, five layers deep, floating on nutrient solution containing each of the respective NaCl concentrations in 300 cm^3 plastic beakers. Before planting, seeds were surface sterilised in 2% bleach (v/v) for five minutes.

The experiment was set up as a completely randomised block design with 5 replicates, each of 6 seeds, in a growth room maintained at 24±1°C, and relative humidity of 70-80%, with 16 hours photoperiod at an intensity of 95 μM $m^{-2}S^{-1}$ PAR (Photosynthetically Active Radiation). To reduce solution evaporation, and maintain humidity the beakers were enclosed within clear, 50 cm x 50 cm x 50 cm plastic chambers. After 10 days growth, 5 randomly taken seedlings from each of the 5 replicates of each accession in each of the treatments plus control were measured for longest root length.

2.2.3. Analysis of data.

Mean absolute and relative root lengths of the 5 seedlings per replicate of each accession in each NaCl concentration were subjected to analysis of variance using MANOVA of SPSS (SPSS for Windows: Advance Statistics, 1994), where

$$\text{Relative root length} = \frac{\text{root length in saline solution}}{\text{root length in control solution}} \times 100$$

Genotypic and phenotypic components of variance were also estimated and broad sense heritability (h^2_B) was determined following Falconer and Mackay (1996). $h^2_B = V_G / V_P$, where V_G and V_P are estimates of the genotypic and phenotypic variances respectively.

The response functions, piecewise linear NOPT 5/NOPT 2, and sigmoid non-linear NOPT 12 (van Genuchten and Hoffman, 1984) were used to estimate Ct, the threshold concentration at which the root length begins to decrease, C0, and C50, the concentrations at which root length reaches zero and 50% of its control value respectively. These data were obtained using the computer programme "SALT" (van Genuchten, 1983).

The "SALT" programme is programmed to convert its option from NOPT 5 to NOPT 2 whenever all the observed data points fall to the right of the fitted threshold, resulting in too small estimates for Ct, and consequently they estimate only the concentration at which root length equals zero (C0).

1. NOPT 5 / NOPT 2, the absolute yield is given by:

$$Y = \begin{cases} Ym & 0 \le C \le Ct \\ Ym - Ym^{s}\,(C-Ct) & Ct < C \le C0 \\ 0 & C > C0 \end{cases}$$

where Y = absolute yield;

Ym = absolute yield under non-saline conditions;

C = average root zone salinity during the growing season;

Ct = threshold concentration at which yield begins to decrease;

C0 = concentration at which yield equals zero;

and "s" is defined as an absolute value of slope of the response function between Ct and C0, and is obtained with the equation

$$s = \sum_{i=1}^{n} (Y_m - Y_i) \sum_{i=1}^{n} (C_i - Ct),$$

where (Ci, Yi) represents the i-th data point ($1 \leq i \leq n$), and "n" is the number of observed data points used in analysis.

2. NOPT 12, a sigmoid-form function given by:

$$Y = \frac{Y_m}{1+(C/C50)^P}$$

where C50 = salinity at which yield decreases by 50 %,

and P is an empirical constant that specifies the steepness of the curve.

2.3.Results.

There were significant differences ($P < 0.001$) in absolute and relative root lengths (Table 2.1) between the 72 maize accessions, and in the reduction in root length due to increased salt concentration. The interaction, accession x concentration was also significant ($P < 0.001$), indicating different responses of different accessions to increasing NaCl concentrations.

There was large variation in salinity tolerance assessed as relative root length (Table 2.2). At the highest treatment, 150 mM NaCl, accessions Sundance and Conquest had the highest relative root values of 59% and 57% respectively. Seven accessions, Lg. 20.80, C 12338, Champ, PI 213714, PI 503567, PI 503568, and PI 508270 had relative root values at 80 mM NaCl in excess of 75%. When response, i.e. relative root length, was assessed as means across three NaCl concentrations, five accessions had mean values of relative root lengths of 66 and 67%, Champ, Lg. 20.80, PI 503567, PI 503568, and Sundance. Accessions Akber, Sadaf, Zea 1072, and Reward by contrast had the smallest relative root mean values across all NaCl concentrations, with mean values between 28 and 33%.

Absolute root length data for 10-day-old seedlings of the 72 maize accessions examined at three NaCl concentrations and control are presented in Appendix 2.1.

The response functions for absolute root length data of three maize accessions, PI 451716, Bozm 1335, and C 235 representing tolerant, moderately tolerant, and the sensitive accessions respectively are presented in Fig 2.1. Estimates of Ct, C0, and C50 (Fig 2.1) clearly show substantial differences in the response of these three representative accessions to NaCl. The estimates of C0 for the 72 accessions are given in Appendix 2.1. The two-piece response function (NOPT 5) fits two linear lines, one, the tolerance plateau with zero slope, and the second indicates the reduction in the character per unit increase in salinity (van Genuchten and Hoffman, 1984). Whilst slope (s) of the regression line did not differ markedly, considerable differences are evident in the tolerance plateau and of the three accessions. Differences among the accessions are also evident for non-linear sigmoid curve (NOPT 12, Fig 2.1). Estimates of Ct for each of the 72 maize accessions are plotted against C50 in Fig 2.2, with each graph subdivided into 4 quadrants, facilitating separation of tolerant and non-tolerant accessions. Tolerant accessions are considered to be those having Ct values greater than 25 mM NaCl and C50 values greater than 120 mM NaCl, this latter concentration chosen because it was considered as a point beyond which stringent selection would be effective for increased tolerance. Overall, high tolerance accessions fall in quadrant I and the most sensitive in quadrant IV. Those accessions having high Ct or C50 estimates only, fell in quadrant II and III respectively. Only three accessions Champ, PI 503567 and PI 503568 fell in quadrant I, having the highest

Ct and C50 estimates. Of the remaining accessions, 45 fell in quadrant IV, confirming the sensitivity to salinity of maize. Twenty accessions had Ct values greater than 25 mM NaCl, but low C50 values. Only four accessions had C50 values greater than 120 mM NaCl, Sundance, Conquest, C 12338, and Lg. 20.80, all having Ct values less than 25 mM NaCl.

Heritability values together with genotypic (Vg) and phenotypic (Vp) variances for absolute and relative root lengths under increasing NaCl concentrations are given in Table 2.3. Genotypic and phenotypic variances, for absolute and relative root length, decreased as the concentration of NaCl in the solution increased. Heritability values were high for absolute root length under control and NaCl concentrations, varying from 0.80 to 0.90. However, for relative root length heritability values were somewhat lower at 60 mM, 0.65, and 80 mM, 0.68, but high, 0.83, at 150 mM concentration of NaCl.

Fig. 2.1. Response functions between NaCl concentrations and root length (cm) of maize seedlings from three representative accessions, tolerant, medium tolerant, and susceptible.

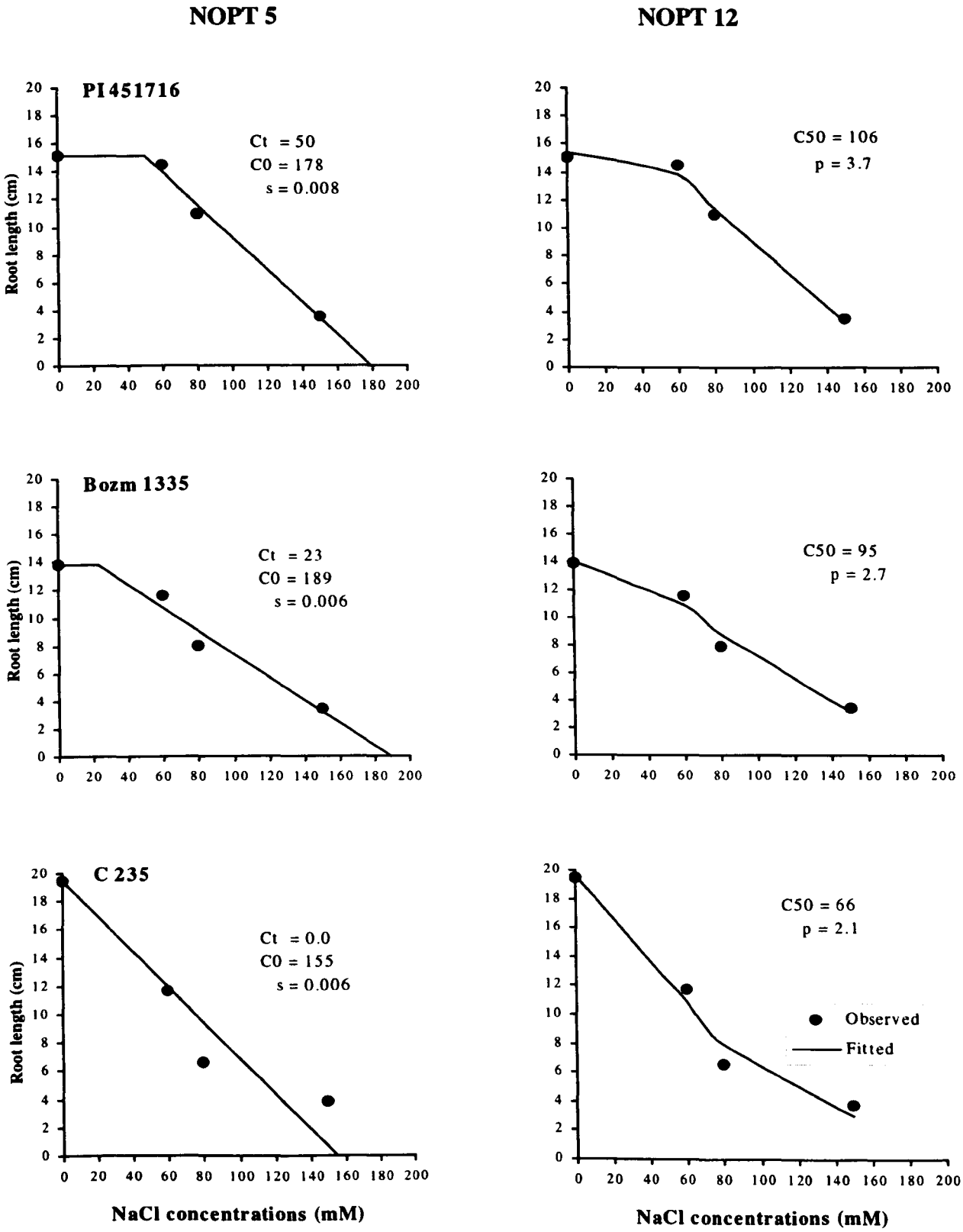


Fig. 2.2. Ct/C50 absolute root length data from 10-day-old seedlings of 72 maize accessions grown at 4 NaCl concentrations plotted against each other.

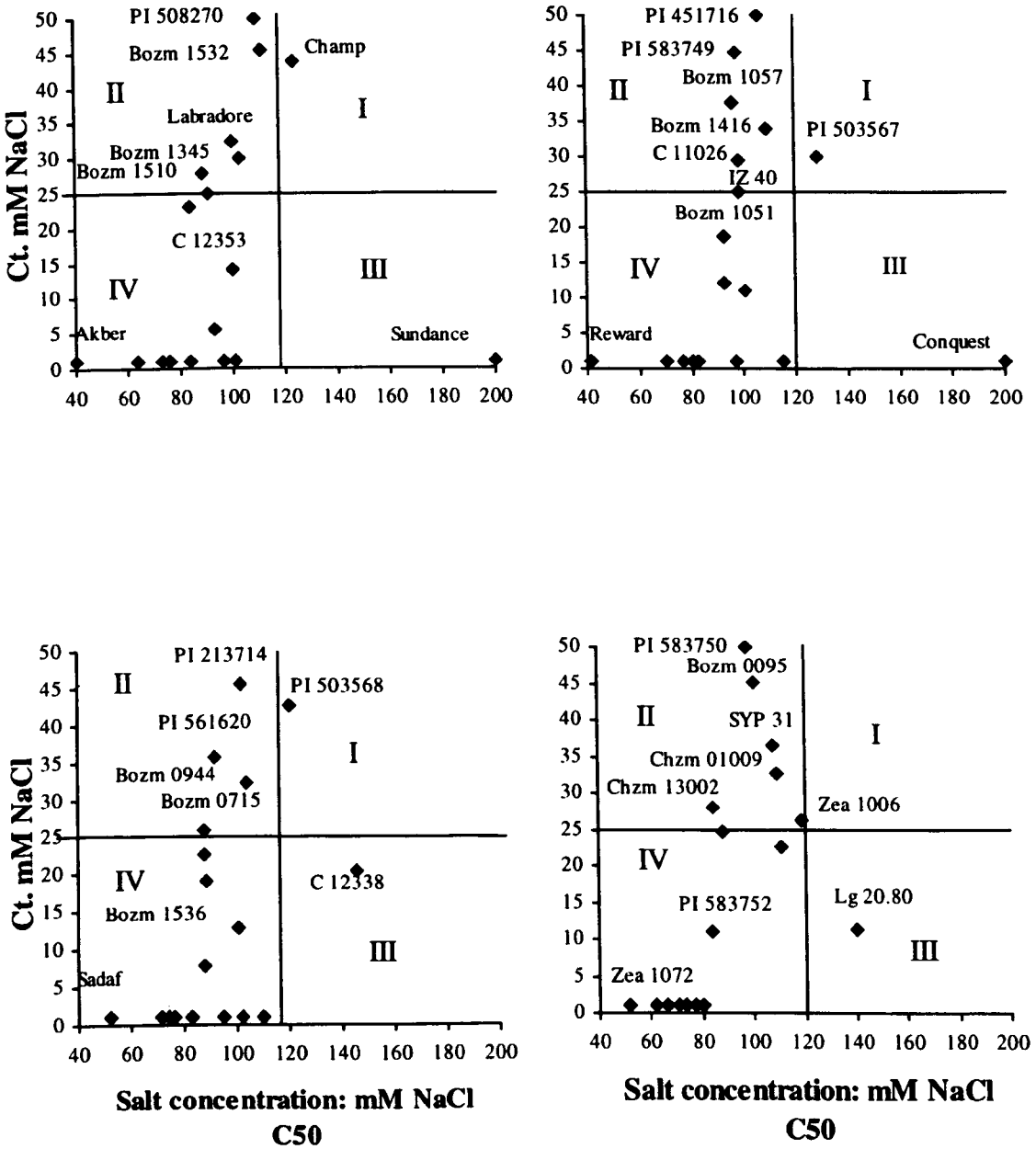


Table 2.1. Mean squares and significances from the analysis of variance of absolute and relative root length data of 10-day-old seedlings of 72 maize accessions grown at four NaCl concentrations.

Sources of variation	Absolute root length (cm)		Relative root length (%)	
	Degrees of freedom	Mean squares	Degrees of freedom	Mean squares
Blocks	4	2.06NS	4	593.35NS
Accessions (Acc.)	71	61.74***	71	1334.15***
Concentrations(Conc.)	3	5367.39***	2	167350.08***
Acc. X Conc.	213	13.59***	142	545.32***
Within + Residual	1042	4.22	769	327.86

***, indicates differences significant at P≤ 0.001, whilst NS, denotes differences which are insignificant.

Table 2.3. Components of variance and broad sense heritability (h^2_B) of NaCl tolerance in *Zea mays* (L.) seedlings at each concentration.

Component	Absolute root length (cm)				Relative root length (%)		
	Control	60 mM	80 mM	150 mM	60 mM	80 mM	150 mM
Vg	31.40	36.03	25.70	6.41	973.05	784.62	468.03
Vp	38.94	40.18	29.84	7.30	1500.48	1152.07	560.46
h^2	0.80	0.90	0.86	0.88	0.65	0.68	0.83

Table 2.2. Relative root length (%) of 10-day-old seedlings of 72 maize accessions grown at 3 NaCl concentrations.

Accessions	60mM	80mM	150mM	Mean	Accessions	60mM	80mM	150mM	Mean
Zea 642	67.58	55.42	33.94	52.31	Reward *	44.17	28.93	23.94	32.94
Zea 671	56.77	43.34	32.01	44.04	Sundance *	70.36	68.12	58.69	65.72
Zea 699	59.10	47.09	30.59	45.59	Champ *	88.26	75.79	37.24	67.10
Zea 769	55.09	45.44	29.73	43.42	Bozm 0095	94.23	66.89	22.19	61.10
Zea 1006	82.12	72.82	36.00	63.65	Bozm 0715	84.70	54.20	18.32	52.41
Zea 1072	45.49	34.09	19.21	32.93	Bozm 0944	80.69	71.18	24.87	58.91
G 800	73.79	57.39	34.12	55.11	Bozm 0999	96.52	42.85	29.12	56.16
Pyramid *	58.63	32.67	36.07	42.46	Bozm 1014	78.83	55.97	18.53	51.11
Labrador *	93.54	55.73	31.89	60.39	Bozm 1052	80.68	57.50	23.33	53.84
Lg. 20.80	78.67	76.68	45.73	67.03	Bozm 1057	91.75	60.74	22.77	58.42
C 88	77.23	57.23	24.28	52.91	Bozm 1335	83.74	57.36	25.14	55.41
C 89	68.14	59.70	31.65	53.16	Bozm 1337	66.95	62.10	17.09	48.71
C 235	60.05	34.05	19.89	37.99	Bozm 1345	80.24	69.86	19.98	56.69
C 10881	58.97	42.88	25.14	42.33	Bozm 1376	70.83	52.50	43.75	55.69
C 10932	69.45	35.67	24.16	43.09	Bozm 1416	87.00	68.35	30.84	62.06
C 11025	67.07	51.01	24.31	47.46	Bozm 1457	65.58	67.84	3.15	45.52
C 11026	86.63	60.71	25.22	57.39	Bozm 1483	79.11	70.44	32.20	60.58
C 12299	56.11	55.32	27.55	46.33	Bozm 1510	87.96	52.38	19.88	53.41
C 12338	83.33	77.32	48.06	69.57	Bozm 1532	93.05	72.01	24.49	63.18
C 12353	77.78	60.89	27.06	55.25	Bozm 1533	73.49	65.15	27.56	55.40
Sadaf *	46.72	30.11	18.19	31.67	Bozm 1536	91.29	48.21	35.99	58.50
Golden *	64.54	41.23	17.07	40.95	Chzm 01001	72.16	60.66	24.29	52.37
Sultan *	61.11	50.46	16.76	42.78	Chzm 01008	50.63	45.81	27.49	41.31
Aghoghi *	57.94	49.24	27.47	44.88	Chzm 01009	85.91	68.34	30.63	61.63
Agati 72 *	52.88	51.29	19.29	41.15	Chzm 03004	75.91	45.95	22.37	48.08
Agati 94 *	70.02	49.36	28.25	49.21	Chzm 13002	93.62	44.60	21.84	53.35
Akber *	33.12	29.87	20.19	27.73	PI 213714	83.45	77.11	11.79	57.45
EV 6085 *	59.27	45.12	27.44	43.94	PI 451716	96.15	72.70	23.33	64.06
IZ 26 *	62.16	44.25	26.00	44.14	PI 503567	83.65	77.14	40.16	66.98
IZ 40 *	90.76	53.91	30.46	58.37	PI 503568	90.22	75.77	34.62	66.87
IZ 46 *	58.42	56.51	26.78	47.24	PI 508270	86.73	81.85	16.34	61.64
IZ 80 *	74.45	51.62	35.36	53.81	PI 561620	88.85	61.78	22.03	57.55
IZ 87 *	75.93	58.77	34.17	56.29	PI 583749	91.81	66.71	17.37	58.63
IZ 7103 *	67.98	67.12	39.33	58.14	PI 583750	95.15	69.41	14.85	59.80
SYP 31 *	83.27	73.61	25.66	60.88	PI 583751	82.49	50.27	14.44	49.07
Conquest *	68.24	61.95	56.76	62.32	PI 583752	69.34	56.51	12.71	46.19

* commercial varieties/ breeder lines; Remainder land races.

2.4. Discussion.

Plant roots are the first organ to become exposed to salinity and root growth is particularly sensitive and rapidly reduced or prevented by salinity (Cramer *et al*, 1988). Salt tolerance is considered to be a developmentally regulated phenomenon, the early seedling stage being the most sensitive (Maas *et al*, 1983), and tolerance at one stage of plant development does not necessarily correlate with tolerance at other developmental stages (Shannon, 1985). However, relative salt tolerance at the seedling stage examined in solution culture persisted through to the mature plant in sorghum (Azhar and McNeilly, 1989), maize (Ashraf and McNeilly, 1989; Maiti *et al*, 1996), alfalfa (Al-Khatib, 1991), and millet (Kebebew and McNeilly, 1995). A sorghum genotype, G.114, identified as the most salt tolerant under field conditions at maturity, was more salt tolerant at the seedling stage than Savanna-5, a lower salt tolerant genotype (Hassneian and Azab, 1993). The technique, assessment in solution culture at the seedling stage, can thus provide a rapid, accurate, and less expensive method of preliminary screening of a large number of accessions for enhanced salt tolerance, provided the tolerance thus assessed is genetically based. Relative NaCl tolerances of 10-days-old seedlings, based upon root length data, clearly showed that such variation does exist in the maize accessions examined and reported herein.

The non-linear least square inversion method developed by van Genuchten and Hoffman (1984) describes the response of crop accessions to soil salinity better than the linear regression, since response to salinity is not a linear function. Maas and Hoffman (1977) suggested that crops tolerate soil salinity up to a

threshold level, above which yields show an approximately linear decrease as salt concentration continues to increase. The absolute root length measurements of 72 maize accessions grown in four NaCl concentrations were analysed following van Genuchten and Hoffman (1984), relative root length data were also used to compare accessions for tolerance to NaCl. Accessions, PI 451716, Bozm 1335, and C 235 representative of tolerant, moderately tolerant, and sensitive accessions, differed significantly for the three tolerance parameters Ct, C50 and C0 (Fig 2.1). Maas and Hoffman (1977) classified maize as a moderately salt sensitive crop, and the Ct/C50 graphs presented in Fig. 2.2 confirm that conclusion, and 63% of the accessions examined with low Ct and C50 values fell in quadrant IV. However, 3 accessions, Champ, PI 503567 and PI 503568 from USA, had high Ct and C50 values (Fig 2.2), and higher mean relative root lengths (Table 2.2) also identified them as salt tolerant. Considerably large differences clearly exist in Ct and C50 estimates, and accessions with high Ct and C50, high Ct and low C50, low Ct and high C50, and low Ct and C50 could readily be identified. No general consistency for tolerance was, however, found between the estimates of Ct and C50. For example, accessions Conquest and Sundance scoring the highest C50 value (200 mM NaCl) had zero Ct, while accessions PI 451716 and PI 508270 with the highest Ct value of 50 mM had a C50 less than 120 mM. These patterns of response functions suggest that different genetic systems may be involved in controlling the inheritance of these tolerance parameters, and looking at the extent of variability within them, it would not seem illogical to assume that each parameter is quantitatively inherited. If the two parameters had been controlled by

quantitative trait loci (QTL) the estimates would have shown a degree of correlation between Ct and C50. Previous studies of these response functions for millet (Kebebew and McNeilly, 1995) and maize (Rao, 1997) also showed no such relationship between Ct and C50. If these parameters are controlled by different genetic systems, tolerant accessions, with high Ct and C50 values, are likely to possess genes for both these characters.

The relative root length data in Table 2.2 allows identification of more tolerant and non-tolerant accessions at different concentrations of NaCl used. Eleven accessions having higher values for Ct, C50, or both, also had the highest mean relative root lengths between 61 and 70%. Accessions having the highest estimate of C50 and the lowest Ct (zero mM), had the greatest relative root lengths at 150 mM, whilst relative root length at 60 and 80 mM was relatively low. By contrast, accessions with higher Ct and comparatively low C50 values had longer relative root lengths at 60 and 80 mM but had shorter roots at 150 mM NaCl. These data clearly indicate that even producing similar mean relative root lengths, accessions with longer relative root lengths at lower salt concentrations had the highest Ct, while accessions with highest relative root length values at high NaCl concentrations had the highest C50 estimates. It follows that in highly saline soils, accessions selected for higher C50 values are more likely to perform better. Nevertheless, it also follows from these data that both Ct and C50 would quantify accession tolerance, and the expression of root growth as a function of NaCl concentrations provides (albeit preliminary), a useful guideline for assessing salt tolerance. Both Ct and C50 have each been suggested as a reference parameter for

selection in previous studies of salinity response functions. For instance, from a study of 24 barley cultivars at the germination stage Martinez-Cob *et al* (1987) suggested that Ct, threshold salinity, was the most appropriate parameter for determining salt tolerance. Salinity tolerance parameters estimated by Kebebew and McNeilly (1995), differed considerably, both between accessions within a species, and between the three species examined, pearl millets, finger millets and tef, reflecting inter-specific differences in tolerance to salinity. It was also suggested that Ct is a useful parameter for assessing salinity tolerance but not for all species. Steppuhn *et al* (1996) concluded that C50 could serve to compare responses of wheat varieties to rooting media, but, from the present results it seems possible to breed crop accessions with high Ct and C50, and simultaneous selection for high Ct and C50 would seem to be more appropriate. However, the selection parameter for enhanced salt tolerance may differ depending upon the sensitivity of the crop and level of salinity.

From a breeding perspective, the value of variability in the character used to assess tolerance to an environmental stress, depends upon the extent the character is determined by genotype. Based on the estimates of broad sense heritability for salt tolerance in seven grasses and four forage species (Ashraf *et al*, 1986a,b; 1987), in two species of minor millets, tef, and finger millet (Kebebew and McNeilly, 1995), and in tomato (Foolad and Jones, 1991; Foolad, 1996a), salt tolerance is genetically based and significant advances in salinity tolerance should be possible using high selection pressure. The heritability estimates for salt tolerance in maize under different NaCl concentrations are similar to those

observed in the other species that have been studied. Heritability values for absolute and relative root length were larger under stress than non-stress conditions (Table 2.3), being highest at 150 mM NaCl. Increased broad sense heritability under increasing salt concentrations, was reported in tomato (Saranga *et al*, 1992; Foolad, 1996b). Saranga *et al* (1992) speculated that increased heritability under increasing salinity levels may be a result of greater genetic variation due to the expression of genes associated with salinity tolerance and/or a smaller environmental variation. Bradshaw and Hardwick (1989) argued that hidden variation, previously unselected, could be uncovered when stress is applied, thus possibly increasing heritability. Relying on the present high estimates of heritability it seems likely that significant advances could be achieved in salt tolerance in maize, provided the greater proportion of these estimates are indeed due to QTL.

Genetic basis of variation for salinity tolerance

Chapter 3

CHAPTER 3

Genetic basis of variation for salinity tolerance

3.1. Introduction.

The problem of soil salinity is well recognised, its devastating effects and consequences on crop production have widely been reported. Variability for salt tolerance, of varied magnitude, within and between species has been found in many cultivated crops.

Plant physiologists have found several mechanisms such as ion exclusion, ion accumulation, production of compatible solutes, and osmotic adjustment, which are suggested to be associated with genetic variation in salt tolerance. Yet their successful use in improving salt tolerance, via physiological selection criteria, is largely non-existent (Noble and Rogers, 1992).

Some progress has been made through the use of simple breeding programmes, and a few cultivars possessing useful degrees of salt tolerance have been developed in some crops; the rate of progress is, however, very slow. A better understanding of the genetic basis of desirable traits involved in the manifestation of improved salt tolerance would perhaps accelerate the pace of progress. Information regarding the genetic basis of salinity tolerance is relatively limited and fragmentary, and insufficient genetic knowledge has severely restricted breeding efforts (Foolad and Jones, 1991 & 1992). Flowers and Yeo (1995) underlined the fact that salt tolerance is a complex character controlled by a number of genes or groups of genes, and involves a number of component traits which are likely to be quantitative in nature. They (Flowers and Yeo, 1995)

anticipated that the importance of salinity as a breeding objective would increase in future.

The available evidence from the various species examined for salt tolerance suggests that both additive and non-additive gene effects are important in controlling the expression of tolerance. For example, Gregoria and Senadhira (1993) found that the Na / K ratio in rice at the seedling stage was governed by both additive and dominance gene effects; two groups of genes were involved, one group was envisaged to control sodium exclusion and the other to control potassium absorption. Ashraf *et al* (1995) reported narrow-sense heritability for salt tolerance in sunflower ranging between 30 and 70%. The estimates of narrow sense heritability of F_2 and F_α generation for grain yield per plant were 75 and 86% respectively and varied between 70 to 95% for ion contents in spring wheat (Ahsan *et al*, 1996). Mano and Takeda (1997) observed no correlation between salt tolerance at germination and the seedling stage in 8 x 8 diallel of barley varieties. At germination non-additive genetic variance was larger than additive variance with a mean degree of dominance as 1.47, while additive genes predominantly controlled tolerance at seedling stage and the mean degree of dominance was 0.52.

In maize, studies on the inheritance of salt tolerance are rather limited. Ashraf and McNeilly (1990) reported considerable variation between and within two cultivars of maize, and obtained an estimate of 0.54 narrow sense heritability from a polycross of eighteen selected lines grown at 180 mM NaCl. They suggested that improvement for salinity tolerance in maize is possible through selection and breeding. A single gene with partial dominance has been reported

for glycinebetaine production, an osmoprotectant under drought and salinity stress, in maize (Grote *et al*, 1994; and Saneoka *et al*, 1995). Rao (1997) employed North Carolina Mating Design II to study the genetic basis of salt tolerance in maize. He found both additive and non-additive gene effects for salt tolerance at seedling stage, the non-additive effect being more important at 60 mM and 80 mM NaCl.

The magnitude of heritable variation for tolerance in any genetic stock has a close bearing on the success of breeding programmes aiming to improve salinity tolerance in crop cultivars. Data presented in Chapter 2 provide evidence for the existence of a considerable amount of variation for salt tolerance among the 72 accessions of maize. Information about the nature of gene action and components of genetic variation is the necessary link between the detection of variation in response to salinity and the breeding of salt tolerant crops. The biometrical designs, North Carolina Model II (Comstock and Robinson, 1952) and Triple Test Cross (Kearsey and Jinks, 1968), used here to assess the genetic basis of salt tolerance based on root length measurements in solution culture at the seedling stage, provide such information.

3.1.1. Data analysis.

The computer package SPSS for windows 6.0 was used for the statistical analysis of root length data of the hybrid progenies following North Carolina Model II and Triple Test Cross procedures.

3.1.1.1. North Carolina Mating Design II.

The NCM II progenies were obtained from crossing *m* males to *n* female parents designated at random. A total of *mn* progenies were thus produced. Root

length data of the *mn* progenies, grown in hydroponic culture, were subjected to analysis of variance following the procedure described by Becker, (1992). The form of analyses of variance and derivations of components of genetic variance are given in Tables 3.1, and 3.2.

Table 3.1. Form of the analysis of variance used for the root length data collected on the full-sib and half-sib families developed by North Carolina Mating Design II (NCM II).

Analysis of Variance (using means of families)			
Source of Variation	**Degrees of Freedom**	**Means Squares**	**Expected mean squares***
Replications (R)	R-1	MS_R	
Males (M)	M-1	MS_M	$\sigma^2e + r\sigma^2mf + rf\sigma^2m$
Females (F)	F-1	MS_F	$\sigma^2e + r\sigma^2mf + rm\sigma^2f$
Males X Females	(M-1) (F-1)	MS_{MF}	$\sigma^2e + r\sigma^2mf$
M-F combination X replicates	(MF-1) (R-1)	MS_I	σ^2e
Analysis of variance (using individual observations)			
Between plots	RMF-1	----	---
Within plots	n...-RMF	MS_W	σ^2w

* r, m, and f, refer to replicates, males, and females, respectively.

Table 3.2. The estimates of genetic components (North Carolina Mating Design II) and their determination.

Genetic component	Estimates	Reference
Additive effects (D_R)	$4(\sigma^2m + \sigma^2f)$	Lawrence (1984)
Non-additive effects (H_R)	$16(\sigma^2mf)$	Lawrence (1984)
Environmental effects (E)	$\sigma^2w-(\sigma^2m + \sigma^2f + 3\sigma^2mf)$	Lawrence (1984)
Additive variance (V_A)	$1/2\ D_R$	Kearsey (1965)
Non-additive variance (V_D)	$1/4\ H_R$	Kearsey (1965)
Genotypic variance (V_G)	$1/2\ D_R + 1/4H_R$	Kearsey (1965)
Phenotypic variance (V_P)	$1/2\ D_R + 1/4H_R + E$	Kearsey (1965)
Potence ratio (P_R)	$(H_R / D_R)^{1/2}$	Wigan (1944)
Narrow-sense heritability (h^2_N)	V_A / V_P	Kearsey (1965)
Broad-sense heritability (h^2_B)	V_G / V_P	Kearsey (1965)

3.1.1.1a. Assumptions.

The underlying assumptions in the derivations of expectations of mean squares, and genetic interpretations of variance for NCM II (Comstock and Robinson, 1952) are as follows:

1. Random choice of the parents mated for production of experimental progenies.
2. Random distribution of genotypes relative to variation and environment.
3. No maternal effects.
4. Regular disomic segregation.
5. Uncorrelated gene distribution.
6. No multiple allelism.
7. No linkage.
8. No non-allelic interaction (epistasis).

Failure to meet some of these conditions will cause characteristic disturbances and would bias the estimates of genetic components of variation.

3.1.1.2. Triple Test Cross.

Kearsey and Jinks (1968) developed the triple test cross (TTC) design as an extension of the North Carolina Mating Design III (Comstock and Robinson, 1952) which was subsequently expanded and modified by Jinks *et al* (1969), and Jinks and Perkins (1970). The primary purpose of the triple test cross design is to unambiguously detect epistasis for quantitatively inherited characters, but it also provides independent tests for the presence of additive and dominance components of genetic variation that are equally precise in the absence of epistasis (Jinks and Perkins, 1970).

The *m* males were crossed to three testers, L_1 (tolerant), L_2 (sensitive), and L_3 (L_1 x L_2). Each *m* male, therefore, had three progenies, and a total of 3*m* triple test cross progenies were produced. The expectations of mean squares and derivations of components of genetic variance are presented in Tables 3.3 and 3.4, respectively.

Table 3.3. Form of the analysis of variance following Triple Test Cross.

Analysis of variance (individual treatments)			
Source of Variation	**Degrees of Freedom**	**Mean Squares**	**Expected mean squares***
Additive (Add)	n-1	MS_s	$\sigma^2w + p\sigma^2r + 3rp\sigma^2s$
Dominance (Dom)	n-1	MS_d	$\sigma^2w + p\sigma^2r + 2rp\sigma^2d$
Epistasis-i type(Epi-1)	n-(n-1)	$MS_{ep\text{-}I}$	$\sigma^2w + p\sigma^2r+6rp\sigma^2ep+frpK^2i$
Epistasis-j & l type(Epi-2)	n-1	$MS_{ep\text{-}jl}$	$\sigma^2w + p\sigma^2r + 6rp\sigma^2ep$
Reps x families	2[(r-1)(f-1)]	MS_{rf}	$\sigma^2w + p\sigma^2r$
Error	[x..t_1-rf]+[x..t_2-rf]	MS_w	σ^2w
Analysis of variance (Combined)			
Additive (Add)	n-1	MS_s	$\sigma^2w + p\sigma^2r + 3rpt\sigma^2s$
Dominance (Dom)	n-1	MS_d	$\sigma^2w + p\sigma^2r + 2rpt\sigma^2d$
Epistasis-i type(Epi-1)	n-(n-1)	$MS_{ep\text{-}I}$	$\sigma^2w + p\sigma^2r+6rpt\sigma^2ep+frptK^2it$
Epistasis-j & l type(Epi-2)	n-1	$MS_{ep\text{-}jl}$	$\sigma^2w + p\sigma^2r + 6rpt\sigma^2ep$
Add x Treatment	(n-1)(t-1)	MS_{st}	$\sigma^2w + p\sigma^2r + 3rp\sigma^2st$
Dom x Treatment	(n-1)(t-1)	MS_{dt}	$\sigma^2w + p\sigma^2r + 2rp\sigma^2dt$
Epi-1 x Treatment	[n-(n-1)][t-1]	$MS_{epi\text{-}it}$	$\sigma^2w + p\sigma^2r+6rp\sigma^2ept+frptK^2it$
Epi-2 x Treatment	(n-1)(t-1)	$MS_{ep\text{-}jlt}$	$\sigma^2w + p\sigma^2r + 6rp\sigma^2ept$
Reps x families	2[(r-1)(f-1)]	MS_{rf}	$\sigma^2w + p\sigma^2r$
Error	[x..t_1-rf]+[x..t_2-rf]	MS_w	σ^2w

*, r, p, n, f, t and x.., refer to replicates, plants, males, families, treatment and individual observations, respectively. While, s and d, and t1 and t2 refer to sums and differences, and control and 80mM NaCl, respectively. K^2i, refers to correction factor.

Table 3.4. The estimates of genetic components (Triple Test Cross) and their determination.

Genetic component	Estimates	Reference
Additive effects (D)	$8\sigma^2s$	Lawrence (1984)
Dominance effects (H)	$8\sigma^2d$	Lawrence (1984)
Environmental effects (E)	$\sigma^2w-(\sigma^2s+\sigma^2d)$	Kearsey and Pooni (1996)
Additive variance (V_A)	$1/2\ D_R$	Kearsey (1965)
Dominance variance (V_D)	$1/4\ H_R$	Kearsey (1965)
Genotypic variance (V_G)	$1/2\ D_R + 1/4H_R$	Kearsey (1965)
Phenotypic variance (V_P)	$1/2\ D_R + 1/4H_R + E$	Kearsey (1965)
Potence ratio (P_R)	$(H_R / D_R)^{1/2}$	Wigan (1944)
Narrow-sense heritability (h^2_N)	V_A / V_P	Kearsey (1965)
Broad-sense heritability (h^2_B)	V_G / V_P	Kearsey (1965)
Direction of dominance (F)	-4 (Cov sd)	Jinks *et al* (1969)

These two biometrical genetic models were applied to assess the mode of inheritance of salinity tolerance in maize accessions, which is the subject matter for this Chapter.

3.2. Material and methods

3.2.1. Parental material.

To study the genetic basis of salt tolerance 21 accessions (origin of the accessions is given in Appendix 1.1) were chosen based on their response to NaCl stress in solution culture (Chapter 2), and were crossed to generate NCM II progenies. Six accessions, Zea 1006, Reward, Sundance, Champ, Lg 20.80, and Akber, and one hybrid, Zea 1006 x Reward, were used as female parents. Sixteen accessions, Golden, SYP 31, Zea 769, C 12338, Bozm 1335, Bozm 1337, Bozm 1345, Bozm 1416, Bozm 1483, Bozm 1532, Bozm 1533, Bozm 1536, Chzm 01008, Chzm 01009, G 800 and EV 6085, were used as male parents. Triple Test

Cross progenies were generated by crossing, Zea 1006 (tolerant), Reward (sensitive), and their F_1 hybrid, Zea 1006 x Reward, with the sixteen male parents.

3.2.2. Crossing block.

The parental accessions were raised in the glasshouse and crossed according to NCM II and TTC mating designs.

3.2.2.1. Growth of the parents.

Seed of the parental accessions were sown in 8-cm pots during February/March 1997 in the glasshouse with temperature ranging from 20-28 °C. Differences in flowering time were expected because of the land race origin of the parents, therefore to ensure the availability of pollen at silk (female inflorescence) emergence of the female parents, the male parents were sown at three different dates with an interval of 10 days. First sowing of the male parents was done 10 days before sowing the female parents, second with the female parents, and third sowing of the male parent was done after another 10 days.

Month old healthy seedlings were then transplanted to 20-cm plastic pots containing John Innes compost No 2 to grow to maturity. Plants were watered daily, and Vitax 1:1:1 (20%N, 20%P, 20%K) liquid fertiliser was supplied every week to promote healthy growth. To ensure controlled pollination, male parents were kept 20 m apart form the female parents and a polythene sheet was also placed as a barrier to unwanted pollen dissemination. The sheet was placed in such a way that it would not cause any hindrance to air circulation, application of water and nutrients, and collection of the pollen.

3.2.2.2. Preparation of the female parents.

Three months after planting, during May 1997, the plants started flowering. Prior to anthesis a day before emergence, in the afternoon, tassels (male inflorescence) were removed manually from the female parents. Plants were rechecked in the early morning to remove any chance leftover tassels as they emerged. The ear shoots were covered with butter paper bags before the emergence of the silks (female inflorescence) from the husk tip. To hold it in place the bag was firmly anchored between the shoot and the auricle of the ear leaf. After 2-3 days of de-tasseling, the silks started appearing under the bag, and were ready for pollination.

3.2.2.3. Pollination.

When the ear shoot was prepared for pollination, the tassels of the male parents were also enclosed within a paper bag to eliminate any contamination by alien pollen. The bag was held in place by a paper clip. In the afternoon, pollen was collected in the paper bag by giving a gentle shake to the tassel. Anther dehiscence occurred into the bag and pollen released by them was transferred to a sterilised petri dish. The pollen was dusted onto the silks of the desired female parent with a soft camel hair brush by lifting the butter paper bag, and the ear shoot was immediately covered again with the same bag and left until seed setting. A small white tag was tied to the ear shoot mentioning the name of the cross and date of pollination. The pollination equipment and hands were sterilised with 100% ethanol before carrying out the next pollination. The pollination was repeated several times to ensure maximum seed setting, and the number of

pollination was marked on the attached tag. Three to four crosses were made per combination to produce sufficient seed of the F_1 families.

3.2.2.4. Harvesting of the seed.

During July, when the husk turned brown and the seeds became hard, the cobs were harvested separately for each cross from each female parent. The seeds were separated from the rachis of the cob, cleaned and counted for the assessment of NaCl tolerance in solution culture. Three accessions, Bozm 1536, Chzm 01008, Chzm 01009, flowered very late, and could not be crossed with the female parents, while the seed number set involving another male parent, Bozm 1533, was insufficient to include in the salinity testing programme. Therefore, the eighty-four NCM II families, and 36 triple test cross progenies were assessed for tolerance to NaCl at the seedling stage.

3.2.3. Assessment of the hybrid progenies in NaCl solutions.

Surface sterilised seeds of the F_1 families were assessed in two treatment solutions containing, 0 (control) and 80 mM NaCl prepared in 1/2 strength Rorison nutrient solution (see Appendix 1.2 for composition of the solution). The screening protocols and growth conditions were similar to those described in (page 19) Chapter 2.

Root lengths of 10 seedlings of each family from each of the three replications, arranged randomly in a complete block fashion, in each treatment were measured after 10 days growth in the treatment solutions. The family means were thus based on 30 root length observations. The measurements of the longest root length in control and 80 mM NaCl were used to examine the genetic basis of

salt tolerance following North Carolina Mating Design II and Triple Test Cross genetic models.

3.2.4. Validity of assumptions.

Parents involved in the NCM II crossing programme were randomly designated as male and female. Out of 21, three female parents were non-random, because of their being used as testers in the triple test cross. In the absence of significant maternal effects, the effect of these non-random parents was assumed minimal and not a significant source of bias in the estimates of genetic variation components. The crosses were made under similar glasshouse conditions over a short period of time, and progenies were assessed in a growth room, with controlled conditions. It can therefore be assumed that the genotype x environmental interaction would also be minimal. In NCM II, maternal effects could be obtained by dividing mean squares for females with mean squares for males, and if present could be avoided by estimating the additive effects from male variance only. Maize is a diploid species with a somatic chromosome number of 20, and its meiotic behaviour is diploid (Poehlman and Sleper, 1995). The model does not provide any test for linkage, multiple allelism, and occurrence of non-allelic interaction. Multiple alleles, if present, would tend to increase the dominance variance but would not affect the additive variance (Robinson *et al* 1955). Epistasis - non-allelic interaction - causes upward bias in dominance variance, and the bias may not be large (Comstock and Robinson 1948). Complete validity of these assumptions is, however, impossible (Comstock and Robinson 1952).

The assumptions for Triple Test Cross are not demanding, and the model is independent of gene correlation, mating system, and allelic frequency (Kearsey and Jinks, 1968).

3.3. Results

3.3.1. North Carolina Model II.

Mean squares due to male and female, and male x female obtained for the NCM II families, following the analysis of variance procedure described by Becker (1992), are given in Table 3.5. Other statistics including computations of the genetic variance components, and narrow and broad sense heritabilities are presented in Table 3.6.

Root length measurements varied significantly ($P \leq 0.001$) among male and female half-sib families at control and 80 mM NaCl, indicating the involvement of additive gene effects in controlling the expression of salt tolerance, which turned out that salt tolerance could increase in response to selection. The ratio MS_f/MS_m was not significant ($P > 0.05$) either at control or 80 mM NaCl, which suggested that maternal effects were not involved to a great extent, in root growth at these NaCl concentrations. Non-significant maternal effects consummate the assumption that the effect of non-randomness of the three female parents used in the crossing programme would be minimal. This finding also suggests that considerations for enhancing salt tolerance in maize should not influence the choice of parents to be used as a female parent in a hybrid-breeding programme.

Significant male x female interaction for absolute root length at control ($P \leq 0.01$) and 80 mM NaCl ($P \leq 0.001$) indicated that progenies in different

maternal families resemble one another more than progeny of the same female, suggesting non-additive gene effects for controlling root length at both concentrations. The components of genetic variation also testified the predominance of non-additive variation as non-additive gene effects, H_R, was larger than additive, D_R, and environmental E effects at control and 80 mM NaCl. Greater than 1.0 values of the potence ratio (P_R) indicated overdominance for absolute tolerance, the values of P_R being 1.16 and 1.38 for root length in control and in 80 mM NaCl, respectively.

The estimates of narrow sense heritability (h^2_N) were 0.41 and 0.51, while the corresponding values of broad sense heritability (h^2_B) for absolute root length in control and 80 mM NaCl were 0.69 and 0.99, respectively. The non-additive effects, H_R, were twice the estimates of D_R, the additive effects, for salt tolerance at 80 mM NaCl and so were the estimates of broad and narrow sense heritabilities (0.99 vs 0.51).

Table 3.5. Analysis of variance (North Carolina Mating Design II) of root length data evaluated at, 0 (control) and 80 mM NaCl.

a: 0 mM NaCl (Control)

Analysis of Variance (using means of families)				
Source of variation	**Degrees of freedom**	**Mean squares**	**V**	**P**
1 Replications (R)	2	4.68	5	NS
2 Males (M)	11	35.89	4	***
3 Females (F)	6	74.71	4	***
4 Males X Females	66	8.30	5	**
5 M-F combination X replicates	166	5.08		
Analysis of variance (using individual observations)				
6 Between plots	251	66.58	7	***
7 Within plots	1669	11.00		

b: 80 mM NaCl

Analysis of Variance (using means of families)				
Source of variation	**Degrees of freedom**	**Mean squares**	**V**	**P**
1 Replications (R)	2	20.58	5	**
2 Males (M)	11	25.80	4	***
3 Females (F)	6	61.45	4	***
4 Males X Females	66	6.25	5	***
5 M-F combination X replicates	166	2.71		
Analysis of variance (using individual observations)				
6 Between plots	251	42.25	7	***
7 Within plots	1641	6.07		

V indicates variance used as the denominator in each significance test, and corresponds to the number ascribed to the various source of variation.

Table 3.6. Estimates of components of variation, and heritabilities (North Carolina Mating Design II) for root length data evaluated at, 0 (control) and 80 mM NaCl.

Genetic components	NaCl concentration	
	0 mM (Control)	80 mM
Additive effects (D_R)	12.60	9.84
Non-additive effects (H_R)	17.12	18.88
Environmental effects (E)	4.64	0.07
Additive variance (V_A)	6.30	4.92
Non-additive variance (V_D)	4.28	4.72
Genotypic variance (V_G)	10.58	9.64
Phenotypic variance (V_P)	15.22	9.71
Potence ratio (P_R)	1.16	1.38
Narrow-sense heritability (h^2_N)	0.41	0.51
Broad-sense heritability (h^2_B)	0.69	0.99

3.3.2. Triple Test Cross.

Analyses of variance for triple test cross progenies are given in Table 3.7, while derivations of other statistics are presented in Table 3.8.

The mean squares due to epistasis indicated that epistasis i type was significant ($P \leq 0.001$) whereas epistasis j + l type was non-significant ($P > 0.05$) for root length at 80 mM NaCl, suggesting additive x additive interaction between genes controlling root length at this concentration (Table 3.7b).

Similarly the combined analysis for root length measurements at two NaCl concentrations revealed significant ($P \leq 0.001$) epistasis i type and non-significant j + l type epistasis (Table 3.7c). The interactions between treatments and both types of epistasis were also significant (Epi-1 x environment and Epi-2 x environment significant at $P \leq 0.001$). This indicated that interaction between non-alleles i.e. additive x additive, and additive x dominance and dominance x dominance controlling root length expression was of different magnitude at 0 mM and 80 mM NaCl concentrations.

Additive and dominance components were worked out irrespective of the significance of epistasis to illustrate their relative magnitude in controlling tolerance. The additive and dominance items were highly significant ($P \leq 0.001$) in the analysis of variance for individual concentrations (Table 3.7a & b) while the additive item was significant at $P \leq 0.001$ and dominance was significant at $P \leq 0.05$ in the combined analysis (Table 3.7c). Because of the presence of epistasis the estimates of the additive effects were biased to an unknown extent, however, their highly significant mean squares indicate their importance in the genetic control of salinity tolerance. The estimates of dominance (H) components were higher than

additive (D) components indicating the preponderance of dominance effects in the expression of salt tolerance. Consequently, the corresponding estimate of potence ratio $(H/D)^{1/2}$ was greater than one (P_R = 1.33) which also indicated higher magnitude of dominance effects compared to additive effects and over dominance for genes governing salt tolerance. The positive but non-significant estimate of F showed that dominance was ambidirectional; alleles for higher and low tolerance were more or less equally distributed among the maize accessions studied. Nevertheless, a high F value of 5.09 with positive sign indicated some degree of directional dominance towards higher tolerance. The additive x treatment interaction was not significant ($P>0.05$) suggesting that the additive effects are not sensitive to the environments. The degree of dominance for those genes controlling salt tolerance was found to be influenced by the environmental changes as the interaction dominance x treatment was significant at $P\leq0.05$ (Table 3.7c).

Table 3.7. Analysis of variance (Triple Test Cross) of root length data evaluated at, 0 (control) and 80 mM NaCl.

a: Absolute root length at 0 mM NaCl

Source of Variation	Degrees of Freedom	Mean Squares	V	P
1 Additive	11	137.38	5	***
2 Dominance	11	106.18	5	***
3 Epistasis-I type	1	1101.00	5	***
4 Epistasis-j & l type	11	76.25	5	*
5 Reps x families	70	30.31	6	***
6 Error	885	8.14		

b: Absolute root length at 80 mM NaCl

Source of Variation	Degrees of Freedom	Mean Squares	V	P
1 Additive	11	97.91	5	***
2 Dominance	11	111.52	5	***
3 Epistasis-I type	1	99.64	5	*
4 Epistasis-j & l type	11	24.38	5	NS
5 Reps x families	70	22.00	6	***
6 Error	906	3.21		

c: Absolute root length at, 0 and 80 mM NaCl

Source of Variation	Degrees of Freedom	Mean Squares	V	P
1 Additive	11	198.91	9	***
2 Dominance	11	55.17	9	*
3 Epistasis-I type(Epi-1)	1	931.55	9	***
4 Epistasis-j & l type(Epi-2)	11	34.28	9	NS
5 Additive x Treatment	11	13.48	9	NS
6 Dominance x Treatment	11	52.13	9	*
7 Epi-1* x Treatment	1	269.08	9	***
8 Epi-2** x Treatment	11	66.35	9	***
9 Reps x families	70 + 70	26.16	10	***
10 Error	885 + 906	5.65		

V, indicates variance used as the denominator in each significance test, and corresponds to the number ascribed to the various sources of variation.
*, Additive x additive, **, additive x dominance, dominance x dominance.

Table 3.8. Estimates of components of variation, and heritabilities (Triple Test Cross) for root length data evaluated at, 0 (control) and 80 mM NaCl.

Genetic component	NaCl concentration	
	0 mM (Control)	80 mM
Additive effects (D_R)	9.52	6.72
Dominance effects (H_R)	10.08	11.92
Environmental effects (E)	5.69	0.88
Additive variance (V_A)	4.76	3.36
Dominance variance (V_D)	2.52	2.98
Genotypic variance (V_G)	7.28	6.34
Phenotypic variance (V_P)	12.97	7.22
Potence ratio (P_R)	1.02	1.33
Narrow-sense heritability (h^2_N)	0.37	0.47
Broad-sense heritability (h^2_B)	0.56	0.88
Direction of dominance (F)	-3.01	+5.09

3.4. Discussion.

Improving salinity tolerance in crop plants through selection and breeding is an active research pursuit, and information about the genetic basis of salt tolerance and its components is an obvious imperative for the breeding of genetically superior crop cultivars.

The North Carolina Mating Design II supplies information about the relative magnitude of additive and dominance effects, degree of dominance, and narrow and broad sense heritabilities. It also includes a test for detecting maternal effects if present. However it does not provide any information about the presence of epistatic effects. The Triple Test Cross analysis is considerably more sophisticated in that it provides tests for the presence of epistatic variation, as well as estimating the additive and dominance components of variation when epistasis is absent.

Most of the biometrical models used to estimate genetic parameters often assume epistasis to be absent or of little importance, although these models rarely provide a valid test of this assumption. Singh and Singh (1976) showed that if the presence of epistasis is overlooked, one would not only lose the information about the implication of epistasis, but would also obtain biased estimates of additive and dominance components of genetic variation, and would thus lead to faulty breeding procedures. Where studies in various crop populations using the TTC/NCM III models have been used, it has shown that epistasis was a significant component of genetic variability for several plant parameters. Moreno (1994) suggested that interaction between genes is an important source of genetic variability and epistatic effects are larger than the quantitative theory implies. In

studying pairs of quantitative trait loci (QTL) in soybean, Lark *et al* (1995) showed that interactions between QTL are frequent and control large effects. In their investigations, an allele at one locus that exhibited little or no variation by itself, had a large effect on height via interaction with other loci.

In the present study, additive x additive epistatic effects were found to control in part the expression of salt tolerance at 80 mM NaCl, and when data from the two NaCl treatments were combined. Absence of significant j + l type epistasis mean squares for root length data (Table 3.7) indicated that additive x dominance, and dominance x dominance types of interaction between genes controlling salt tolerance was either not present or was of a relatively smaller magnitude. From an analysis of an F_2 population using the triple test cross, Singh *et al* (1986) found additive x additive epistasis to be the major contributor to the genetic variation for the morphological and yield traits in field pea. Azhar and McNeilly (1988), from a diallel analysis of sorghum cultivars, also reported non-allelic interaction for the expression of salt tolerance at 150 and 200 mM NaCl. Significant epistatic effects for yield and yield components from a triple test cross study in maize were found by Wolf and Hallauer (1997). They concluded that epistasis in maize would not greatly affect commercial breeding because present commercial maize breeding is effective in selecting favourable epistatic gene combinations. Simultaneous inbreeding and evaluation allow the fixation of favourable epistatic effects in maize inbreds that have excellent specific combining ability. The development of source populations by crossing related inbreds and recycling elite inbreds to form new source populations helps to maintain and accumulate favourable epistatic gene combinations. From a triple

test cross analysis of pearl millet accessions Singh *et al* (1991) also suggested the use of significant epistatic effects in cross-pollinated crops. Therefore, additive x additive epistatic effects revealed in the present studies for salinity tolerance in maize, are clearly of use in developing inbred lines with improved tolerance following hybridisation and selection procedures as suggested by Subbaraman and Sree Rangasamy (1989). Thus the genes with additive effects and their interaction could be fixed in a homozygous line. These results also indicated that the information derived from those models assuming no epistasis about genetic systems controlling a trait, would not be complete and be likely to be misleading.

In the absence of epistasis, analysis of variance for sums and differences provide direct tests of the significance of the additive and dominance components respectively. Whilst j and l type epistatic effects were not detected in the current investigations, in the presence of additive x additive epistasis, the estimates of additive (D) and dominance effects (H) would be biased, and no precise conclusions can therefore be drawn about the relative importance of these components in controlling NaCl tolerance. The variance of the sums of the TTC design provide more satisfactory estimates of the additive component of genetic variance even in the presence of duplicate and complementary types of epistasis (Pooni and Jinks, 1979). Jinks (1981) implied that the estimate of D is independent of the H component, but it would be biased by the presence of epistasis, and furthermore, Jinks (1981) and Kearsey (1980) suggested that D would still provide the best source of prediction of variance of the recombinant inbred population. In the results reported here both additive and dominance gene effects were significant for the expression of salt tolerance, the latter was higher in

magnitude under stress. Jinks and Perkins (1970) showed that irrespective of the presence of epistasis, the estimates of D, H, and F will change to different extents over environments if different kinds of gene action are not equally sensitive to the environments. In the present case the estimates of H did not change much in control and NaCl stress, but the differences due to the two environments for the estimates of D and F were considerable. It follows that additive gene effects are relatively more sensitive to the environment than non-additive effects. On the other hand, in the combined TTC analysis of variance, the additive x treatment interaction was not significant, indicating non-sensitivity of additive effects to the environments. However, additive x additive, and additive x additive x treatment interactions were highly significant (Table 3.7c). It appears therefore, that the additive effects *per se* are not sensitive to the environment, but the sensitivity of the additive x additive epistasis to NaCl treatments greatly influenced the estimates of D, the additive effects. It also appears from these results that the contribution of the epistatic effects to the estimates of D, was of different magnitude in control and NaCl stress. The dominance by treatment interactions was barely significant, and epistasis-2 (additive x dominance, and dominance x dominance) x treatment was highly significant (Table 3.7c) but epistasis (j + l type) *per se* was not significant, and the difference in the dominance component (H) in control and treatment was small. It thus seems that epistatic action of genes is more sensitive to the environmental differences than are the additive and dominance effects. Furthermore, the epistatic effects and their significant interaction with the treatments detected in the current analysis, support the suggestion that salinity tolerance is a complex phenomenon. Significant epistasis

x environment interactions has previously been reported in tobacco (Jinks *et al*, 1973) and in maize (Martin and Hallauer, 1976). The estimates of F were non-significant in any treatments, and were smaller in magnitude relative to D and H, therefore, any dominance, or epistasis confounded with the dominance, cannot have much of a directional element (Jinks and Perkins, 1970).

The NCM design II does not provide any information about epistatic effects and these effects were assumed to be absent (Comstock and Robinson, 1948 and 1952). Assuming the absence of epistasis the non-additive effects H_R can be taken as dominance effects (Kearsey *et al*, 1987). The additive (D_R) and dominance (H_R) effects estimated from the NCM design II were higher than the corresponding estimates of D and H derived from the Triple Test Cross. A degree of relationship between the components of genetic variation, for root length data at two NaCl treatments, obtained from the two models, was found. The dominance components in both treatments were higher than the additive components in NCM II and TTC analyses. In the NCM II analysis the estimates of dominance components were reasonably consistent in the two treatments, and also in the TTC analysis. The dominance effects were twice the estimates of additive effects under NaCl stress in both analyses. Additive components under stress were lower than in control, whilst the differences between the estimates in the two treatments were similar in magnitude in NCM II and TTC analyses. Theoretical studies (Jinks, 1983) have shown that both D and H components will be inflated by epistasis, and hence the effect on the dominance ratio $(H/D)^{1/2}$ would be small. In the present case both models revealed overdominance for the expression of salt tolerance at 80 mM NaCl, and the estimates for degree of dominance obtained

from NCM II (1.38) were remarkably similar to those obtained from TTC (1.33). It seems that H was inflated relatively more than D thus giving a high dominance ratio. Devey *et al* (1989) drew similar conclusions in *Lolium* for the estimates of degree of dominance obtained from TTC analyses from plants grown under two environmental conditions, namely drills and open plots at two locations.

Success of any breeding programme depends upon the efficiency with which selection can be performed, and estimates of heritability provide a useful guide for improvement in the character under progression. It is uncommon to estimate heritability from the analysis of triple test crosses because the testers used are the extreme selection lines and would not predict the response to selection (Kearsey and Jinks, 1968). However, Kearsey *et al* (1987) and Devey *et al* (1989) in *Lolium,* and Pooni *et al* (1994) in *Nicotiana tabacum,* estimated heritabilities from the analysis of triple test crosses. The values were found to be considerably low for the various characters examined. The heritability values from the present TTC analysis were estimated to compare with those obtained from the NCM II, despite estimates of heritability having notoriously high standard errors (Lawrence, 1984), and subject to considerable variation among different populations (Falconer and Mackay, 1996). Nevertheless, the estimates of heritability may be used to predict progress through selection (Liang *et al*, 1972), but considerable caution is necessary in their interpretation (Falconer and Mackay, 1996).

The estimated values of narrow sense heritabilities at 80 mM NaCl from the two analyses were moderate and were reasonably consistent. Broad sense heritabilities were twice the estimates of narrow sense heritabilities, which

confirms that genes with epistatic and dominance properties predominantly control salt tolerance. The estimate of broad sense heritability obtained from the TTC (0.88) was relatively smaller than the value obtained from NCM II (0.99), which was expected because of higher environmental effects in TTC analysis. The evidence that salt tolerance in maize has a narrow sense heritability of about 50% supports the view, as obtained from data of different sources in the previous Chapter, that selection for improved salt tolerance among accessions is feasible.

Variability in aluminium and manganese tolerance among maize accessions

Chapter 4

CHAPTER 4

Variability in aluminium and manganese tolerance among maize accessions

4.1. Introduction.

Aluminium and manganese toxicity are common in acid soils, their solubility increasing where soil pH is below 4.5, and represent problems to plant growth. More than half of the non-irrigated arable lands in the world are acidic (Duncan *et al*, 1983) and about 8 million ha of this land is under maize cultivation (Borreo *et al*, 1995). When lime ($CaCO_3$) is applied the toxic species of aluminium (Al^{3+}), and manganese (Mn^{2+}), precipitate in the form of their oxides, which are non toxic to plants, being replaced by Ca^{2+} on the cation exchange sites (S. Pandey, pers. comm.). The most widely used practice to overcome Al and Mn toxicity in acid soils has been the addition of lime but it can be less efficient, more expensive, labour intensive, and ecologically unsuitable, compared with using tolerant cultivars. Inter- and intra-specific differences in tolerance to aluminium and manganese have been reported in a number of crop species including maize (Urrea-Gomez *et al*, 1996), and a winter wheat germplasm, OK91P648, with increased tolerance to Al has recently been released (Smith *et al*, 1997) for cultivation on acid soils.

A number of methods have been used for the identification of the initial impacts of aluminium and manganese on plants. Solution culture is the most commonly used medium for examining Al and Mn tolerance, which provides easy access to root systems, tight control over nutrient availability and pH, and non

destructive measurements of tolerance (Little, 1988; Scott and Fisher, 1989). Foy *et al* (1978) showed that excess Al affects plant root growth. Instead of relying on root measurements, Polle *et al* (1978) proposed a simple and quicker method of haematoxylin staining for quantifying Al tolerance in plants. They recommended their method for large scale screening of seedling materials, using simple visual assessment of haematoxylin staining of roots grown in a series of Al concentrations in solution culture. However more recent evidence, for example, Hill *et al* (1989), Aniol (1990), Duque-Vargas *et al* (1994), Shuman and Wilson (1994) and Foy (1996) confirmed reduction in root growth as the most obvious consequence of aluminium toxicity. An acceptable relationship has been shown between solution culture results and plant reaction in sand culture and acid soils (Horst, 1983; Hill *et al*, 1989 and Ring *et al*, 1993).

In contrast to Al^{3+} excess, Mn^{2+} was shown by Foy *et al* (1978) to cause chlorosis and necrosis of leaves, but more recently Wilkinson and Duncan (1994) have reported that root growth is also affected by manganese in sorghum. They (Wilkinson and Duncan, 1994) also found interaction between H^{+} and Mn^{2+} with additive and antagonistic effects on root length of different sorghum genotypes.

Variation in the tolerance of wheat, triticale, cotton, and flax (Foy, 1983), to manganese has been shown, and in soybean differential Mn tolerance was found to be heritable (Heenan *et al*, 1981). The superior manganese tolerance of maize has been found to be associated with reduced transport of manganese from roots to shoots (Benac, 1976), and Al^{3+} inhibits the uptake of Mn^{2+} in plant shoots (Clark, 1977 and Pintro *et al*, 1996). Solution culture screening has been considered useful for increasing tolerance to soil acidity in different crops. For

example, Camargo *et al*, (1992) assessed 23 durum wheat accessions for aluminium tolerance in nutrient solution and confirmed their tolerance rankings in field studies on acid soils in Brazil.

The objectives of the study described in this chapter were (1) to determine concentrations of Al^{3+} and Mn^{2+} for effective screening for aluminium and manganese tolerance; (2) to screen 72 maize accessions of diverse origins for tolerance to Al^{3+} and Mn^{2+}; (3) to assess the individual and combined impacts of aluminium and manganese on 5 maize accessions.

4.2. Material and methods

4.2.1. Experimental conditions.

Maize accessions were screened against Al^{3+} and Mn^{2+} in a controlled temperature growth room at 16/8 hours (light/dark), 24±1^{0}C, 60-70 % relative humidity, and at a light intensity of 95 µM $m^{-2}S^{-1}$ PAR.

Seed were surface sterilised with 2% bleach for five minutes and then germinated on moist paper towels after thorough washing with running tap water. Upon germination the seeds were then grown for seven days in 0.1 strength Rorison solution (Hewitt, 1966) supported on five layers deep black alkathene beads in plastic troughs containing 10 litres of solution. The pH of the treatment solutions was adjusted and maintained at 4.0±0.5 throughout the duration of the experiment. Aluminium was added as Al_2 (SO_4) $_3$16H_2 O and manganese as $MnSO_4.4H_2O$. The experiment was of split plot design throughout, with concentrations as the main plots and accessions as sub-plots.

Scheme for visual scoring of manganese toxicity symptoms in maize accessions.

Score	Percentage of leaf area which is chlorotic and necrotic
1	0
2	< 15
3	16 – 30
4	31 – 45
5	46 – 60
6	61 – 75
7	> 75

4.2.2. Accession response to aluminium.

This experiment was carried out to determine an aluminium concentration, which would readily separate susceptible and more tolerant accessions, and subsequently to screen for increased aluminium tolerance. The experiment had 5 replications, each containing 6 maize seedlings, and length of the longest root was measured. Germinated seeds of two accessions, G 800 known to be moderately aluminium tolerant, and Lg 20.80 known to be susceptible to aluminium were grown for 7 days across 8 aluminium concentrations, 0.11, 0.22, 0.33, 0.44, 0.52, 0.88, and 1.10 mM Al plus control without aluminium. Relative root length was used for comparison of accessions, where,

$$\text{Relative root length (\%)} = \frac{\text{Root length in treatment solution}}{\text{Root length in control solution}} \times 100$$

4.2.3. Accession response to manganese.

This experiment was carried out to determine which manganese concentration would distinguish manganese susceptible and manganese tolerant accessions. Germinated seeds of two accessions, Lg 20.80 known to be moderately Mn tolerant, and a Mn susceptible accession, Golden, were grown at four Mn concentrations, 0, 1.0, 2.0, and 4.0 mM for 7 days. The experiment had 3 replicates, each having 10 seedlings per Mn concentration. Chlorosis and/or necrosis of the shoots were rated, 1, (zero), to 7, (severe) visually for all seedlings (see back of page 62).

4.2.4. Screening for aluminium, and manganese tolerance.

Seventy-two maize accessions were assessed for tolerance to aluminium and to manganese separately. 0.22 mM aluminium, and 2.0 mM manganese were

used, with a control solution having no Al or Mn. Root length measurements, and visual ratings of chlorosis and/or necrosis were recorded from 10 seedlings from each of the three replications. Absolute and relative root length data were used to compare maize accessions for tolerance to aluminium.

4.2.5. Accession response to aluminium, manganese, and aluminium + manganese.

This experiment was designed to examine individual and combined impacts of aluminium, and manganese. Germinated seeds of five accessions namely, Sultan, Lg 20.80, Sundance, Zea 1072, and Champ were grown for 7 days at four combinations of, 0, and 0.22 mM Al, and, 0, and 2.0 mM Mn. These accessions were chosen based on their tolerance rankings in the previous experiment (4.2.4). Length of longest root, and visual ratings for shoot chlorosis and/or necrosis were recorded from 10 seedlings in each of the three replicates.

4.2.6. Statistical analysis.

Mean absolute and relative root length data per replication, and log transformed visual ratings were statistically analysed using the Repeated Measure Anova Model of SPSS (SPSS for Windows: Advanced Statistics).

4.3. Results

4.3.1. Aluminium tolerance.

Increasing Al concentrations caused a significant ($P \leq 0.001$) decrease in the relative root lengths (Table 4.1) of both accessions G 800 and Lg 20.80 ($P \leq 0.001$). Relative root length was reduced to 60% at 0.11 mM Al^{3+} the lowest concentration used. At 0.52 mM, and higher aluminium concentrations root length of the two accessions did not decline further, and both had similar root lengths. A

clear difference in seedling root length was noticed between Al-tolerant G 800, and Al-sensitive Lg 20.80, accessions at 0.22 mM Al^{3+} (Fig 4.1). Considerable differences in Al induced inhibition of root elongation were observed among maize accessions grown in nutrient solution with 0.22 mM Al^{3+}. Absolute and relative root lengths of the seedlings of 72 maize accessions grown in a control and aluminium containing nutrient solutions are given in appendix 4.1. For simplicity and ease in data interpretation, a sub sample of 25 accessions including the most and least tolerant has been extracted and is presented in Table 4.6. Mean squares for absolute root length at, 0, and 0.22 mM Al^{3+} are given in Table 4.3, while mean squares for relative root length are presented in Table 4.4.

Accessions differed significantly ($P \leq 0.01$) for both absolute and relative root lengths in response to Al^{3+} (Table 4.3 & 4.4). The interaction between concentrations and accessions (significant at $P \leq 0.001$) for absolute root length indicated that root growth patterns of the accessions were not similar in control and Al solution. Seedlings grown in control solution without added Al and/or Mn grew well and developed normally, and did not show any toxicity and/or deficiency symptoms. Although absolute root length differed between accessions when grown without Al, the root lengths of seedlings grown with Al were generally shorter.

Generally, accessions, which produced longest roots in the Al containing solution, had the highest relative root lengths. Three land races from Bolivia, Bozm 1335, Bozm 1536, and Bozm 1337, showed high aluminium tolerance, having absolute and relative root lengths of 8.98 cm and 80%, 9.03 cm and 78%

and 9.05 cm and 77% respectively at the given level of Al toxicity, and two other accessions Zea 642, from Bulgaria, and Chzm 01008, from Chile, also had relative root lengths greater than 70%. Zea 769, from Italy, had the highest absolute and relative root length at 0.22 mM Al, the values being, 10.91 cm and 82%, respectively. The most sensitive accessions Chzm 13002, Lg 20.80, Akber, and Sultan, had relative root lengths of 20%, 20%, 20%, and 22% respectively (Table 4.6).

4.3.2. Manganese tolerance.

Leaf chlorosis and/or necrosis, the symptoms of manganese toxicity on shoots became more severe as Mn^{2+} levels increased in the nutrient solution. Visual rating (VR) data for leaf chlorosis and/or necrosis were transformed (Log_{10}) before being subjected to analyses of variance. Analysis of variance for the preliminary experiment (Table 4.2) revealed highly significant ($P \leq 0.001$) differences between concentrations, between the two maize accessions, and different responses of the two accessions to Mn^{2+} (concentration x accession significant at $P \leq 0.001$). The accession Golden from Pakistan was very susceptible to manganese at 1.0 mM and had complete shoot necrosis at 4.0 mM Mn^{2+} (Fig 4.2). Separation of susceptible and tolerant accessions by degree of leaf chlorosis was most effective at 2.0 mM Mn^{2+}.

Mean VR data (Log_{10}) of leaf chlorosis and/or necrosis of the 25 maize accessions out of 72 assessed at 2.0 mM manganese are presented in Table 4.6. The mean visual rating data of the 72 accessions are given in Appendix 4.1. Analysis of variance of the Log_{10} transformed data (Table 4.4) showed significant

differences ($P \leq 0.001$) between accessions at 2.0 mM manganese. Of the 72 accessions, five, Chzm 01009, Zea 769, Champ, Bozm 0715 and Lg 20.80, showed considerably less leaf chlorosis than the other 67 accessions with VR values of 2.3, 2.7, 2.7, 2.8 and 2.9 respectively. By contrast, the accessions Golden, Zea 699, and Chzm 03004 had greatest leaf damage VR values being between 6 and 7.

4.3.4. Tolerance to aluminium + manganese

4.3.4.1. Assessment based on relative root length.

Analysis of variance for relative root lengths of five maize accessions assessed for individual and combined tolerances are given in Table 4.5, while mean relative root length data of the five accessions are presented in Table 4.7. Analysis of variance indicated significant ($P \leq 0.001$) main effects due to treatments and accessions, as well as a significant ($P \leq 0.01$) treatment x accessions interaction for relative root length. These results suggested that accessions produced different root lengths in response to different treatments. At 0.22 mM Al alone, (Table 4.7) the relative mean root lengths of the accessions were 31% Sundance, 30% Zea 1072, 29% Champ, 22% Sultan, and 20% Lg 20.80. Relative root length was increased in the solution containing both Al (0.22 mM) and Mn (2.0 mM). The increment in relative root lengths was however similar in all the accessions except Champ where relative root length was increased from 29% at 0.22 mM Al alone to 59% in the combined solution. Manganese appeared to reduce the Al toxicity on the maize seedlings in the combined solution.

4.3.4.2. Assessment based on leaf chlorosis and/or necrosis.

No visible symptoms of leaf chlorosis and/or necrosis were observed at control and at 0.22 mM aluminium. Analysis of variance presented in table 4.5 indicated significant differences ($P \leq 0.001$) between treatments and the five accessions, and treatment x accession interaction. Which suggested that treatments produced different degrees of leaf chlorosis and/or necrosis in the accessions. VR estimates (Table 4.7) were similar for Sultan and Champ when grown in solutions with 2.0 mM Mn alone and in the Al + Mn mixture of 2.0 mM Mn and 0.22 mM Al, whilst a significant reduction in VR was observed for the accessions Sundance and Zea 1072. Leaf chlorosis symptoms however became more severe for Lg 20.80 when grown in the combined solution (VR = 3.6) than in Mn alone (VR = 2.9).

Table 4.1. Analysis of variance of relative root length data of 10-day-old seedlings of two maize accessions grown at 8 Al concentrations.

Source of variation	Degrees of freedom	Mean squares
Blocks	4	346.10*
Al^{3+} Concentrations (Conc.)	6	3823.18***
Within + Residual	16	91.01
Accessions (Acc.)	1	462.07***
Conc. X Acc.	6	140.06**
Block x Acc.	4	216.03**
Within + Residual	16	29.69

*, **, ***, Indicates differences significant at P≤ 0.05, 0.01, 0.001, respectively, whilst NS, denotes differences which are not significant. This convention is followed in all subsequent Anova tables.

Table 4.2. Analysis of variance of visual rating (log transformed) of leaf chlorosis and/or necrosis of 10-day-old seedlings of two maize accessions grown at 4 manganese concentrations.

Source of variation	Degrees of freedom	Mean squares
Blocks	2	0.0002^{NS}
Mn^{2+} Concentrations (Conc.)	3	0.87***
Within + Residual	6	0.002
Accessions (Acc.)	1	0.23***
Conc. X Acc.	3	0.04***
Block x Acc.	2	0.002^{NS}
Within + Residual	6	0.002

Table 4.3. Analysis of variance of absolute root length (cm) data of 10-day-old seedlings of 72 maize accessions grown at 2 aluminium concentrations.

Source of variation	Degrees of freedom	Mean squares
Blocks	2	58.89^{NS}
Al^{3+} Concentrations (Conc.)	1	5433.49**
Within + Residual	2	19.31
Accessions (Acc.)	71	15.52**
Conc. X Acc.	71	7.33***
Block x Acc.	142	3.27^{NS}
Within + Residual	142	3.09

Table 4.4. Analysis of variance of relative root length and visual rating data of 10-day-old seedlings of 72 maize accessions grown in solution culture.

	Relative root length (%) at 0.22 mM (Al)		Visual rating (log transformed) at 2.0 mM (Mn)	
Source of variation	Degrees of freedom	Mean squares	Degrees of freedom	Mean squares
Blocks	2	363.35^{NS}	2	0.01*
Accessions	71	582.17**	71	0.03***
Residual	136	194.34	137	0.004

Table 4.5. Analysis of variance of relative root length and visual rating data of 10-day-old seedlings of five maize accessions grown at 4 treatment combinations of Al and Mn.

	Relative root length (%)		Visual rating (log transformed)	
Source of variation	Degrees of freedom	Mean squares	Degrees of freedom	Mean squares
Blocks	2	82.02^{NS}	2	0.003^{NS}
Treatments (T.)	2	12626.60***	3	1.87***
Within + Residual	4	42.38	6	0.002
Accessions (Acc.)	4	496.74***	4	0.05***
T. x Acc.	8	232.36**	12	0.02***
Block x Acc.	8	158.89*	8	0.001^{NS}
Within + Residual	16	59.35	24	0.001

Fig. 4.1. Relative root length (%) of 10-day-old seedlings of two maize accessions grown at 4 concentrations of Al.

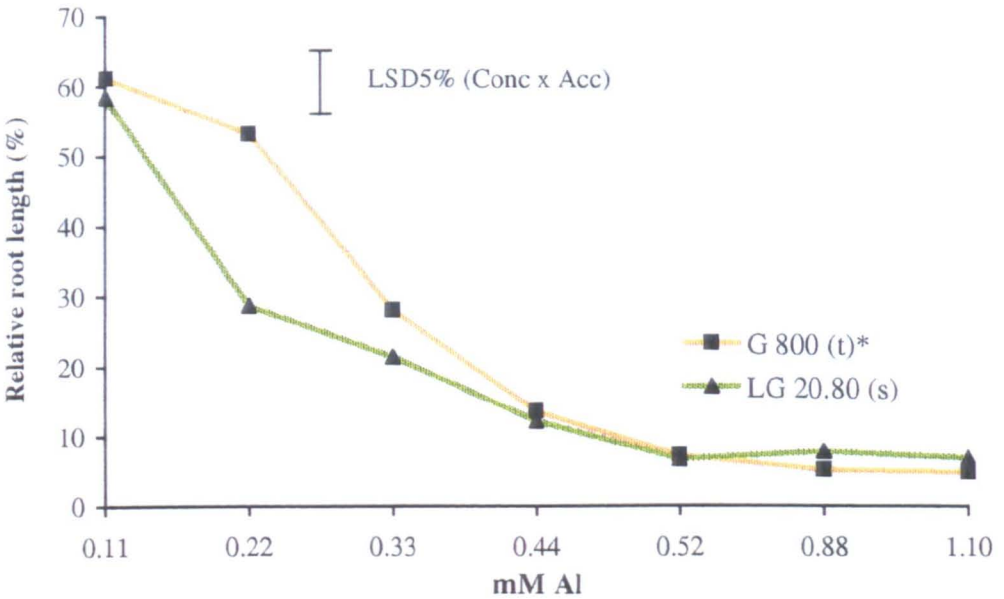
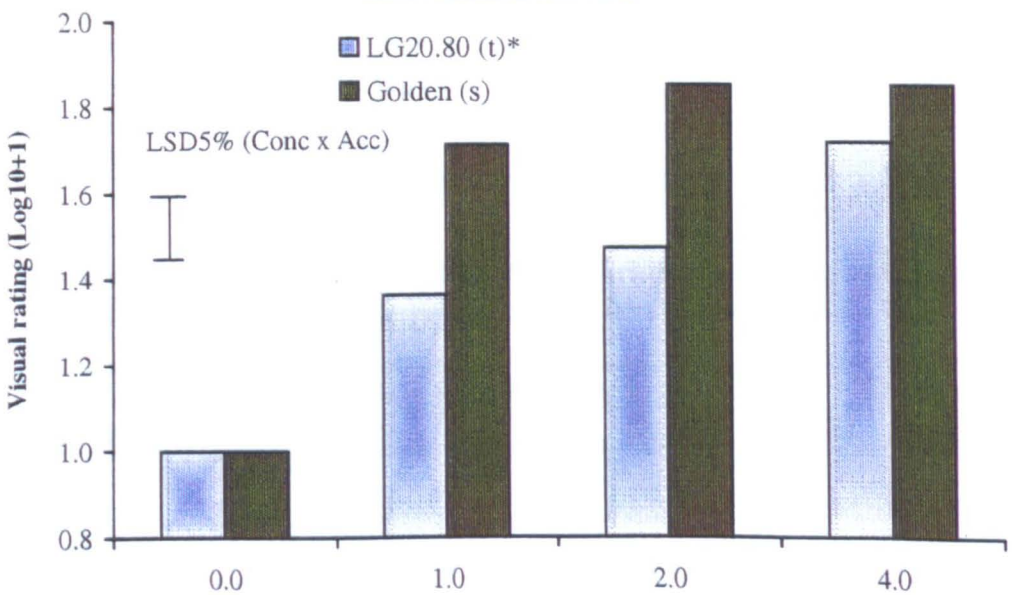


Fig. 4.2. Visual rating of leaf chlorosis and/or necrosis damage on 10-day-old seedlings of two maize accessions grown at 4 concentrations of Mn.



* t = tolerant, s = susceptible

Table 4.6: Relative root length (%) and visual rating of 10-day-old seedlings of 25 maize accessions grown at Al and Mn concentrations.

Accessions	Absolute root length (cm)		Relative root length (%)	Visual rating
	Control	0.11 mM Al	0.11 mM Al	2.0 mM Mn
Bozm 0715	16.67	7.78	46.67	2.85
Bozm 1330	15.06	8.77	58.23	4.47
Bozm 1335	11.28	8.98	79.61	4.94
Bozm 1337	11.75	9.05	77.02	4.86
Bozm 1376	12.05	7.71	63.98	4.99
Bozm 1483	15.92	7.98	50.13	5.33
Bozm 1510	13.02	8.91	68.43	5.10
Bozm 1511	14.80	8.40	56.76	5.37
Bozm 1536	11.57	9.03	78.05	4.81
Chzm 01008	12.19	8.78	72.03	3.33
Chzm 01009	13.67	5.44	39.80	2.28
Chzm 03004	11.12	3.13	28.15	6.00
Chzm 13002	9.10	1.80	19.78	4.50
Reward	9.92	2.47	24.90	4.97
Champ	15.93	4.66	29.25	2.68
Zea 642	12.11	8.80	72.67	3.30
Zea 699	13.53	8.11	59.94	6.27
Zea 769	13.3	10.91	82.03	2.66
Zea 1072	10.35	5.50	30.43	5.85
Lg 20.80	17.34	3.53	20.36	2.95
Akber	9.30	1.90	20.43	4.60
EV 6085	11.57	5.54	47.88	5.97
Golden	14.19	4.01	28.26	7.00
Sultan	14.23	3.15	22.14	5.00
SYP-31	14.01	5.16	36.83	5.80

Table 4.7: Relative root length and visual rating of 10-day-old seedlings of 5 maize accessions grown in Al and Mn alone, and Al + Mn.

Accessions	Relative root length (%)		Visual rating	
	Al	Al + Mn	Mn	Al + Mn
Sultan	22.03	32.76	5.00	5.17
Lg 20.80	20.14	32.94	2.93	3.56
Sundance	31.05	39.21	5.30	3.92
Zea 1072	30.39	40.06	5.85	5.50
Champ	29.47	59.41	2.68	2.77

4.4. Discussion.

Aluminium and manganese are frequently together present and affect potential crop production of agricultural land where soil pH is below 4.5. Whilst liming reduces toxic effects of these elements, it is not economically feasible to lime the large areas affected by Al and Mn. Therefore, there is a considerable need to find crop accessions which could grow and guarantee a threshold production in the presence of these metals. Both absolute and relative root length measurements have been used to rank crop accessions against aluminium toxicity in solution culture. In soybean, Sartain and Kamprath (1978) found it more desirable to express root growth on a relative rather than on an absolute basis. In contrast however, from a study of heavy metal tolerance in *Agrostis capillaris,* Humphreys and Nicholls (1984) suggested that root length in control and heavy metal stress was under independent genetic control, and the tolerance index/ relative root length assessment may lead to confusing/misleading interpretation of the results of crossing programmes. Nonetheless, relative root lengths of seedlings grown in nutrient solution with added aluminium has successfully been used as a selection criterion with a close relationship to adult plant performance in cowpea (Horst, 1983), and more recently in forage crops by Ring *et al*, (1993). Both absolute and relative root length data identified the same accessions as aluminium tolerant and non-tolerant in the present case.

Whereas excess aluminium restricts root growth, (Clark, 1977), excess manganese causes chlorosis and/or necrosis of plant shoots (Foy *et al*, 1978). In the current assessment of maize accessions, considerable variation was observed for tolerance to aluminium and manganese, relative root lengths decreasing

markedly with increasing Al concentrations (Fig 4.1) and visual symptoms of manganese toxicity increased as Mn in solution increased (Fig 4.2). Fig 4.1 and 4.2 clearly show that screening seedlings under a wide range of Al and Mn concentrations would indicate the range in tolerance existing among the genotypes. However, a large number of accessions using numerous stress levels would involve considerable time, labour, and expenditure. Knowledge of the probable range in tolerance would, however, allow choosing a single stress level for assessing large numbers of accessions with some rational basis. Data obtained on the preliminary assessment of maize accessions with known tolerance across a range of Al and Mn concentrations indicated marked differences in tolerance on more than one stress level, and any of these could be selected for screening maize accessions for aluminium tolerance. However, the stress levels, 0.22 mM Al^{3+} and 2.0 mM Mn^{2+}, separated the tolerant and non-tolerant accessions most effectively based on relative root length and leaf chlorosis and/or necrosis. These concentrations were found to be the most logical choice for screening the 72 maize accessions in solution culture.

Considerable variation was observed in aluminium and manganese tolerance among the maize accessions assessed as has previously been reported for Al tolerance between and within 10 Gramineae species including maize, (Wheeler, 1995) and for Mn tolerance in 29 cowpea genotypes (Horst, 1983), screened in solution and sand culture.

The evidence of Foy *et al* (1988), Scott and Fisher (1989), and Ring *et al* (1993) in crop plants supports the conclusion that tolerance to aluminium and manganese is not correlated physiologically and is thus independently inherited.

Foy *et al* (1973) found that the highly Al tolerant wheat variety Atlas 66 was sensitive to Mn, and *vice versa* for Monon, a variety known to be tolerant to Mn, and from a study of rice cultivars Nelson (1983) showed that tolerance to one metal did not confer resistance to the other metal. Similar conclusions can be drawn from the present findings (Table 4.6) as no relationship was obvious between accession tolerance to Al and Mn, and it therefore follows that co-tolerance to these two elements would not be expected, except where maize has been grown on low pH soils for several generations, and the necessary genetic variability for both Al and Mn tolerance were present. Of the 72 accessions examined, only one, Zea 769, exhibited co-tolerance to aluminium and manganese. The Mn tolerant accessions, Lg 20.80, Champ, Chzm 01009, and Bozm 0715 were relatively more sensitive to aluminium. These results again illustrate that tolerance to Al and Mn does not necessarily coincide, and different genetic bases and physiological mechanisms are involved in tolerance to the two metals.

Manganese is the second most important toxin in the acidic soils after aluminium (Foy, 1973), and the effect of excess manganese has been shown to decrease on *Phalaris aquatica* as aluminium increased, whilst root length inhibition by Al was reduced with the addition of manganese at 40, 80, and 150 ppm (Culvenor, 1985). In the current study the toxic effects of Al and Mn alone were slightly moderated when the two metals were both present, as observed in accessions Sundance and Zea 1072 where leaf chlorosis symptoms were less severe when they were grown in Al + Mn than in Mn alone. Reduction in manganese toxicity symptoms in these accessions may be due to Al induced

inhibition of Mn uptake in plant shoots as reported by Clark (1977) and Pintro *et al* (1996). Root growth inhibition caused by Al alone was slightly reduced in all the 5 accessions grown in Al + Mn solution. Ring *et al* (1993) suggested that soil microorganisms reduce the Al toxicity by increasing the rhizosphere pH near the root surface in the presence of excess manganese, but this would not be possible in short term bioassays of root length in nutrient solutions. Although considerable evidence exists that Mn reduces Al toxicity, a clear physiological explanation is still lacking.

It would seem from the above data that evaluation of relative root length and leaf chlorosis and/or necrosis, at the seedling stage, respectively in low ionic strength nutrient solution with added Al (0.22 mM) and Mn (2.0 mM), may be useful for preliminary screening of large numbers of maize or other species accessions where nutrient strength solution would be varied based upon preliminary experiments, such as that made here. Results of the present studies also suggest that progress in increasing Al and Mn tolerance could possibly be made provided the extent of variability observed among the maize accessions is genetically based. The genetic basis of variability for Al and Mn tolerance in maize is examined in Chapter 5.

Genetic basis of aluminium and manganese tolerance

Chapter 5

CHAPTER 5

Genetic basis of aluminium and manganese tolerance

5.1. Introduction.

The plant genetic improvement approach to combat the problem of aluminium and manganese toxicity in acid soil is a rather ignored means to increase economical food production, as are, but to a less degree, control of pollution, and conservation of soils, water, and energy.

A breeding programme for improving adaptation and productivity of crop cultivars on acid soils, as in general with any other, rests upon the identification and characterisation of sufficient genetic variability for tolerance to Al and Mn, and understanding the degree and nature of the inheritance of such tolerance.

Broad differences in Al and Mn tolerance have been documented among and within many plant species. The genetically controlled differences provide almost unlimited opportunities for producing superior cultivars for a particular stressful condition. The extent of genetic variability which existed for Al and Mn tolerance in 72 maize accessions has been discussed in Chapter 4.

The genetic control of Al and Mn tolerance in crop plants is poorly understood, and is clouded with conflicting evidence of simple vs. complex inheritance. For instance, Kerridge and Kronstad (1968) observed two distinct classes of tolerance in the F_4 population of a cross between two genotypes of wheat grown in a 0.06 mM Al solution, and based on seedling root growth in solution culture they came to the conclusion that Al tolerance was simply inherited. Rhue *et al* (1978) found 3:1 segregation in the F_2 generation, and 1:1

segregation in the back-cross generations of maize inbred lines grown in a 250 μM Al solution, and they also concluded that Al tolerance was controlled by a single dominant gene. However, additional evidence in maize from these authors indicated the involvement of a multiple allelic series in the control of Al tolerance. From an examination of the F_1 and back cross progenies of maize inbred lines, Garcia *et al* (1979) also concluded that a single major gene, with dominance for tolerance, with the possibility of modifiers, controlled Al tolerance. However two dominant genes were found to control Al tolerance in Atlas 66, an Al tolerant wheat variety, at 0.22 mM Al (Camargo, 1981).

Dessureaux (1959) reported that tolerance to excess Mn in alfalfa was heritable, and controlled by additive genes with little or no dominance. In lettuce species Mn tolerance however was reported to be controlled by one to four genes (Eenik and Garretsen, 1977). From tested F_2 progenies of four soybean varieties, differing in Mn tolerance, by growing them in a Mn toxic nutrient solution, Brown and Devine (1980) found that control of tolerance to excess Mn was multigenic rather than monogenic. F_1 and F_2 progenies of a cross between Mn tolerant (Lee) and Mn sensitive (Bragg) cultivars of soybean were screened in solution culture containing 275 μM Mn by Hennan *et al* (1981). The F_1 plants exhibited moderate Mn toxicity symptoms whilst the F_2 generation showed a continuous distribution in the symptoms that were skewed towards tolerance. In another study of the F_6 progeny from a cross of Amredo (tolerant) and Bragg (susceptible), a bi-model distribution was reported for Mn tolerance in soybean. It was concluded that a single gene with the possible involvement of minor genes controlled Mn tolerance in soybean.

More recently quantitative inheritance has been reported for acid soil tolerance (mainly Al and Mn, Foy *et al*, 1978) in maize. Magnavaca *et al* (1987) grew plants of six generations (P1, P2, F_1, F_2, BC_1, and BC_2), derived from a set of six crosses between Al tolerant and non-tolerant inbred lines of maize in nutrient solution. The continuous and unimodal distribution of plants within this F_2 population, suggested Al tolerance to be a quantitatively inherited trait. They also found some evidence that dominance was towards non-tolerance, but it was not consistent. Duque-Vargas *et al* (1994) estimated genetic components of variation for yield and yield related characters in maize following North Carolina Design I mating system, across acidic soils. They reported additive x environment interaction to be the most important component of genetic variance for all traits studied. Borreo *et al* (1995) suggested the preponderance of non-additive gene effects in NCM II progenies of a tropical maize population tested on acidic soils. The estimates of heritability, based on half-sib family means, were 0.39 ± 0.14 for yield, 0.43 ± 0.14 for days to silk, 0.66 ± 0.13 for ear height, 0.47 ± 0.14 for ears per plant and 0.48 ± 0.14 for ear rot across the acid soil environments.

Reciprocal differences, cytoplasmic effects, are not frequently reported because many times without any type of prior test, they are assumed to be absent even though they may in fact exist. In the presence of such differences, means of reciprocal families are altered and upon genetic analysis as such, these estimated parameters may be biased to an unknown extent (Patel and Bains, 1984). Evidence, whilst limited, on the contribution of nuclear genes for Al and Mn tolerance is available, while information on the magnitude of reciprocal effects (cytoplasmic inheritance) for tolerance to these elements is even less. Dessureaux

(1960) found that reciprocal differences for Mn tolerance occurred in alfalfa crosses. These differences were evident in number of trifoliate leaves, dry matter production, and unifoliate leaf areas, but were absent for leaf chlorosis. Maternal effects for manganese tolerance has also been reported by Brown and Devine (1980) in F_2 generation of soybean varieties. From an examination of F_2 progenies from six crosses between tolerant and sensitive cultivars of rice, Camargo (1984) recorded partial dominance for Al sensitivity with significant maternal effects. He also recorded significant additive effects, with narrow sense heritability lying between 0.50 and 0.87, depending upon the test concentration of Al.

More information on the role of cytoplasmic effects, and additive and non-additive genetic variance for aluminium and manganese tolerance would provide a strong basis to effectively breed and select maize cultivars and hybrids better adapted to acid soil conditions.

The study reported in this Chapter determines the relative importance of cytoplasmic effects, and additive and non-additive effects in the inheritance of aluminium and manganese tolerance in maize accessions using the North Carolina Design II mating system (Comstock and Robinson, 1952).

5.2. Material and methods

5.2.1. Plant material.

Crossing of 18 maize accessions namely, Zea 1006, Zea 1006 x Reward, Sundance, Lg 20.80 and Akber, designated as female, and Golden, SYP 31, Zea 769, C 12338, Bozm 1335, Bozm 1337, Bozm 1345, Bozm 1416, Bozm 1483, Bozm 1532, Bozm 1533, G 800 and EV 6085, designated as male produced the hybrid plant material used in these studies. The origin and sources of accessions

are given in Appendix 1.1. The choice of parents was based on tolerance rankings in response to increased Al, measured as relative root length, and Mn tolerance scored as the intensity of leaf chlorosis and/or necrosis, in solution culture exploited in Chapter 4.

5.2.2. Mating design and crossing procedure.

The North Carolina Design II mating system of Comstock and Robinson, (1952) was used to examine the inheritance of tolerance to Al and Mn in maize. Plants of five female and thirteen male parents were grown in 20-cm diameter plastic pots in the glasshouse during February/ March 1997, and were control-crossed according to the crossing plan. Details about raising the crossing block and crossing procedure have been outlined in (page 41) Chapter 3.

5.2.3. Assessment of the hybrid progenies in solution culture.

The response of 65 F_1 hybrids for tolerance to excess aluminium and manganese separately was assessed under controlled environmental conditions similar to those as described in Chapter 4. After surface sterilisation with 2% bleach for five minutes, 30 seeds each of 65 families were grown on rafts of black alkathene beads floated on treatment solutions prepared in 0.1 strength Rorison solution as described in Chapter 4. Measurements for longest root length were recorded from each of the 10-day-old seedlings grown at 0, and 0.11 mM Al per replication. Seedlings grown at 1.0 mM Mn treatment were scored for leaf chlorosis and/or necrosis, 1 (zero) to 7 (severe) toxicity, as described in Chapter 4.

The experimental design was split-plot with treatments as main plots and hybrids as sub-plots. Three replications were arranged in a randomised complete

block design. The initial pH of treatment solutions was adjusted to 4±0.5, monitored daily and adjusted to 4±0.5 if needed with 1N HCl or NaOH.

5.24. Statistical analysis.

Root length data for each of the seedlings from each replication of 65 hybrids and mean root length per replication, were subjected to analysis of variance separately as absolute root length in control and 0.11 mM Al, and relative root length at 0.11 mM. Leaf chlorosis and/or necrosis data scored visually from each seedling and their means per replication were transformed as Log_{10} before performing analysis of variance. Analysis of variance was performed following Becker (1992), while derivations of genetic components of variation were made according to Kearsey (1965) and Lawrence (1984). The computer package SPSS for windows, Release 6.0, was used for this purpose. The format for analysis of variance and derivations of components of variation are given in Chapter 3.

5.3. Results

5.3.1. Tolerance to excess aluminium

5.3.1.1. Absolute tolerance.

The mean squares used in the estimation of components of genetic variation for absolute root length at, 0 (control), and 0.11 mM Al are presented in Tables 5.1a-c.

Examination of the analysis of variance tables revealed highly significant ($P < 0.001$) differences between males, and between females at both Al concentrations. The male x female interaction was also significant, whilst probability levels were different in control ($P < 0.001$), and in 0.11 mM Al ($P <$

0.05). It follows that both additive and non-additive effects were involved in controlling tolerance to aluminium at 0.11 mM. Female and male mean square ratio (MS_f/MS_m) was significant for absolute root length at 0.11 mM Al, which indicated the involvement of some extra chromosomal inheritance for Al tolerance. Variance between females, a possible source of extra-chromosomal variation, was therefore not included for the estimation of additive effects, and these effects were estimated from between male variance only (Comstock and Robinson, 1952).

Significant variance among male and female indicated the pronounced role of additive genetic variation in the expression of root length under Al stress. Estimates of genetic components (Table 5.2) revealed non-additive effects (H_R) greater than additive (D_R) and environmental effects (E) for absolute tolerance. Involvement of non-additive genetic effects was also evident for absolute tolerance, as female x male interaction was significant at $P < 0.001$.

The estimates of both narrow sense (h^2_N) and broad sense (h^2_B) heritabilities 0.28 & 0.65, were greater in control conditions than at 0.11 mM Al (0.44 & 0.98). Broad sense heritability values were higher than narrow sense heritability, as would be expected, because a significant proportion of non-additive components accounted for in the estimation of genotypic variance at both Al concentrations, and cytoplasmic effects at 0.11 mM Al, might have further inflated the genotypic variance.

9.3.1.2. Relative tolerance.

Relative root length varied significantly between male ($P < 0.01$) and between female ($P < 0.001$) half-sib families in the NCM II anova (Table 5.1c),

suggesting heritable variation for Al tolerance in maize accessions. A significant difference between the magnitude of the male and female components of variance (MS_f/MS_m was significant at $P < 0.001$) produced a large variance component associated with maternal effects. Therefore, additive effects were estimated from the inter-male variance only.

The estimates of both narrow sense heritability (h^2_N) and broad sense heritability (h^2_B) for relative root length are shown in Table 5.2, in which components of genetic variation and derivations of these components are also given. Non-additive genetic effects were not detected for relative root length, the female x male interaction being non-significant ($P < 0.05$). However the possibility of non-additive effects for relative tolerance to aluminium cannot be ruled out as additive (D_R) and non-additive (H_R) effects were nearly equal, 220 vs. 219, and the F value for MS_{mf}/MS_l borders on significance. Nevertheless, narrow sense heritability shows more additive than non-additive variation for the character, and was estimated as 0.30, whilst broad sense heritability was 0.44.

Table 5.1. North Carolina Mating Design II analysis of variance of root length data evaluated at 0 (control) and 0.11 mM Al.

a) Absolute root length at 0 mM Al (Control)

Analysis of Variance (using means of families)				
Source of variation	**Degrees of freedom**	**Mean squares**	**V**	**P**
1 Replications (R)	2	8.22	5	NS
2 Males (M)	12	35.71	4	***
3 Females (F)	4	92.35	4	***
4 Males X Females	48	11.01	5	***
5 M-F combination X replicates	128	4.10		
Analysis of Variance (using individual observations)				
6 Between plots	194	55.54	7	***
7 Within plots	1064	10.99		

b) Absolute root length at 0.11 mM Al

Analysis of Variance (using means of families)				
Source of variation	**Degrees of freedom**	**Mean squares**	**V**	**P**
1 Replications (R)	2	24.06	5	**
2 Males (M)	12	16.05	4	***
3 Females (F)	4	221.40	4	***
4 Males X Females	48	7.27	5	*
5 M-F combination X replicates	128	4.96		
Analysis of Variance (using individual observations)				
6 Between plots	194	64.86	7	***
7 Within plots	1094	11.25		

c) Relative root length at 0.11 mM Al

Analysis of Variance (using means of families)				
Source of variation	**Degrees of freedom**	**Mean squares**	**V**	**P**
1 Replications (R)	2	543.98	5	NS
2 Males (M)	12	858.02	4	**
3 Females (F)	4	15297.87	4	***
4 Males X Females	48	444.55	5	NS
5 M-F combination X replicates	128	403.46		
Analysis of Variance (using individual observations)				
6 Between plots	194	3750.81	7	***
7 Within plots	945	660.60		

V, indicates variance used as the denominator in each significance test, and corresponds to the number ascribed to the various source of variation.

Table 5.2. Estimates of components of variation and heritabilities for absolute and relative root length data evaluated at, 0 (control), and 0.11 mM Al.

Genetic components	Absolute root length (cm)		Relative root length (%)
	Control (0 mM)	0.11 mM	0.11 mM
Additive effects (D_R)	14.96	4.72	220.56
Non-additive effects (H_R)	36.85	12.32	219.20
Environmental effects (E)	0.35	2.87	211.54
Additive variation (V_A)	7.48	2.36	110.28
Non-additive variation (V_D)	9.21	3.08	54.80
Genotypic variation (V_G)	16.69	5.44	165.08
Phenotypic variation (V_P)	17.04	8.31	376.62
Potence ratio (P_R)	1.56	1.61	0.99
Narrow-sense heritability (h^2_N)	0.44	0.28	0.30
Broad-sense heritability (h^2_B)	0.98	0.65	0.44

5.3.2. Tolerance to excess manganese.

The NCM II progenies of maize accessions showed a wide range in manganese tolerance assessed by leaf chlorosis and/or necrosis (Appendix 5.1). At the concentration of 1.0 mM Mn used to screen the hybrid material, all families showed at least some Mn toxicity symptoms.

Log transformed values of leaf chlorosis and/or necrosis at 1.0 mM Mn were analysed according to North Carolina Model II following Becker, 1992. Highly significant ($P < 0.001$) differences were observed between males, and between females (Table 5.3), which indicated the presence of additive genetic variation for manganese tolerance at this concentration. Significant ($P < 0.001$) male x female interaction, and similar estimates of additive (V_A) and dominance (V_D) variation (Table 5.4), i.e. 0.01, indicated that non-additive genetic variation is equally important in the control of Mn tolerance.

Quantitative environmental effects (E, 0.002) appeared much smaller than additive (D_R, 0.02) and non-additive (H_R, 0.03) effects in the control of manganese tolerance at this concentration. The ratio between male and female mean squares (MS_f/MS_m) was not significant, suggesting the absence of maternal effects for Mn tolerance in maize.

The difference between the genotypic ($V_G = 0.02$) and phenotypic ($V_P = 0.022$) variances was also very small, and thus resulted in a higher estimate of broad sense heritability 0.91, twice the value of narrow sense heritability (V_A/V_P), as would be expected on account of equal contribution of additive (V_A) and dominance (V_D) variation in the total genotypic variance. Nevertheless, narrow

sense heritability of 0.45 is quite reasonably high for dependable selection to increased Mn tolerance.

Table 5.3. Analysis of variance (North Carolina Mating Design II) of log transformed ratings of leaf chlorosis and/or necrosis at 1.0 mM Mn.

Analysis of Variance (using means of families)				
Source of variation	**Degrees of freedom**	**Mean squares**	**V**	**P**
1 Replications (R)	2	0.0004	5	NS
2 Males (M)	12	0.05	4	***
3 Females (F)	4	0.10	4	***
4 Males X Females	48	0.01	5	***
5 M-F combination X replicates	128	0.002		
Analysis of Variance (using individual observations)				
6 Between plots	194	0.07	7	***
7 Within plots	1063	0.01		

Table 5.4. Estimates of components of variation and heritabilities for leaf chlorosis and/or necrosis evaluated at 1.0 mM Mn.

Genetic component	**Visual ratings**
Additive effects (D_R)	0.02
Non-additive effects (H_R)	0.03
Environmental effects (E)	0.002
Additive variation (V_A)	0.01
Non-additive variation (V_D)	0.01
Genotypic variation (V_G)	0.02
Phenotypic variation (V_P)	0.022
Potence ratio (P_R)	1.22
Narrow-sense heritability (h^2_N)	0.45
Broad-sense heritability (h^2_B)	0.91

5.4 Discussion.

Knowledge about the extent of the genetic basis of tolerance is essential for efficient crop plant breeding where improvement in aluminium and manganese tolerance is to be sought as a means to increase more economic crop production on acid soils.

The major goal of the quantitative genetic methods is to partition the phenotypic variance for a trait in a way that isolates additive variance from non-additive and other sources of variation. Additive variance is, however, rarely completely isolated from other components particularly when maternal and/or cytoplasmic effects are involved in the phenotypic expression of a character.

In such a situation the NCM II analysis may be used with advantage, where additive variance can be estimated from the inter-sire variance component as $8\sigma^2m$ instead of jointly from inter-sire and inter-dam, $4(\sigma^2m + \sigma^2f)$, variances (Comstock and Robinson, 1952). The NCM II also provides the most efficient means of testing the significance of additive, non-additive, and maternal effects in the analysis of variance. A large number of male and female parents can be included in the crossing programme, and above all, data interpretation is straightforward. The design however has the disadvantage of providing no test of non-allelic interaction.

Results of the present study indicated the involvement of maternal, and additive and non-additive gene effects in the expression of aluminium tolerance. Maternal effects observed in the current set of data are consistent with those of Cross and Hammond (1982) who also reported reciprocal differences for ear moisture in maize, and recommended that this factor should be taken into

consideration while choosing parents for making hybrids. By contrast, Mann *et al* (1981) found no significant reciprocal effects for yield, days to silk, and ear height in a diallel study of maize genotypes evaluated in three environments. They concluded that lack of significant reciprocal effects might be caused by low variability for the traits studied, due to continuous selection within the parents and to lack of precision in the data collected. Recently, however, Borrero *et al* (1995) used the NCM II mating design to study the inheritance of acid soil tolerance, and reported significantly greater variance for females than males for grain yield in maize when grown in acid soil conditions.

Chloroplasts, mitochondria and other plastids contain DNA, and cytoplasmic inheritance and nuclear-cytoplasmic interaction are generally accepted by most biologists (Rao and Fleming, 1978). The cell wall, plasma membrane, and vacuoles have been found to play a role in excluding Al from the roots or detoxifying it after it has entered the plant (Taylor, 1995), suggesting involvement of the cytoplasm in providing resistance to Al. Inheritance of stress tolerant traits, which are of evolutionary significance, is often simple and largely of additive nature (Hoffman and Pearson, 1991). It may be argued that seed size could be a possible source of maternal effects at the seedling stage. In the current assessment however, where seedlings in the three treatments were grown from the same seed samples, non-significant maternal effects under non-stress, and significant maternal effects under Al stress for both absolute and relative root length, clearly indicates some involvement of extra chromosomal inheritance for Al tolerance at the seedling stage. It is thus, not unlikely that some hypostatic enhancers present in the cytoplasm, as suggested by Schat and ten Bookum (1992)

for copper tolerance in *Silene vulgaris*, may interact with nuclear genes to modify the expression of tolerance. Nonetheless, out of the several mechanisms explaining Al tolerance (Taylor, 1991), one mechanism could be more important than another at a specific stage of plant development (Magnavaca, 1987), and simple genetic control of Al tolerance might exist at specific growth stages.

Prediction of the rate of evolutionary change in traits showing maternal effects, relying solely on the nuclear genome, requires estimates of additive genetic variance or narrow sense heritability that exclude cytoplasmic sources of variation. Female variances were therefore not included for the estimation of additive gene effects and additive effects were estimated from inter-male variance only. It is clear from the estimation of the genetic parameters that genes having both additive and dominance effects on Al tolerance are important for absolute and relative root length at 0.11 mM aluminium (Table 5.2). Dominance effects were greater than additive effects for absolute Al tolerance. For relative Al tolerance dominance contributed equally to the genotypic variance, as did the additive effects (Table 5.2). The additive portion of genetic variance could be fixed through recombination and selection in a breeding programme while dominance effects could be best utilised in hybrid combinations. Therefore, the type of gene action revealed is clearly advantageous in hybrid breeding programmes aiming to improve Al tolerance in maize. Data obtained from half diallel progenies evaluated in soil and solution culture by Gourley *et al* (1990) showed that both additive and non-additive gene effects were responsible for Al tolerance in sorghum. However, from a series of crossing experiments these authors (Gourley *et al*, 1990) found that the type of gene action controlling Al

tolerance is partially dependent upon the technique used for the measurement of tolerance, upon the genotype used, and upon the degree of Al stress.

With complete dominance at every locus controlling the character under consideration, the potence ratio would have been 1, with partial dominance, between 0 and 1 and, with over-dominance, greater than 1 (Wigan, 1944). Potence ratio estimated as $(D_R/H_R)^{1/2}$ indicated overdominance for absolute Al tolerance, but complete dominance for relative tolerance, the values being 1.6 and 0.99 respectively. For quantitatively inherited traits, the potence ratio may not be a true estimate of the degree of dominance in the presence of non-allelic interaction. Therefore, these estimates should be interpreted with care in the present case as the NCM II design used does not provide any information about non-allelic interaction, and involvement of epistatic effects cannot be ruled out for characters which are complex in their inheritance.

When the total phenotypic variation was partitioned into genotypic and environmental components, the proportion of genetic variation 0.98 was greater in control than under 0.11 mM Al stress, being 0.65 for absolute root length and 0.44 for relative tolerance (Table 5.2). The narrow sense heritabilities were moderate and similar for absolute (0.28) and relative tolerance (0.30). In previous studies, genetic variation for aluminium tolerance in sorghum was found to be entirely due to genes with additive effects, and narrow sense heritability was 0.78 (Boye-Goni and Marcarian, 1985). Narrow sense heritabilities for root and shoot lengths in the same crop as 0.31 and 0.80 in acid soil culture, and 0.72 and 0.65 in aluminium saturated solution were found by Gourley *et al* (1990). Narrow sense heritability, estimated using half-sib family means, averaged 0.36 for agronomic traits in

maize grown on acidic soils (Duque-Vargas *et al*, 1994). Heritability values are not consistent, and are liable to change under different environments (Falconer and Mackay, 1996). Therefore, the genotypic values and quantitative environments must be defined together (Comstock and Moll, 1963) and be interpreted with caution (Falconer and Mackay, 1996).

Maternal effects did not appear in the current studies for Mn tolerance assessed as leaf chlorosis and/or necrosis symptoms at 1.0 mM manganese. The intensity of these Mn toxicity symptoms has successfully been used to rank soybean accessions for Mn tolerance with comparable results in solution and sand culture (Horst, 1983). Involvement of maternal effects has previously been suggested in the expression of Mn tolerance in alfalfa (Dessureaux, 1960) and soybean (Brown and Devine, 1980). Mann *et al* (1981) concluded that insignificant maternal effects could result because of low variability in the parents, and lack of precision in the data collection. The conclusion however, does not apply here as the parental material used in the crossing programme was of diverse origin (Appendix 1.1), and contained considerable variation (Chapter 4 and Appendix 4.1) for manganese tolerance, and the data were collected with every possible precision. The current results clearly indicate that maternal effects for Mn tolerance were either not involved or were too small to be detected at the manganese concentration used. Genes with additive and non-additive effects were equally important for the manifestation of leaf chlorosis and/or necrosis used to determine Mn tolerance. Non-additive effects were however more evident. This property of the genes is clearly advantageous for hybrid breeding programmes.

Because of significantly higher non-additive variance, epistatic effects are likely to be present in the phenotypic expression of this trait. Multigenic inheritance is indicated by the present estimates of genetic components, and has previously been advocated by Brown and Devine (1980) for Mn tolerance in soybean. Non-allelic interaction would not be surprising in polygenic characters, Mn tolerance in the present case. However as stated by Falconer and Mackay (1996) non-allelic interactions involving larger numbers of loci contribute so little that they can be ignored. The degree of dominance, as indicated by potence ratio 1.22, for the appearance of manganese toxicity symptoms is overdominance. Heterosis in Mn tolerance has previously been observed in sorghum and tolerance was reported to be partially dominant to susceptibility (Saadan, 1991). The terms 'dominant' and 'recessive' should, however, be used cautiously for tolerance related traits because of possible variation in performance of the F_1 at different levels of stress (Foy *et al*, 1988).

It is the phenotype of individuals which is being directly measured and it depends upon the degree of genetic determination, broad sense heritability, and the extent to which the phenotype is determined by the genes transmitted from the parents, narrow sense heritability, that determine how far the character is hereditary (Falconer and Mackay, 1996). Considerably high estimates of broad sense heritability (0.91) obtained for leaf chlorosis suggested that Mn tolerance in these maize accessions is genetically determined. The estimates of narrow sense heritability (0.45) is half the magnitude of broad sense heritability, which again suggested that variation in Mn tolerance is predominantly due to genes with dominance effects with possible epistatic effects. Nonetheless, the reasonably

high narrow sense heritability of 0.45 indicates the importance of genes with additive effects in the character expression, and suggests that dependable selection could be made based on these estimates. Camargo (1983) also reported high heritabilities in repose to both Al and Mn, and concluded that selection for tolerance in early segregating generations should be effective in wheat.

In conclusion, the information obtained in this Chapter indicates that both additive and non-additive genetic variation are involved in the genetic control of both aluminium and manganese tolerance. Inheritance of aluminium tolerance appeared to be partially influenced by some cytoplasmic effects. The presence of reciprocal differences and/or cytoplasmic effects should not be ignored, since these effects would inflate additive effects and the resulting estimate of narrow sense heritability.

The physiology of aluminium and manganese tolerance

Chapter 6

CHAPTER 6

The physiology of aluminium and manganese tolerance

6.1. Introduction.

Acid soil problems, mainly aluminium and manganese toxicities, can be ameliorated by modifying the soil, the plant, or both (Foy, 1983). The probability of success in any of these approaches would be greatly increased with a complete understanding of the physiological and biochemical mechanisms adapted by plants for tolerance to these toxicities.

Plants adapted to acid soils utilise a variety of mechanisms to cope with the toxic effects of aluminium and manganese. Taylor (1991) explained six different hypotheses, which could account for aluminium tolerance through exclusion, and another five hypotheses for internal tolerance mechanisms. In determining plant tolerance to manganese toxicity the majority of evidence (Foy, 1984; Carver and Ownby, 1995) suggested that high internal tolerance is more important than exclusion. Unlike Al, Mn is an essential element as (1) it participates in various reactions during respiration and photosynthesis, (2) manganese and magnesium have biological and chemical similarities, and Mn^{2+} may enter the plant by the same absorption pathway used by Mg, so that any exclusion process would run the risk of a likewise reduction in Mg uptake and transport (R. B. Clark, Pers. Comm.). Thus, while exclusion mechanisms seem to play a role in Al tolerance, Mn tolerance is based almost entirely on internal tolerance mechanisms (Carvey and Ownby, 1995). However, this is a general conclusion that tolerant plants must either be able to

reduce the absorption of these toxic elements or have some means of detoxifying the aluminium and manganese after these have been absorbed by the plant roots.

Enhanced accumulation of organic metabolites has been noted in plants undergoing various biotic and abiotic stresses, and several examples are given in this paragraph. Possingham (1956) reported accumulation of proline in the leaves of tomato plants grown with mineral nutrient deficiencies. Under high Al^{3+} concentrations amino acids increased 68% in aluminium tolerant peas but only 6% in aluminium sensitive cultivars of pea (Klimashevskii *et al*, 1970). High accumulation of proline in plant tissues was used as a trait to evaluate drought resistance in barley (Singh *et al*, 1972) and in sorghum (Sivaramakrishnan *et al*, 1988). Increased cytoplasmic acidity was found to increase free proline formation in maize (Goring, 1982). Increased levels of sucrose in bushbean, rice, soybean and cotton have been reported in response to NaCl stress by Rathert (1984). Al-Karaki *et al*, (1996) studied the effects of phosphorus nutrition and water stress on proline accumulation in sorghum and bean, and found that both plant species had higher proline accumulation when water-stressed than when under non water-stressed conditions. However they differed in their response to added phosphorus. Sorghum leaves had the highest proline when grown with high phosphorus, but bean leaves had the highest proline when grown with decreased phosphorus.

Accumulation of organic metabolites has been observed in plants under such different stresses, and from the evidence available it may be associated generally with plant survival under stress conditions. Likewise, accumulation of these metabolites also might be involved as a non-specific mechanism for aluminium and manganese tolerance. The present experiments were undertaken to

determine whether accumulation of organic metabolites is a general response to stress conditions, and seek any relationship of these factors with differential tolerance of the maize genotypes found to vary in resistance to aluminium and manganese toxicity.

6.2. Materials and methods

6.2.1. Plant material and culture conditions.

Five accessions of maize found with different tolerance to Al and/or Mn in Chapter 4 were used in this experiment. They were, accession Zea 769 tolerant to Al and Mn, Bozm 1335 tolerant to Al, Chzm 01009 tolerant to Mn, Golden sensitive to both Al and Mn, and Reward sensitive to Al. Seeds of these accessions were grown in plastic troughs containing 10 litres of solution as described in (page 62) Chapter 4. The layout of the experiment was as split plot design, treatments, 0 (control), 0.22 mM Al, and 2.0 mM Mn, being in main plots and accessions in sub plots. pH of the treatment solutions was maintained at 4.0±0.5.

6.2.2 Measurements of organic solutes.

Seedlings used for the measurement of organic solute contents were harvested after they had grown for 7 days in the treatment solutions. Fresh root and shoot from 10-days-old seedlings from each replicate were cut into small pieces, weighed, and placed separately in glass vials. 10 cm^3 of 80% (v/v) ethanol was added to each vial containing the sample, and heated at 60 ^{o}C for 30 minutes. The extract was filtered and the volume made up to 20 cm^3 with 80% (v/v) ethanol. This aqueous ethanol extract was then used for the assay of the organic metabolites.

6.2.2.1. Amino acids.

Total amino acids were determined by the colorimeter method of Rosen (1957). Ninhydrin reagent (Sigma Chem. Co.) was diluted in the ratio of 1 part reagent to 3 parts 80% (v/v) ethanol. In glass test tubes, 1 cm^3 ninhydrin was added to 1 cm^3 ethanol extracts of roots and shoot, and incubated for 15 minutes in a water bath at 90 ^{o}C. Samples were cooled and the volume made up to 10 cm^3 with 50% (v/v) n-propanol, mixed and left for colour development. Optical density at 570 nm, using a Linear Readout Grafting Spectrophotometer, was used to determine total amino acids.

For a calibration curve standard solutions were prepared with glutamic acid at 0, 0.02, 0.04, 0.06, 0.08, 0.10, 0.20, 0.60 and 1.0 mM.

6.2.2.2. Proline.

Free proline was assayed using acid ninhydrin reagent following Troll and Lindsley (1955). Acid-ninhydrin reagent was prepared by dissolving 1.259 g ninhydrin powder in 30 cm^3 glacial acetic acid and 20 cm^3 6 M orthophosphoric acid. 2 cm^3 of the aqueous homogenate sample, as used for total amino acids, was transferred into test tubes and 2 cm^3 of acid-ninhydrin was added. With marbles on top, test tubes were maintained at 95 ^{o}C for one hour in a water bath. Marbles were removed and test tubes were cooled at room temperature. 4 cm^3 toluene was added to each replicate and thoroughly mixed using a test tube rota mixer. After separation of solution layers, the toluene layer was carefully removed and placed in glass cuvetes, and absorption was determined at 518 nm using a spectrophotometer. Proline concentrations were determined from a standard curve and calculated on a

fresh weight basis. L-proline (Sigma Chem. Co.) was used to make standard solutions at concentrations of, 0.01, 0.03, 0.06, 0.08, 0.10 and 0.20 mM.

6.2.2.3. Carbohydrates.

Carbohydrates were determined following the anthrone method of Plummer (1987). Anthrone reagent was made up by dissolving 1 g anthrone in 500 cm^3 concentrated sulphuric acid. Ethanol extracts, as used for amino acids and proline assay, were 10 times diluted with 80% (v/v) ethanol for the assay of carbohydrates. 2 cm^3 anthrone reagent was taken in test tubes and tubes were placed in an ice bath. The diluted aqueous extract was added drop by drop down the sides of test tubes to avoid charring of the samples, and left to mix the contents. When fully mixed tubes were then incubated in a water bath at 90 ^{o}C for 15 minutes, cooled and absorbance read at 625 nm. Standard curves were calibrated using sucrose at 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mM concentrations as standard solutions.

Data for the accumulation of organic metabolites were statistically analysed using Repeated Measure Analysis of Variance model of SPSS (SPSS for Windows, Release 6).

6.3. Results

6.3.1. Amino acids.

The analysis of variance presented in Table 6.1a showed that the accessions differed significantly ($P< 0.001$) in the accumulation of amino acids in both shoots and roots. Concentrations of amino acids in shoots and roots of the 5 maize accessions in response to Al and Mn stress are presented in Fig 6.1a,b.

Control seedlings grown without Al and Mn, accumulated comparatively less amino acids. The highest accumulation in shoots and roots of the seedlings

occurred when accessions were grown with excess Al. The response of the accessions to different treatments differed significantly for both shoot and root data (accession x treatment interaction significant at $P< 0.01$). The concentration of amino acids in shoots and roots of Zea 769, which is tolerant to both Al and Mn, was highest at 0.22 mM Al whilst the Al sensitive accessions, Golden and Reward had the same amino acid contents as Bozm 1335, the Al only tolerant accession. The Mn only tolerant accession, Chzm 01009, contained less amino acids than the rest including Golden, the Mn sensitive accession, in both roots and shoots when grown at 2.0 mM Mn.

6.3.2. Proline.

Accessions differed significantly in accumulation of proline in shoots ($P < 0.01$) and roots ($P < 0.001$), however a greater proportion of proline was retained in the roots (Fig 6.2a,b). Differences between treatments were significant for root data ($P < 0.01$) but were non significant ($P > 0.05$) for shoot data. Whilst the pattern of proline accumulation was different in the 5 accessions in Al and Mn treatments; the interaction, accession x treatments being significant ($P < 0.05$) for both shoot and root data (Table 6.1b). The largest accumulation of proline was observed at 0.22 mM Al in the roots of Zea 769, tolerant to both Al and Mn, followed by the Al tolerant accession Bozm 1335. The Al sensitive accession, Reward accumulated almost the same concentrations of proline in all the three treatments in both shoots and roots. In the control and, Al and Mn treatments the differences in root proline contents of Golden, non-tolerant to Al and Mn, were also non significant (Fig 6.2b). The Al tolerant accessions, Bozm 1335 and Zea 769 accumulated relatively higher shoot proline when grown with 0.22 mM Al. At 2.0 mM Mn, the intolerant

accession, Golden accumulated significantly higher and lower proline contents respectively in shoots and roots.

6.3.3. Carbohydrates.

There were significant ($P < 0.001$) differences between accessions in the accumulation of carbohydrates in both shoots and roots (Table 6.1c). Accumulation differed significantly between accessions in different treatments (accessions x treatments interaction significant at $P < 0.05$, shoots and $P < 0.001$, roots). Root carbohydrate concentrations were higher than shoots in all the 5 maize accessions. The Al tolerant accessions, Zea 769 and Bozm 1335, accumulated higher concentration of carbohydrates than the sensitive accessions under Al and Mn stress in both shoot and root. Carbohydrate accumulation in Chzm 01009 (Mn sensitive) was relatively lower than the rest of the accessions at 0.22 mM Al, but at 2.0 mM Mn, carbohydrate content of Chzm 01009 was higher, although non significantly ($P > 0.05$), than the Mn sensitive accession. Carbohydrate content in shoots and roots of Reward did not differ across treatments.

Table 6.1. Analysis of variance of concentrations of amino acids, proline and carbohydrates in shoots and roots of 10-day-old seedlings grown in nutrient solution with, 0 (control), 0.22 mM Al and 2.0 mM

a: amino acids

Sources of variation	Degrees of freedom	Mean squares	
		Shoot	Root
Blocks (B)	2	0.22^{NS}	6.44*
Treatments (T)	2	84.39**	53.48***
Within + Residual	4	5.29	0.94
Accessions (Acc)	4	21.58***	8.41***
Acc x T	8	15.70**	5.22**
Acc x B	8	2.54^{NS}	0.87^{NS}
Within + Residual	16	2.88	1.00

b: proline

Sources of variation	Degrees of freedom	Mean squares	
		Shoot	Root
Blocks (B)	2	0.24^{NS}	2.06^{NS}
Treatments (T)	2	3.17^{NS}	37.85**
Within + Residual	4	0.73	1.25
Accessions (Acc)	4	1.95**	20.49***
Acc x T	8	0.84*	6.16**
Acc x B	8	0.04^{NS}	2.96^{NS}
Within + Residual	16	0.33	2.16

c: carbohydrates

Sources of variation	Degrees of freedom	Mean squares	
		Shoot	Root
Blocks (B)	2	16.56^{NS}	16.98^{NS}
Treatments (T)	2	489.36**	944.51***
Within + Residual	4	16.65	12.75
Accessions (Acc)	4	319.42***	536.47***
Acc x T	8	70.16*	149.11***
Acc x B	8	15.00^{NS}	21.60^{NS}
Within + Residual	16	24.37	14.12

Fig.6.1. Amino acid concentrations in shoots and roots of 10-day-old seedlings of maize accessions grown in nutrient solution with 0, (control), 0.22 mM Al and 2.0 mM Mn.

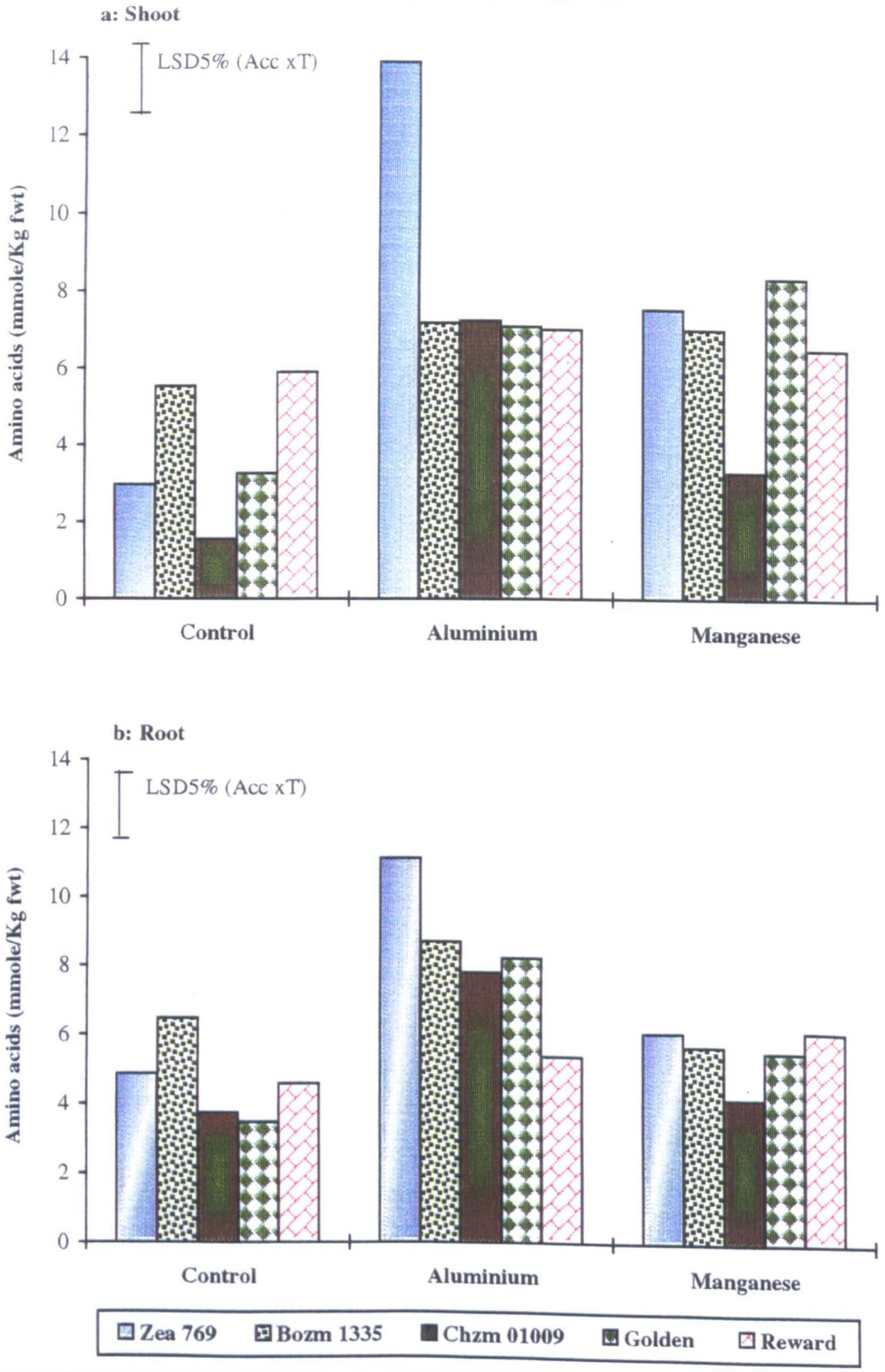


Fig.6.2. Proline concentrations in shoots and roots of 10-day-old seedlings of maize accessions grown in nutrient solution with 0, (control), 0.22 mM Al and 2.0 mM Mn.

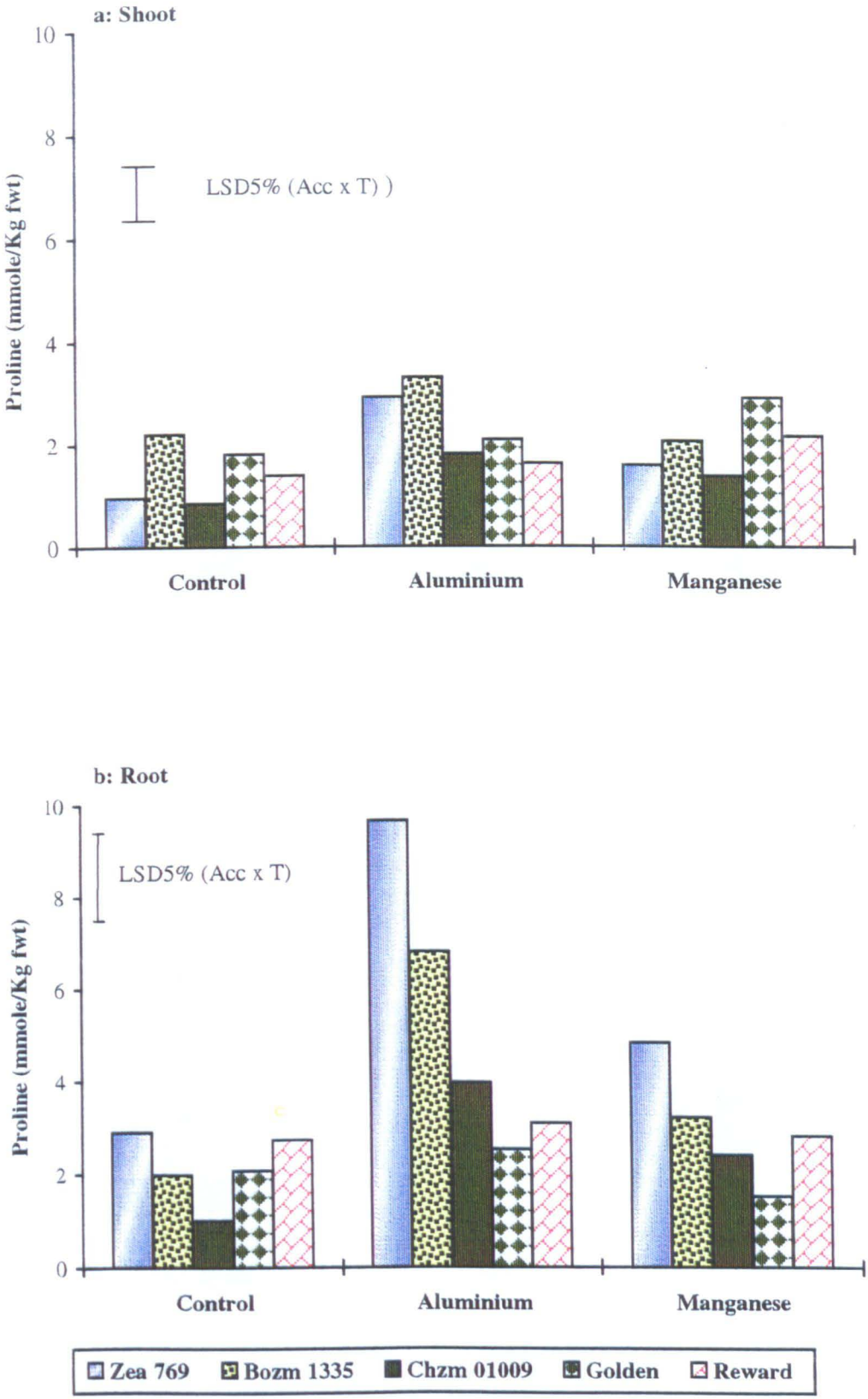
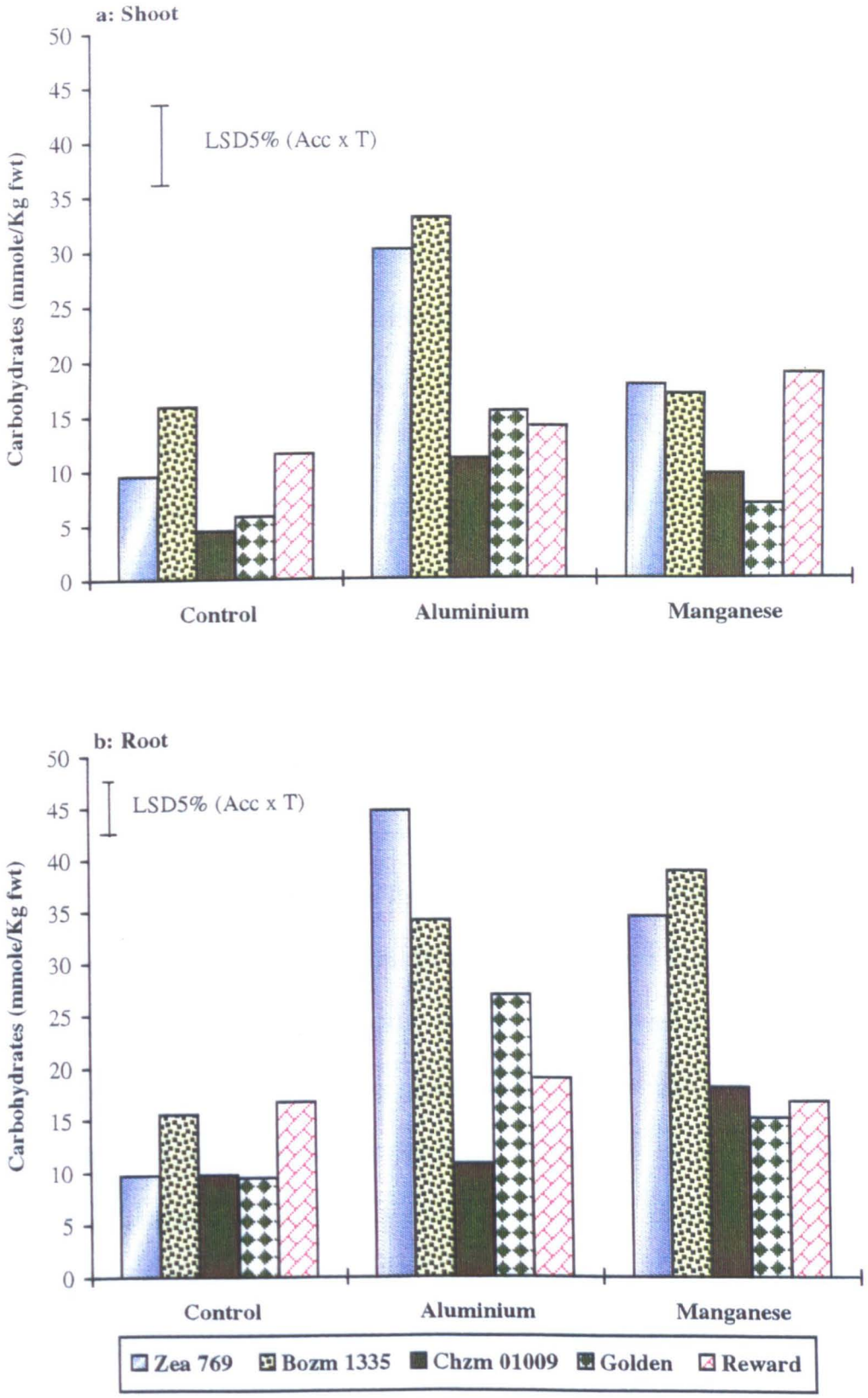


Fig.6.3. Carbohydrate concentrations in shoots and roots of 10-day-old seedlings of maize accessions grown in nutrient solution with 0, (control), 0.22 mM Al and 2.0 mM Mn.



6.4. Discussion.

Heavy metal ions accumulate in different parts of the plant after they are absorbed by the root system, often resulting in retardation of plant growth. This could be due to their interference with the activities of a number of enzymes essential for normal metabolism and developmental processes (Van Assche and Clijsters, 1990). In a recent review Bohnert *et al*, (1995) concluded that the means whereby all organisms tolerate abiotic stress, to some degree, is by accumulating compatible solutes which include nitrogen-containing compounds (proline, other amino acids, and polyamines) and hydroxyl compounds (sucrose, polyols, and oligosaccharides).

Higher concentrations of amino acids in the roots of Al-tolerant cultivars, when grown with excess aluminium have been reported in pea (Klimashevskii *et al*, 1970) and sorghum (Cambraria *et al*, 1983). Strogonov (1973) showed that in pea plants necrosis, caused by salt poisoning, is normally accompanied by an increase in amino acid contents. Manganese toxicity also causes chlorosis and/or necrosis of leaves and plants grown with excess Mn^{2+} may also accumulate amino acids. The Al and Mn tolerant accession Zea 769 used in this study accumulated more amino acids both in shoots and roots than the other tolerant and non-tolerant accessions under excess Al and Mn treatments. Amino acid accumulation observed in the present study is not in complete agreement with the previous studies in pea (Klimashevskii *et al*, 1970) and sorghum (Cambraria *et al*, 1983), where an increase in amino acid content was observed in the roots of Al-tolerant plants. The Al tolerant accession, Bozm 1535, and Mn tolerant accession, Chzm 01009, accumulated amino acids no higher than the non-tolerant accessions, when grown

with added Al and Mn. The amino acid concentrations in shoots and roots of Chzm 01009 were rather lower than Golden, the Mn sensitive accession, when grown in Mn solution. Various hypotheses have been formulated previously and tested to explain the mechanism of aluminium and manganese tolerance (Foy, 1984; Taylor, 1991), but the exact mechanism(s) by which plants tolerate the toxic effects of these substances are still not clear, probably due to their varied effects in plants. Foy *et al*, (1978) suggested that different species and varieties within a species may adopt different physiological mechanisms for tolerance to aluminium, and manganese.

Proline holds an important position among the 20 naturally occurring amino acids, conferring particular properties upon proteins due to its unique structural properties (Yaron and Naider, 1993). Proline has been considered to play an important role in osmoregulation (Aspinall and Paleg, 1981), protecting enzyme denaturation (Paleg *et al*, 1984), acting as a reservoir of carbon and nitrogen source (Fukutoku and Yamada, 1982), stabilising the machinery of protein synthesis (Kandpal and Rao, 1985) and regulating the cytosolic acidity (Venekamp, 1989). Klismashevskii (1983) concluded that free proline accumulation could be used as a physiological based test for plant stress response to metal toxicity in roots. Increased levels of Al enhanced proline in the roots of sorghum genotypes (Galvez *et al*, 1991) and proline increased in *Cajanus cajan*, *Vigna mungo*, and *Triticum aestivum* cultivars under heavy metal stress (Ali and Saradhi, 1991). However from a recent study of sorghum genotypes Zaifnejed *et al*, (1997) reported that proline did not increase in shoots and roots of plants grown with 200, 400, and 600 μM Al. Proline concentrations in the shoots of the 5 maize accessions examined did not change with Al and Mn treatments but proline contents increased especially, in the

roots of tolerant accessions grown with excess Al and Mn. Proline concentrations were highest in the Al tolerant accessions Zea 769 and Bozm 1535, in high Al treatment. Aluminium toxicity can damage roots to the extent that they cannot absorb adequate water and nutrient uptake is reduced while Al and Mn both cause nutrient imbalance in the plants (Foy, 1984). It is possible that increased accumulation of proline in the roots of plants grown with Al and Mn might be a non-specific or an indirect response to Al and Mn toxicity caused by nutrient imbalance. However larger increases of proline in the roots of tolerant accessions in contrast to the sensitive accessions, suggest that proline accumulation might be involved in conferring tolerance to Al and Mn toxicity.

Increased concentrations of carbohydrates were found in the shoots and roots of Zea 769 and Bozm 1535 in response to Al treatment, and Zea 769 and Chzm 01009 in response to Mn treatment with a greater increase noted for the tolerant genotypes. A similar response has been shown by Cambraia *et al*, (1983), who reported that an Al-tolerant hybrid of sorghum accumulated greater concentrations of soluble sugars than a sensitive hybrid. They also found that soluble sugar contents increased in both hybrids with Al treatment until 2 ppm and then remained nearly constant.

In conclusion, the increase in organic metabolites in response to aluminium and manganese stress supports the view, as obtained from data of different sources that accumulation of these metabolites may be a general response to stress conditions. Synthesis of organic metabolites may be one of the non-specific physiological mechanisms that follow a stereotypic pattern, irrespective of the nature of stress factors (Larcher, 1995).

A feasibility study for the use of molecular markers (RAPDs) for salinity tolerance

Chapter 7

CHAPTER 7

A feasibility study for the use of molecular markers (RAPDs) for salinity tolerance

7.1. Introduction.

Breeding for salt tolerance is often hampered by complexity of the trait, which is affected by a number of interacting plant and environmental factors. The apparent quantitative nature of inheritance of the genes conferring tolerance has further complicated breeding efforts (Flowers *et al*, 1997). One approach to facilitate the selection and breeding for complex traits such as salt tolerance is the identification and utilisation of simply inherited genetic markers that are genetically associated with the trait(s) of interest (Stuber *et al*, 1992; Dudley, 1993; Foolad *et al*, 1995). Molecular markers tightly linked to genes of interest allow the simultaneous selection for several traits and the rapid elimination of undesirable characteristics introduced from the donor parents following the initial hybridisation, thereby reducing both the number of generations required to introgress a gene and the extent of "linkage drag" (Dudley 1993). To increase the efficiency of artificial selection for several traits, genetic/molecular markers alone or combined with traditional phenotypic methods can be used through indirect selection or marker assisted selection. Molecular genetic markers are increasingly finding applications in plant breeding programmes and genetic studies. They are useful for fingerprinting varieties, establishing phylogenies, tagging desirable genes, determining similarities among inbreds and mapping plant genomes.

The development of molecular techniques, restriction fragment analysis by Southern blotting and DNA hybridisation, and *in vitro* amplification of DNA by polymerase chain reaction have significantly contributed to the development of DNA-level genetic markers for constructing linkage maps.

Through the development of restriction fragment length polymorphism (RFLP) markers in the early eighties, indirect selection in plant breeding using DNA markers became technically feasible. Restriction fragment length polymorphism is variation between individuals in the lengths of DNA fragments produced by digestion with restriction endonuclease. RFLPs have been used extensively to develop genomic maps (Caetano-Anolles *et al*, 1991), establish linkages to traits (Osborn *et al*, 1987), develop phylogenetic trees (Song *et al*, 1988) and tag chromosomes (McGrath *et al*, 1990). These markers have the advantages that they are phenotypically neutral, codominantly inherited, non-specific to growth stage, and practically limitless in number. However detection of RFLPs by Southern blotting is often laborious, time consuming, and expensive, and requires large amounts of high molecular weight high quality DNA for each individual assay. In addition the RFLP procedure involves the use of radioactive material.

With the introduction of polymerase chain reaction (PCR) technology (Saiki *et al*, 1988) several alternate strategies such as minisatellite, microsatellite, and random amplified polymorphic DNA (RAPD) become available to the scientists to generate genetic markers (Williams *et al*, 1990; Caetano-Anolles *et al*, 1991).

Minisatellites are not randomly distributed in the genome (Royle *et al*, 1988; Drinkwater *et al*, 1990; Georges *et al*, 1990), and they are technically difficult to adopt in PCR-based assays (Decorte *et al*, 1990). Therefore the use of minisatellite markers was overtaken by the discovery of microsatellites.

Microsatellites are DNA sequences containing a simple tandemly repeated motif composed of 1-6 base pairs (Weber and May, 1989) and, as with minisatellites, polymorphism is due to variation in the numbers of tandem repeats. Microsatellite markers have contributed enormously to generate mapping and analysis of complex traits in animals (Georges *et al*, 1993; Hearne *et al*, 1992; Andersson *et al*, 1994).

Random amplified polymorphic DNA (RAPD) markers are well suited to high throughput systems required in plant breeding because of its simplicity and relatively low cost (Williams *et al*, 1990). RAPD markers are anonymous DNA fragments generated by PCR amplification of DNA at multiple loci using a single short oligonucleotide of arbitrary sequence (Welsh and McClelland, 1990; Williams *et al*, 1990). Electrophoresis of the resultant DNA fragments in an agarose gel reveals band patterns that are characteristic of the sequence of the primer and/or the template.

Genetic analysis using RAPDs is attractive because (1) the prior nucleotide sequence of the template is not required, (2) a universal set of primers can be used for all species, (3) it permits simultaneous investigation of multiple loci in a single PCR reaction, (4) no probe libraries, radioactivity or Southern transfers required, (5) very minute quantities of DNA samples are required to act as template in the PCR, and (6) the process can be automated. The limitation to

the use of RAPD markers is that they are dominant. However Williams *et al* (1990) indicated that it could be overcome by using more than one closely linked marker.

Available data indicate that RAPD technology is suitable for studies of genetic diversity and DNA fingerprinting (Welsh *et al*, 1991; Wilde *et al*, 1992), for rapid identification of markers linked to important plant genes (Martin *et al*, 1991; Klein-Lankhorst *et al*, 1992), and for the construction of high density genetic maps (Reiter *et al*, 1992). From RAPD analyses of an F_2 population of rice, Haiyuan *et al* (1998) demonstrated that a single major dominant gene controls salt tolerance in rice. Foolad and Chen (1998) identified favourable QTLs for salinity tolerance from RAPD marker studies in an interspecific cross of tomato. They suggested greater chances of recovering transgressive segregants for salt tolerance in the progenies, which can be identified through RAPD markers.

The present preliminary studies were undertaken to test the feasibility of using RAPDs on the plant material generated in this project. Specifically, polymorphism was sought in two salt tolerant accessions, and a small sample of their F_2 progeny using a limited number of random10-mers.

7.2. Materials and methods

7.2.1. Plant material.

Two salt tolerant accessions, Zeo1, from Zeneca Seeds, UK and Sundance, from USA, and their F_2 population provided the material for experimentation. The accessions were crossed by Rao (1997) by artificial hand pollination in the glasshouse during summer, 1996. The crossing procedure is described in Chapter

3. The F_2 population was derived by polycrossing a group of 50 F_1 plants in isolation in polythene tunnels at Ness Botanical Garden, Wirral, Cheshire.

7.2.2. Extraction of DNA.

Genomic DNA of each of the parental accessions and 16 randomly selected F_2 progeny was extracted from the leaves of three-week-old seedlings grown in 8-cm pots in a heated glasshouse. Leaves were harvested from single seedlings of each parent and the F_2 progeny. The Phytopure™ DNA isolation Kit (Scotlab) was used for genomic DNA extraction according to the manufacturer's instructions.

The procedure was as follows:

7.2.2.1. Breaking the cell wall.

a) One gram sample (fresh weight) of leaf tissue was weighed and frozen in liquid nitrogen, and stored at - 20 ^{0}C.

b) Frozen leaf tissue samples were ground in a mortar and pestle under liquid nitrogen until homogenised and a fine powder was obtained.

c) The powder was immediately transferred into a polypropylene centrifuge tube using a chilled spatula.

7.2.2.2. Cell Lysis.

a) 4.6 ml of Reagent 1 was added and thoroughly mixed ensuring that all reagent ingredients were fully mixed

b) 1.5 ml of Reagent 2 was added.

c) The tube was inverted several times until a homogeneous mixture was obtained.

d) The mixture was incubated at 65 ^{0}C in a shaking water bath for 10 minutes.

e) The sample was placed on ice for 20 minutes.

7.2.2.3. DNA extraction.

a) The sample was removed from ice and 2 ml cold isoamyl alcohol ($CHCl_3$: IAA{24:1}) was added, which had been stored at -20 ^{0}C.

b) 200 µl of Nucleon Phytopure DNA extraction resin suspension was added, the resin was shaken vigorously before addition.

c) The sample was shaken at room temperature on a tilt shaker for 10 minutes.

d) Centrifuged at 1300 g for 10 minutes.

e) Holding the tube vertically, and without disturbing the nucleon resin suspension layer, only the DNA containing upper phase above the Nucleon Phytopure resin layer (brown in colour) was transferred into a fresh centrifuge tube, using a pasteur pipette.

7.2.2.4. DNA precipitation.

a) One volume of cold isopropanol was added to the tube.

b) The tube was gently inverted several times until the DNA precipitated.

c) The mixture was centrifuged at 4000 g for 5 minutes to pellet the DNA.

d) The DNA pellet was washed with 70% ethanol to remove the residual salts.

e) The sample was centrifuged at 4000 g for 5 minutes to pellet the DNA.

f) The supernatant was discarded leaving the pellet in the tube.

g) The DNA pellet was air-dried either by inverting the tubes or vacuum dried for 15 minutes. In either case, care was taken not to over dry the pellet, which would make it difficult to dissolve.

h) The DNA was resuspended in 400 µl sterilised distilled water, and stored at - 20 ^{0}C.

7.2.3. Digestion of the DNA preparation with RNase.

To obtain RNA-free DNA, RNase A was added to the resuspended DNA samples, and incubated at 37 ^{0}C for 30 minutes.

7.2.4. Quantification of DNA.

Nucleic acids were quantified by measurement of optical density (OD) in a Perkin-Elmer Lambda 2 UV/VIS Spectrometer. An absorbance of 1 at 260 nm wavelength corresponds to 50 μg ml^{-1} for double stranded DNA, 37 μg ml^{-1} for single stranded DNA and 33 μg ml^{-1} for oligonucleotides. Contaminating protein absorbs at 280 nm and thus the ratio of absorbance at 260 nm to 280 nm gives an indication of purity. DNA having an OD 260 nm/280 nm of around 1.8 is usually required for most molecular techniques.

7.2.5. RAPD analysis

7.2.5.1. Primers.

RAPD analyses were performed using four random 10 base pair primers synthesised by Perkin-Elmer Applied Biosystems UK. The primers were characterised by an arbitrary sequence, while satisfying the imposed condition of 50-70% G + C content and no internal repeats. Sequences of the primers used in these studies are given in Table 7.1.

7.2.5.2. DNA amplification.

Polymerase chain reaction (PCR) was carried under a variety of conditions for reproducible DNA amplification. PCR was tested with respect to, template, source of Taq DNA polymerase, $MgCl_2$ and primer concentrations, final volume of the reaction, denaturation and annealing temperatures, PCR tubes, different thermal cyclers, and number of cycles during the optimisation process.

7.2.5.3. Standard polymerase chain reaction (PCR).

The amplifications were performed in a PTC-100™ Programmable Thermal Cycler (MJ-Research). PCR amplifications were performed in a total volume of 10 µl in 0.5 ml thin walled PCR tubes. *Ampli*Taq® DNA Polymerase (Perkin-Elmer) was used for amplification. The PCR buffer obtained from Perkin-Elmer already contained Magnesium chloride ($MgCl_2$) therefore no additional $MgCl_2$ was added. dNTPs were made into 2.5 mM mixes from different stocks (Boehringer Mannheim). The template DNAs and primers were all dissolved in sterile distilled water (SDW), rather than TE (Tris EDTA), to avoid any inhibitory effect of EDTA (Ethylen Diamine Tetra Acetate). Sterile distilled water for PCR reactions was prepared by autoclaving 15 ml aliquots of double distilled water (Purite Super Still, Purite) in acid washed glass universals. Acid washing of the glassware ensures that the water is free from detergent. A bottle of SDW which was used successfully in an initial PCR was divided into 1 ml aliquots and stored at –20 ^{0}C for subsequent use. The PCR mix (90 µl) was constituted as follows:

1) SDW	make to 90.0 µl
2) 10x PCR buffer	10.0 µl
3) dNTPs mix (2.5 mM stock)	8.0 µl
4) Primers (0.2 µM)	2.0 µl
5) *Ampli*Taq® DNA Polymerase (2.5 Units/100 µl)	0.5 µl

This was mixed and dispensed into ten 9 µl aliquots before the addition of 1 µl of DNA template (20 ng/µl) to each tube.

To prevent losses through evaporation in the thermal cycler, the 10 µl reaction mix in each tube was overlaid with mineral oil (Sigma Chemicals Co.). The reaction was then allowed to proceed through a temperature cycle, which caused denaturation of the template DNA, annealing of single stranded primers to exposed template, followed by the extension of new polynucleotide from the anchored primer. Continuous cycling results in a logarithmic increase of the DNA fragment defined by the primers used.

A typical temperature profile and cycle was as follows:

1 – Initial denaturation	94 ^{0}C for 60 seconds
2 – Denaturation	94 ^{0}C for 10 seconds
3 – Primer annealing*	29 ^{0}C for 30 seconds
4 – Primer extension	72 ^{0}C for 60 seconds
5 – Cycles back to 2	40 times
6 – Final extension	72 ^{0}C for 5 minutes
7 – Stage	4 ^{0}C for 15 hours

* The annealing temperature of each primer was selected on the basis of GC ratio according to the following formula.

Annealing temperature = $\{2\,^0C(A + T) + 4^0C(G + C)\} - 5^0C$

7.2.6. RAPD product separation.

RAPD products were visualised with ethidium bromide after electrophoresis on 2% (w/v) agarose gels. Electrophoresis was carried out in 58 x 20 x 25 horizontal gel tanks (Gibco-BRL). The agarose was dissolved in 1x TAE (Tris Acetate EDTA) buffer (Sambrook *et al*, 1989) by heating in a microwave oven set on medium power. To maintain the concentration of the gel and buffer,

the flask containing the agarose suspension was weighed prior to heating and losses through evaporation were made up with sterile distilled water. The molten agarose was cooled down to about 55 ^{0}C, and poured into a levelled mould and a comb was placed in it. For DNA staining, ethidium bromide was included in the molten agarose prior to pouring, to a concentration of 5 μg ml^{-1}. Ethidium bromide affects the electrophoretic mobility of DNA, in its presence, the greater the concentration of DNA the faster it migrates. The gels were submerged in 1x TAE buffer, and amplified DNA samples were loaded in $^{1}/_{10}$ volume of loading buffer. Care was taken not to load the oil but the amplified DNA. Gels were run at 80-100 volts for a period that allowed the desirable separation.

The stained DNA was visualised under UV light and photographed using a video camera (Hitachi).

7.3. Results

7.3.1. DNA extraction.

The extraction of clean, high quality DNA in large quantities is the first prerequisite for most of the DNA profiling techniques. The Puregene™ DNA Isolation Kit (Gentra) and Phytopure™ DNA Isolation Kit (Scotlab) were tested during preliminary experimentation for DNA extraction.

The Puregene kit is designed for DNA extraction from a wide variety of organisms. The expected yield 30-300 μg DNA was not consistent using the large-scale method, which required a 300 mg tissue sample, and the DNA extracted through this protocol was of low quality, and demanded extra centrifugation and time for cleaning. Whilst larger tissue samples (1 g) were required for the protocol using the Phytopure kit, reasonable quantities of DNA

were obtained which were suitable for PCR analysis. Therefore DNA from all samples was extracted using the Phytopure kit.

The Phytopure system is designed specifically for plants, especially those species in which polysaccharide contamination presents a significant problem. Polysaccharides are removed through centrifugation after their binding to a special resin. The DNA yield of the 20 samples examined ranged from 500 to 950 µg per gram of fresh tissue. After digestion with RNAase OD ratios (260 nm/280 nm) of the DNA samples were between 1.7 and 1.8. The DNA obtained through the Phytopure method was used in RAPD analyses without any further purification or cleaning.

7.3.2. Optimisation of amplification conditions.

Four random 10-mer primers were used for PCR amplification and identification of polymorphic markers. During optimisation, several cycling conditions and reaction components were tested in order to obtain an optimal RAPD protocol. Different DNA patterns were produced with *Taq* DNA polymerase (Boehringer Mannheim), *AmpliTaq* Gold (Perkin-Elmer) and *AmpliTaq* (Perkin-Elmer). By taking into consideration cost and reproducible amplification, the optimum concentration was obtained from reactions using 5 units of *AmpliTaq* DNA polymerase. Tests of reaction components also included different concentrations of DNA template, primer and dNTPs. The optimal reaction conditions were obtained with 10 µl volume and consisted of 20 ng of template, 0.2 µM Primer, 200 µM dNTPs, and 1x PCR buffer ($MgCl_2$ included, from Perkin-Elmer).

7.3.3. Characterisation of RAPDs.

The inheritance of RAPD markers was studied by sampling 16 F_2 progenies derived from the cross Zeo 1 x Sundance. The results presented here (Table 7.1, Fig 7.1) are limited for practical reasons by the small number of F_2 progeny surveyed with the RAPD procedure using only 4 primers.

Table. 7.1. Sequence of primers used in RAPD analysis, annealing temperature and number of bands.

Name	Sequence (5′ to 3′)	Annealing temperature	Number of bands	
			Monomorphic	Polymorphic
OPA01	CAGGCCCTTC	29 ^{0}C	1	1
OPA13	CAGCACCCAC	29 ^{0}C	1	6
OPB08	GTCCACACGG	29 ^{0}C	-	7
OPC06	GAACGGACTC	27 ^{0}C	1	4

Fig. 7.1. shows the RAPD banding patterns of the amplified samples obtained by using four 10-mer primers. Lanes 2 and 3 represents the two parents, Zeo 1 and Sundance respectively, and lane 4 to 19 represents the 16 F_2 progenies. Lane 1 and 20 represents the 12 Kb ladder used as a marker. None of the 4 decamers gave satisfactory banding in the gels with all the genotypes. The number of amplified fragments ranged from 1 to 7 (Table 7.1). The size of the amplified fragments that could be scored ranged from 1.6 to 4.0 kb.

The banding pattern generated by the primer OPA01 was not very informative (Fig 7.1a). It displayed monomorphic profiles or very low level of variation, unsuited to the discrimination of genotypes. F_2 sample number 10 (lane

13) did not work, and bands generated by sample number 15 and 16 (lane 18, 19) were faint and not clear. However, the parent Zeo 1, which did not show any band with the other three primers was amplified with OPA01 primer.

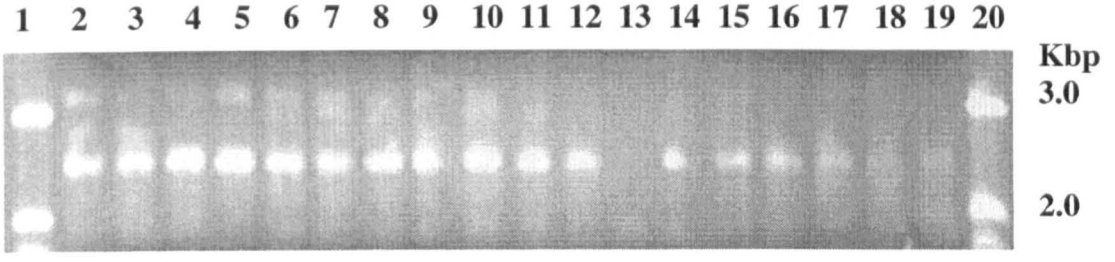
The primer OPA13 revealed polymorphism (Fig. 7.1b), although it did not work in one of the parents, Zeo 1 (lane 2), and three F_2 samples (lanes 9, 11, 13). It generated one monomorphic and 6 polymorphic band. A heteroduplex band was observed in the F_2 sample numbers, 13, 15 and 16 (lane 15, 18, 19), showing codominant nature of the marker.

The amplification of the DNA samples with the primer OPB08 was very poor; it did not work in about 50% of the samples including the two parents. However, it can been seen from Fig. 7.1c that OPB08 revealed polymorphism among the F_2 progeny. Seven polymorphic bands were scored in the 8 samples that worked. Whilst both parents did not work, the heteroduplex bands were observed in the F_2 progeny (lane 9, 12, 13, 18) suggesting codominance for the marker.

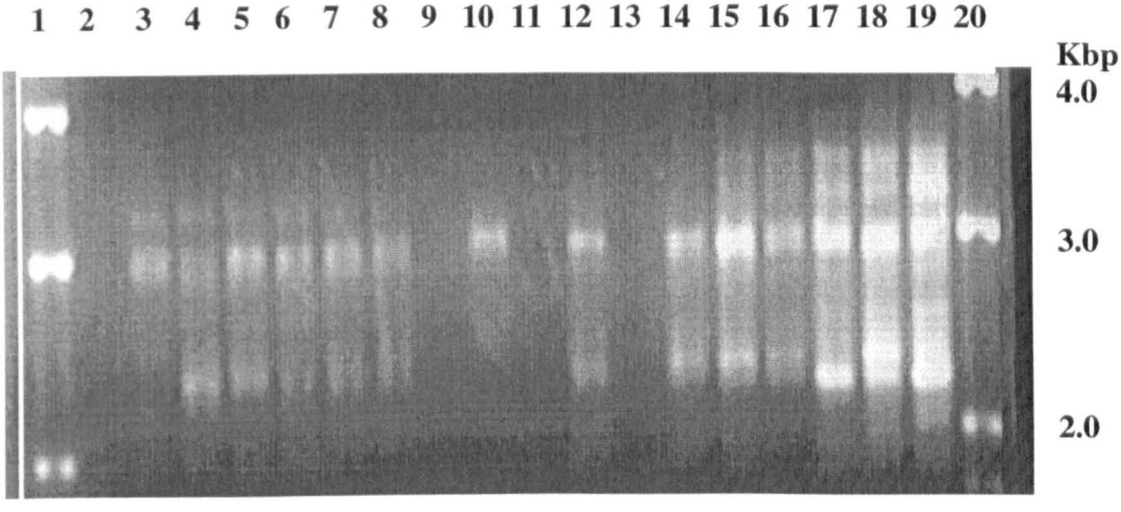
The banding pattern revealed by Primer OPC06 is shown in Fig 7.1d. OPC06 worked in one of the parents Sundance, and 13 samples of the F_2 population. The banding pattern generated by the primer showed polymorphism. Six bands were scored for polymorphism. A dominant band can be clearly observed in the population, and heteroduplex band was also observed in samples 6 and 12 (lane 9, 16).

Fig. 7.1. RAPD patterns of F_2 population of a cross between two salt tolerant accessions, using different primers.

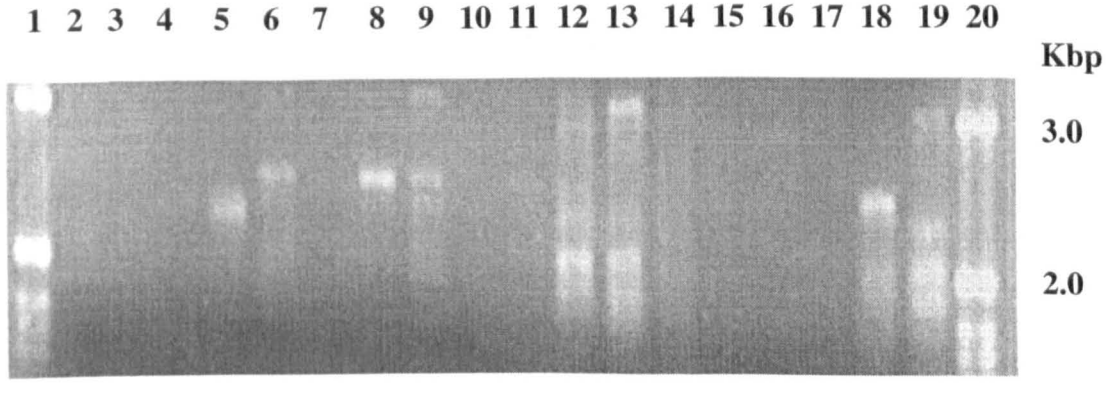
a) OPA 01.



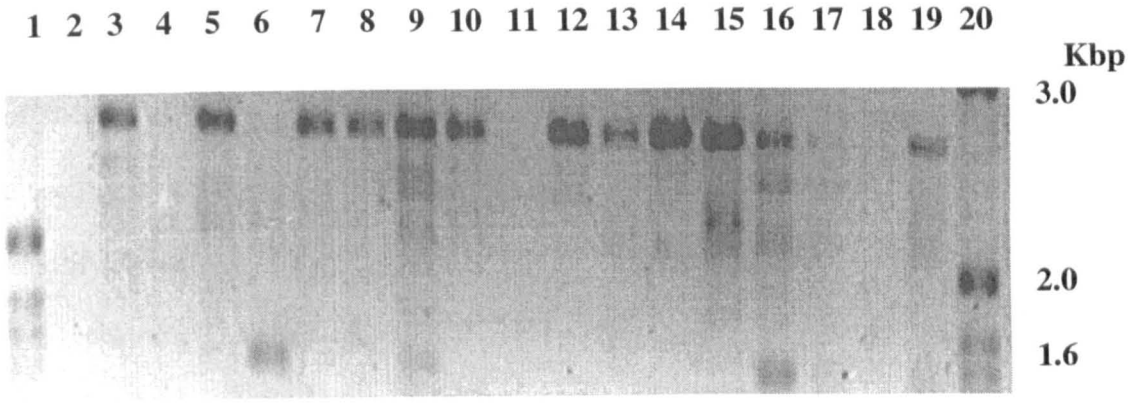
b) OPA 13



c) OPB 08



d) OPC 06



Legends (Fig, a-d).

Lanes 1and 20.	**Marker**
Lane 2.	**Parent 1 (Zeo 1)**
Lane 3.	**Parent 2 (Sundance)**
Lanes 4 to 19.	**F_2 progeny**

Kbp, Kilo base pairs.

7.4. Discussion.

Genomic analysis of RAPDs has been well documented in a wide variety of species including plants (Welsh *et al*, 1991). The methodology is very flexible since, by changing the amplification conditions a single primer can conveniently amplify from few to numerous different sequences to produce amplification fingerprinting (Caetano-Anolles *et al*, 1991). RAPD markers used by Markan *et al* (1993) for genetic analysis revealed extensive DNA polymorphism in maize. In their studies, about 75% of the tested markers generated discrete amplification products and one third of these produced bands polymorphic between the two maize lines used. On the basis of their data they (Marsan *et al*, 1993) suggested that RAPD analysis is suitable for the construction of genetic maps in maize.

The four primers used in the present studies, for generating RAPD patterns were chosen based upon their consistency of amplification strength assessed by Fritsch *et al* (1993), who characterised 480 primers (10-mer) for RAPD analysis in flowering plants.

Based on a limited data obtained from the present results it is not possible to make any conclusive remarks about the suitability of RAPD markers in the genetic analysis of salinity tolerance in maize. However, the level of polymorphism obtained using only 4 random primers is encouraging, compared with the 40 primers tested by Huff (1997) for investigating inter- and intra-cultivar variation in perennial rye grass, of the 40, only 2 generated polymorphic information. Similarly in a study involving much larger screening of primers, Bai *et al* (1997), found that of 420 primers, only 36 could be used to assess genetic diversity amongst populations of North American ginseng (*Panax quinquefolius*).

In the current endeavour, all the primers revealed at least some degree of polymorphism in the samples that worked. Some samples did not work even with different amplification conditions. The failure of PCR amplification in these samples may be attributed to poor quality of the template. Kepinski (1997) also experienced such problem during his DNA profiling studies in *Anthoxanthum odoratum*. Fritsch *et al* (1993) found that they could often improve amplification strength and consistency by simply furthering the purification of the DNA template. The number and size of the DNA fragments generated strictly depends on the nucleotide sequence of the primer used and the source of the DNA (Ferreira and Keim, 1997), and 100% matching between primer and template DNA was found necessary to obtain amplification in maize (Marsan *et al*, 1993). Therefore, a minor mismatch between the template and the primer might have been the cause of amplification failure in these maize samples.

RAPD markers are inherited in a Mendelian fashion, but unlike other DNA markers, which are codominant, RAPDs are commonly inherited as dominant markers, where the presence of a particular band is dominant, and its absence is recessive (Tingey and del Tufo, 1993). Codominant markers are comparatively rare (Schulz *et al*, 1994). Whilst, one of the parents Zeo1 did not work, heteroduplex bands which seem to be non parental were observed in the profiles generated by the primers, OPA13, OPB08 and OPC06, which are associated with codominant RAPDs. Davis *et al* (1995) also identified codominant RAPD markers in chickpea and strawberry populations mapped and suggested that these markers are valuable because of their genetic information

content. According to Cai *et al* (1994) these non-parental heteroduplex bands could originate from intra-allelic interaction at an amplifiable region.

Consistency and reproducibility of RAPD patterns has been questioned by many molecular biologists (e.g. Devos and Gale, 1992; Reidy *et al*, 1992). The sensitivity of the RAPD technique to change in experimental parameters is well known (Devos and Gale, 1992; Reidy *et al*, 1992; Munthali *et al*, 1996). Several cycling conditions and reaction components were tested in these studies in order to obtain an optimal RAPD protocol. The RAPD PCR amplifications were extremely sensitive, and a single change in the concentration of reaction component and/or thermal cycling parameters appeared to be altering the RAPD patterns significantly. RAPD patterns even appeared to be machine specific. The sensitivity of PCR amplifications is perhaps not surprising because PCR amplifications are temperature dependent, and any variation in PCR components would not provide the optimum temperature required for the de-naturation and annealing, thereby resulting into an altered product. Nevertheless, once optimised, the RAPD has the potential of providing an effective and convenient method to generate molecular markers, which could be utilised in marker aided selection of complex traits.

General discussion

Chapter 8

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General discussion

Environmental stresses, whether salinity, drought, aluminium, and/or manganese toxicity which have already plagued vast productive areas of agriculture land, are a potential threat to crop agriculture all over the world. World food production *per caput* more than kept pace with population growth over the period 1950-1980 (Evans, 1993). This statistic has not, however, continued its previous steady rate of increase over the last two decades (Flowers and Yeo, 1995). Current projections of world population are a vital challenge to the agriculture scientists to maintain food production at a level that does not reduce food supply on a *per capita* basis. This highlights the importance of maintaining the productivity of the existing good arable land, and improving the productivity of those soils suffering from salinity, drought, and aluminium, and manganese toxicity, and other growth limiting factors. The engineering and agronomic means for correcting affected soils are often technically difficult and costly - if possible at all. It follows therefore, that utilisation of the biological potential for selection and breeding for tolerant cultivars, as has been found useful to combat the devastating effects of salinity (Shannon, 1985), and aluminium and manganese (Blum, 1985), seems to be the obvious choice.

For successful exploitation of the biological potential, three important prerequisites have to be met. First, the most suitable, cheap, and fast technique for identifying tolerance must be available for screening large numbers of plants. Second, appropriate genetic variability among plant material must exist and

sources of tolerance must be identified. Third, the mode of inheritance of tolerance must be known.

The work described in this thesis was directed to assess genetic variability in maize accessions for tolerance to salinity and aluminium toxicity, based on root growth measurements, to manganese toxicity based on the intensity of leaf chlorosis and/or necrosis, and to provide information about the mode of inheritance of these tolerances. Preliminary attempts have been made to link some physiological parameters with aluminium and manganese tolerance, and some preliminary studies for the feasibility of using molecular markers for tolerance breeding.

Crop scientists are continuously searching for criteria to select plants tolerant to any given soil stress. Tolerance is a phenotypic trait, and requires the stressful conditions for its expression. The most obvious medium for screening plants for tolerance to soil stresses is a particular soil itself. However, screening large numbers of plant material under field conditions, is generally not applicable because of the inevitable non-uniformity of the environments, and confounding effects of other variables such as pH, soil temperature. A screening method that could provide quantitative information, objectively over short time periods, would increase the precision and information content of measurements and decrease interference by environmental effects (Yeo, 1994). Water culture methods used in the series of experiments for assessing variability at the seedling stage to tolerance to salinity, and aluminium and/or manganese seem to achieve these objectives. The method provides uniform conditions and easy access to the entire root system, tight control over nutrient availability and pH, and non-destructive

measurement of tolerance (Scott and Fisher, 1989). Roots and shoots of the plants grown in the solutions can be used for chemical/physiological analyses, and plants can be easily transferred to other media (Epstein *et al*, 1980).

Evaluation of crop genotypes for salinity and mineral stress tolerance during the juvenile stage has been found useful in a number of crops species. It has been shown that tolerance to salinity and aluminium stress at the seedling stage also reflects improved tolerance at adult plant level. For example, Camargo *et al* (1992) assessed 23 wheat accessions for aluminium tolerance using solution culture, and confirmed their tolerance rankings in field studies on acid soils in Brazil. Relative salinity tolerance of seedlings was shown to reflect the tolerance of adult grain sorghum plants (Hassanein and Azab, 1993). Variation in maize seedling response to salinity has also been shown to reflect potential grain yield at maturity (Maiti *et al*, 1996).

Rapid screening tests, based on identifying early differences in growth response to salinity (or drought) have been considered useful/desirable for quantifying tolerance in crop accessions and breeding material (Blum, 1988). Salinity rapidly inhibits root growth, and hence capacity for uptake of water and essential mineral nutrients from the soil, and consequently may be used as an index in characterising plant genotype for salinity tolerance (Neumann, 1995). Relative root length, and salinity tolerance parameters characterising root length response of the 10-day-old seedlings to four NaCl concentrations, obtained by using a non-linear least square inversion method, were used to assess salinity tolerance in 72 maize accessions (Chapter 2). The non-linear least square inversion method was developed by van Genuchten and Hoffman (1984), with

several options applicable to different kinds of data. Two models NOPT 5 and NOPT 12 used in this study, have been considered useful for quantifying salinity tolerance at the seedling stage in two species of millet and tef (Kebebew and McNeilly, 1995), in 52 accessions of maize (Rao, 1997), and in other crops at various growth stages. NOPT 5 provides estimate of absolute response curves, the threshold salt concentration, Ct, at which root growth begins to decrease, and the concentration at which root length equals to zero, C0. NOPT 12 provides an estimate C50 of the salt concentration at which root length reduces to 50% of its control length or any other character that is easily measured. Combining these two techniques has provided useful information for the assessment of salinity tolerance in the maize accessions examined. From the estimates of Ct and C50, and relative root length presented in Chapter 2, it is clear that there is considerable variability for salt tolerance in the 72 maize accessions examined. Plotting the estimates of Ct for each of the accession against C50, with each graph subdivided into 4 quadrants (see Chapter 2) effectively separated the tolerant and non-tolerant accessions. It appeared from the patterns of response functions that the two tolerance parameters, Ct and C50, are controlled by different genetic systems in the maize accessions examined. From the extent of variability within the parameters it appears that each is controlled by QTL.

Three accessions, Champ, PI 503567, and PI 503568, which had high Ct and C50 values and higher mean relative root lengths, were identified as the most salt tolerant. Both Ct and C50 have been proposed as reference parameters for selection in the previous studies (e.g. Martinez-Cob *et al*, 1987; Kebebew and McNeilly, 1995; Steppuhn *et al*, 1996). The present estimates of response

functions suggest that selection of accessions possessing genes both for high Ct and C50 would be worthwhile for enhancing salinity tolerance in maize.

Information about the mode of inheritance of tolerance is obviously a necessary link between the existence of variability and selection, and breeding for improved salt tolerance in any crop. Root growth measurements in salinised solution culture were used again in investigations to estimate the genetic control of salt tolerance at the maize seedling stage, as has been effectively used previously in several species to assess the genetic basis of salt tolerance, as in rice (Moeljopawiro and Akehashi, 1981), sorghum (Azhar and McNeilly, 1988), wheat (Ashraf and McNeilly, 1991), and pearl millet (Kebebew and McNeilly, 1996).

In cross-pollinated crops, maize in this particular case, an accession tolerant to a particular stress may either be utilised directly after screening, or to create new genetic variability for the development of genetically superior inbred lines, which could be used to synthesise commercial hybrids and/or a synthetic variety. The development of inbred lines involves the exploitation of additive genetic variance, while the others depend on the relative magnitude of additive, dominance, and epistasis components (Jinks *et al*, 1969). An understanding of the nature of genetic variance (additive, dominance, epistatic) and their interaction with non-heritable factors, allow the breeders to make more accurate decisions about breeding methodologies and selection techniques. The use of two biometrical techniques, NCM II, and TTC (Chapter 3) provided reasonably comprehensive information about the inheritance of salt tolerance at the seedling stage in maize, from maternal effects to epistasis. Whilst maternal effects were not

appeared to be involved to any great extent, significant epistatic effects for root growth at 80 mM NaCl confirmed the complex nature of the inheritance of salt tolerance, as was also suggested by Azhar and McNeilly (1988) for salinity tolerance in sorghum. The high values of broad sense heritability obtained from TTC and NCM II for salt tolerance in maize accessions revealed that variation in root growth in response to salinity was largely genetically based. The estimates of narrow sense heritability indicated that 50% of this genetic variability was additive and the other 50% due to dominance and epistasis interactions. The indication from the present results that the direction of dominance is towards increased salinity tolerance, confirms the views of Azhar and McNeilly (1988), Ashraf and McNeilly (1990) and Al-Khatib *et al* (1994b). The nature of gene action revealed suggests that prospects of improving the salinity tolerance in maize through hybrid breeding are considerable.

In most acid soils, aluminium and manganese toxicities go hand in hand, but are not correlated physiologically (Foy *et al*, 1988; Scott and Fisher, 1989; Ring *et al*, 1993). Whilst aluminium toxicity restricts root growth, manganese toxicity causes chlorosis and necrosis of shoots. A single concentration for aluminium and manganese toxicity tests was determined by assessing pairs of maize accessions with known tolerances, across a range of Al and Mn concentrations, which were then used to examine variability in 72 maize accessions for tolerance to these metals (Chapter 4).

Clearly, increasing aluminium concentrations reduced the root growth, and increasing Mn concentrations accelerated the onset and intensity of leaf chlorosis and necrosis. It was also found that there was considerable variability in tolerance

to Al and Mn among the maize accessions examined. The absence of any relationship for tolerance to aluminium and manganese, provided the evidence that the two metals are not "related physiologically". Only one accession, Zea 769 from 72 accessions was co-tolerant to both aluminium and manganese. Whilst, available genetic and physiological data (Foy *et al*, 1988; Scott and Fisher, 1989; Ring *et al*, 1993) indicate that there is no association between aluminium and manganese tolerance, the possibility of incorporating both types of tolerance in one genotype has been suggested by Foy *et al* (1973) and Blum (1988). Interestingly, the toxic effects observed in aluminium and manganese alone were slightly moderated when the two metals were both present in the growing solution, suggesting a positive interaction between them. Such interaction has also been observed in forage crops by Ring *et al* (1993).

Successful exploitation of such variability as has been found here is only possible if the variation observed for aluminium and manganese is genetically based. The North Carolina design II mating system and analysis (Chapter 5) provide preliminary information about the genetic basis of variability in aluminium and manganese tolerance in maize. This clearly indicated that the observed variability for tolerance to the two metals is genetically based. Genetic variation for the characters used to assess variability, appeared to be influenced by genes with additive and dominance effects, dominance effects being predominant. Maternal effects were found in the inheritance of root growth capacity under aluminium stress, but not in the control conditions, nor for the manganese toxicity symptoms under manganese stress. It seems from these results that along with nuclear genes, some cytoplasmic enhancers (Schat and ten Bookum, 1992) are

involved in the expression of aluminium tolerance. Borrero *et al* (1995) also observed maternal effects in the inheritance of acid soil tolerance in maize. It is true that with additivity, the best variety would be a pureline, but with additivity one should not expect heterosis. Therefore, the preponderance of dominance effects observed in the inheritance of aluminium and manganese tolerance is clearly advantageous in the breeding programmes aiming to exploit heterosis for tolerance to these metals.

Physiological and biochemical mechanisms of stress tolerance in general, and aluminium and manganese tolerance in particular, are still not well defined, despite these being actively investigated in many parts of the world (Foy, 1997). Increased understanding of these processes would potentially be able to lead to the development of crop cultivars with improved tolerance. Blum (1988) has suggested that the use of physiological parameters should simplify the genetics and breeding procedures. The physiology of plant responses to salinity and their relation to salinity tolerance have been much researched and frequently reviewed in recent years (e.g., Munns, 1993; Larcher, 1995). Synthesis of organic solutes (amino acids, proline, and carbohydrates) is a common feature observed in plants experiencing various stressful situations. How far these are related to stress tolerance however is still not clear. In the present study (Chapter 6) it was shown that both aluminium and/or manganese tolerant and non-tolerant accessions accumulated more organic solutes under aluminium and manganese stress than control. Generally, tolerant accessions accumulated more organic solutes than the non-tolerant accessions.

Hoffman and Pearson (1991) suggested two approaches for investigating genetic variation for stress responses. One approach starts by partitioning the phenotypic variation in stress response traits into genetic and non-genetic components, as an initial step in characterising the genetic variation. This is the standard approach used in quantitative genetics and does not assume any knowledge about the loci contributing to the genetic variance. In the present studies, this approach was followed, basically, to gain information about the genetic basis of tolerance to salinity, and Al and Mn toxicity, in maize. An alternative approach starts with a genetic polymorphism or variation in a trait with a known genetic basis involved in stress tolerance. This approach was also used to study polymorphism for salt tolerance through the RAPD technique (Chapter 7). Whilst the level of polymorphism obtained using a limited number of primers and small population size was reasonable, it became increasingly and frustratingly clear that the chosen technology was extremely sensitive to the DNA amplification conditions. It also became clear that the application of RAPDs in this study was severely hampered by the quality of the DNA obtained from different plant samples. DNA amplification through this technique required specific conditions, sometimes not possible to achieve. RAPD patterns obtained by one thermal cycler were not reproducible on the other, even when using the same DNA amplification protocols. This is a common problem associated with RAPD analysis, and concerns about the reproducibility of RAPD band patterns have been shown by most molecular biologists (e.g., Devos and Gale, 1992; Reidy *et al*, 1992; Munthali *et al*, 1996). It became clear later in the project, that the microsatellite technique is much more robust and reproducible because of the use

of relatively long primers in amplification. A further advantage is that microsatellite markers are codominant and show Mendelian inheritance, allowing their use in linkage analyses as well as in population studies and breeding programmes. Quantitative genetic methods can be applied to the QTL data generated by microsatellite analyses. Further work and resources would have allowed exploitation of this technique, which has the potential of providing more precise information about the genetic basis of salinity tolerance.

In conclusion, to feed the increasing world population, crop accessions have to be developed which can grow and guarantee a threshold production on vast areas affected by soil salinity and acidity. This series of experiments demonstrated systematically that variability in maize accessions for tolerance to salinity, Al, and Mn does exist, and clearly this variability has a significant component of genetic variation, which is heritable. It also appeared that land race material, as was used here, is likely to provide more useable variability for tolerance to soil stresses. Furthermore, the suggestions made by Epstein *et al* (1980), Blum (1985) and McNeilly (1990), that progress in stress tolerance based on seedling assays is possible through selection and breeding, with or without background knowledge of the physiological and biochemical mechanisms involved in stress tolerance, seems to be the most cost effective and valid. The use of physiological and molecular techniques may well have much to offer, as aids to breeding for stress tolerance in future, but their use in present day breeding does not yet exist. However, looking at the slow progress in terms of breeding stress tolerant varieties, it seems that collaborative efforts of a team of plant breeders, physiologists and molecular biologists is perhaps needed in future. In the

immediate future, breeding programmes aiming to improve stress tolerance in crop species should continue to exploit existing variability present in the crop varieties and/or land races and wild relatives, through selection and breeding. Epistasis is one of the major sources of variability in natural populations, this component should not be ignored in determining the modes of inheritance and devising breeding methodologies. The synthetic system of variety constitution and commercial hybrid breeding in cross-pollinated crops such as maize, would be effective in selecting favourable epistatic gene combinations involved in a character expression.

Furthermore, the increasing understanding of the physiological and molecular bases of stress tolerance should provoke more active research using genetic engineering. Future work should include more emphasis on the engineering of metabolic pathways into crop plants, and the development of transgen ic plants to be used as a new source material for use in conventional breeding programmes. The use of transgenic plants would not only be a step forward towards improving stress tolerance in the crop plants, but their use in physiological studies would also increase our understanding of stress response mechanisms.

References

REFERENCES

Ahmed, S. 1990. Soil salinity and water management. In: *Proc. of the Indo-Pak workshop on soil salinity and water management.* pp. 3-18. PARC: Islamabad.

Ahsan, M., Wright, D. and Virk, D.S. 1996. Genetic analysis of salt tolerance in spring wheat (*Triticum aestivum* L.). *Cereal Res. Communication*, 24 (3), 353-360.

Ali, A. and Saradhi, P.P. 1991. Proline accumulation under heavy-metal stress. *J. Plant Physiol.*, 138(5), 554-558.

Al-Karaki, G.N., Clark, R.B. and Sullivan, C.Y. 1996. Phosphorus nutrition and water stress effects on proline in sorghum and bean. *J. Plant Physiol.*, 148, 745-751.

Al-Khatib, M. 1991. An assessment of the potential for improving salt tolerance in alfalfa, *Medicago sativa* (L.). Ph.D. Thesis, The University of Liverpool, Liverpool, UK.

Al-Khatib. M., McNeilly, T. and Collins, J.C. 1994a. Between and within cultivar variability in salt tolerance in lucerne, (*Medicago sativa* L.). *Gen. Res. And Crop Evolution*, 41, 159-164.

Al-Khatib. M., McNeilly, T. and Collins, J.C. 1994b. The genetic basis of salt tolerance in Lucerne (*Medicago sativa* L.). *J. Genet & Breed.* 48, 169-174.

Andersson, L., Haley, C.S., Ellegren, H., Knott, S.A., Johansson, M., Andersson, K., Anderssoneklund, L., Edforslilja, I., Fredholm, M., Hansson, I.,

Hakansson, J. and Lundstrom, K. 1994. Genetic-mapping of quantitative trait loci for growth and fatness in pigs. *Science*, 263, 1771-1774.

Aniol, A. 1990. Genetics of tolerance to aluminium in wheat (*Triticum aestivum* L Thell). *Plant and Soil*, 123, 223-227.

Ashraf, M. and McNeilly, T. 1988. Variability in salt tolerance of nine spring wheat cultivars. *J. Agron. and Crop Sci.*, 160, 14-21.

Ashraf, M. and McNeilly, T. 1989. Effect of salinity on some cultivars of maize. *Maydica*, 34, 179-189.

Ashraf, M. and McNeilly, T. 1990. Improvement of salt tolerance in maize by selection and breeding. *Plant Breeding*, 104(2), 101-107.

Ashraf, M. and McNeilly, T. 1991. A potential source of variation for salt tolerance in spring wheat. *Hereditas,* 115, 155-120.

Ashraf, M., McNeilly, T. and Bradshaw, A.D. 1986a. The potential for evolution of salt tolerance in seven grass species. *New Phytol.*, 103, 299-309.

Ashraf, M., McNeilly, T. and Bradshaw, A.D. 1986b. The response of selected salt-tolerant and normal lines of four grass species to NaCl in sand culture. *New Phytol.*, 104, 453-461.

Ashraf, M., McNeilly, T. and Bradshaw, A.D. 1987. Selection and heritability of tolerance to sodium chloride in four forage species. *Crop Sci.*, 27, 232-243.

Ashraf, M., Zafar, Z.U. and O'Leary, J.W. 1995. Genetic-variation for salt tolerance in sunflower (*Helianthus annuus* L.). *Hereditas*, 123, 141-145.

Aspinall, D. and Paleg, L.G. 1981. Proline accumulation: Physiological aspects. In: D. Aspinall and L.G. Paleg (Eds.). *The physiology and biochemistry of drought resistance in plants*. pp. 215-228. Acad. Press, Sidney.

Azhar, F.M. and McNeilly, T. 1987. Variability for salt tolerance in *Sorghum bicolor* (L.) Moench under hydroponic conditions. *J. Agron. and Crop Sci.*, 159, 269-277.

Azhar, F.M. and McNeilly, T. 1988. The genetic basis of variation for salt tolerance in *Sorghum bicolor* (L.) Moench seedlings. *Plant Breeding*, 101, 114-121.

Azhar, F.M. and McNeilly, T. 1989. The response of four sorghum accessions/ cultivars to salinity during whole plant development. *J. Agron. and Crop Sci.*, 163, 33-43.

Bagheri, A., Paul, J.G., Langridge, P. and Rathjen, A.J. 1995. Genetic-distance detected with RAPD markers among selected Australian commercial varieties and boron-tolerant exotic germplasm of pea (*Pisum sativum* L). *Molec. Breeding*, 1(2), 193-197.

Bai, D.P., Brandle, J. and Reeleder, R. 1997. Genetic diversity in North American ginseng (*Panax quinquefolius* L) grown in Ontario detected by RAPD analysis. *Genome*, 40(1), 111-115.

Becker, W. A. 1992. *Manual of Quantitative genetics*. 5th ed. Acad. Enterprises, Pullman, W. A.

Benac, R. 1976. Response of sensitive (*Arachis hypogaea*) and tolerant (*Zea mays*) species to different concentrations of manganese in the environment. *Cah ORSTOM Ser.*, 11, 43-51.

Benson, G.O. and Pearce, R.B. 1987. Corn perspective and culture. In: S.A. Watson and P.E. Ramstad (Eds.). *Corn chemistry and technology.* pp. 169-231. AACC, St Paul.

Bianchi-Hall, C.M., Thomas, E., Carter, Jr., Rufty, T.W., Arellano, C., Boerma, H.R., Ashley, D.A. and Burton, J.W. 1998. Heritability and resource allocation of aluminium tolerance from soybean PI 416937. *Crop Sci.*, 38, 513-522.

Blum, A. 1985. Breeding crop varieties for stress environments. *CRC, Crit. Rev. Plant Sci.*, 2(3), 199-238.

Blum, A. 1988. *Plant breeding for stress environments.* CRC. Press. Inc. Boca Raton, Florida. USA.

Bohnert, H.J., and Jensen, R.G. 1996. Metabolic engineering for increased salt tolerance - The next step. *Austr. J. Plant. Physiol.* 23, 661-667.

Bohnert, H.J., Nelson, D.E. and Jensen, R.G. 1995. Adaptations to environmental stresses. *Plant Cell*, 7(7), 1099-1111.

Borreo, J.C., Pandey, S., Ceballos, H., Magnavaca R. and Bahia Filho, A.F.C. 1995. Genetic variances for tolerance to soil acidity in a tropical maize population. *Maydica*, 40, 283-288.

Boye-Goni, S.R., and Marcarian, V. 1985. Diallel analysis of aluminium tolerance in selected lines of grain sorghum. *Crop. Sci.*, 25, 749-752.

Bradshaw, A.D. and Hardwick, K. 1989. Evolution and stress – genotypic and phenotypic components. *Biolo. J. of Linnean Soci.*, 37, 137-155.

Bradshaw, A.D. and McNeilly, T. 1981. *Evolution and Pollution.* Edward Arnold, London.

Breburda, J. 1990. Development of agricultural yield levels and soil K-status in Eastern and Western Europe. In: *Development of K fertiliser recommendations.* pp. 17-35. International Potash Institute, Switzerland.

Brown, J.C. and Devine, T.E. 1980. Inheritance of tolerance or resistance to manganese toxicity in soybean. *Agron. J.*, 72, 898-900.

Caetano-Anolles, G., Bassam, B.J. and Gresshoff, P.M. 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Bio. Technol.*, 9, 553-557.

Cai, Q., Guy, C.L. and Moore, G.A. 1994. Extension of the linkage map in citrus using random amplified polymorphic DNA (RAPD) markers and RFLP mapping of cold-acclimation- responsive loci. *Theor. and Appl. Genet.*, 89(5), 606-614.

Camargo, C.E.O. 1981. Wheat breeding. I. Inheritance of tolerance to aluminium toxicity in wheat. *Bragantia*, 40, 33-45.

Camargo, C.E.O. 1983. Wheat breeding. III. Evidence of genetic control over tolerance of toxic manganese and aluminium in wheat. *Bragantia*, 43, 91-103.

Camargo, C.E.O. 1984. Genetic evidence of aluminium tolerance in rice. *Bragantia*, 43, 95-110.

Camargo, C.E.O., Santos, R.R. and Jr, A.P. 1992. Durum wheat: Tolerance to aluminium toxicity in nutrient solution and in the soil. *Bragantia*, 51, 69-76.

Cambraria, J., Galvani, F.R. and Estevao, M.M. 1983. Effects of aluminium on organic acid, sugar and amino acid composition of the root system of sorghum (*Sorghum bicolor* L. Moench). *J. Plant Nutri.*, 6(4), 313-322.

Carver, B.F. and Ownby, J.D. 1995. Acid soil tolerance in wheat. *Adv. Agron.*, 54, 117-173.

Chowdhury, M.A., Moseki, B. and Bowling, D.J. 1995. A method of screening rice for salt tolerance. *Plant and Soil*, 171, 117-132.

Clark, R. B. 1977. Effect of aluminium on growth and mineral elements of Al - tolerant and Al - intolerant corn. *Plant and Soil*, 47, 653-662.

Clark, R.B. and Duncan, R.R. 1993. Selection of plants to tolerate soil salinity, acidity, and mineral deficiencies. *International Crop Sci.*,1, 371-379.

Comstock, R.E. and Moll, R.H. 1963. Genotype-environment interactions. In: W.D. Hanson and H.F. Robinson (Eds.). *Statistical genetics and plant breeding*. Natl. Acad. Sci. pp. 164-196. Washington, D.C.

Comstock, R.E. and Robinson, H.F. 1948. The components of genetic variance in populations of biparental progenies and their use in estimating the average degree of dominance. *Biometics*, 4, 254-266.

Comstock, R.E. and Robinson, H.F. 1952. Estimation of average dominance of genes. *Heterosis*, Ch. 30. Iowa State College Press. USA.

Cramer, G. R., Epstein, E., and Läuchli, A. 1988. Kinetics of root elongation of maize in response to short-term exposure to NaCl and elevated calcium concentration. *J. Exp. Bot.*, 39, (208) 1513-1522.

Cross, H.Z. and Hammond, J.J. 1982. Plant density effects on combining abilities of early maize synthetics. *Crop Sci.*, 22, 814-817.

Culvenor, R.A. 1985. Tolerance of *Phalaris aquatica* L lines and some other agricultural species to excess manganese and the effect of aluminium on manganese tolerance in *P. aquatica. Aust. J. Agric. Res.*, 36(5), 695-708.

Davis, T.M., Yu, H., Hagis, K.M. and McGowan, P.J. 1995. Template mixing - a method of enhancing detection and interpretation of codominant RAPD markers. *Theor. and Appl. Genet.*, 91(4), 582-588.

Decorte, R., Cuppens, H., Marynen, P. and Cassiman, J.J. 1990. Rapid detection of hypervariable regions by the polymerase chain reaction technique. *DNA Cell Biol.*, 9, 661-469.

Dessureaux, L. 1959. Heritability of tolerance to manganese toxicity in lucerne. *Euphytica*, 8, 260-265.

Dessureaux, L. 1960. Inheritance of manganese tolerance in alfalfa crosses. *Plant and soil*, 13, 114-122.

Devey, F.D., Hayward, M.D., Kearsey, M.J., McAdam, N.J. and Eggleston, M.P. 1989. Genetic analysis of production characters in *Lolium*. II. Triple test cross analysis of drill and plant performance. *Plant Breeding*, 103, 63-72.

Devos, K.M. and Gale, M.D. 1992. The use of random amplified polymorphic DNA markers in wheat. *Theor. and Appl. Genet.*, 84(5-6), 567- 572.

Downton, W.J.S. 1984. Salt tolerance of food crops: Perspectives for improvement. *CRC, Crit. Rev. Plant Sci.*, 1, 183-201.

Drinkwater, R.D., Hediger, R. and Hetzel, D.J.S. 1990. Polymorphism and location of minisatellite sequences in sheep. In: *Proc. of 4th World Congress on Genetics Applied to Livestock Production.* pp. 132. Edinburgh, Scotland.

Dudal, R. 1976. Inventory of the major soils of the world with special reference to mineral stress. In: Wright, M.J. (Ed.). *Plant adaptation to mineral stress in problem soils.* pp. 2. Cornell University, Ithaca, NY.

Dudley, W.J. 1993. Molecular markers in plant improvement: manipulation of genes affecting quantitative traits. *Crop Sci.*, 33, 660-668.

Duncan, R.R., Clark R.B. and Furlani, P.R. 1983. Laboratory and field evaluation of sorghum for response to aluminium and acid soils. *Agron. J.*, 75, 1023-1026.

Duque-Vargas, J., Pandey, S. Granados, G., Ceballos, H. and Knapp, E. 1994. Inheritance of tolerance to soil acidity in tropical maize. *Crop Sci.*, 34, 50-54.

Eenik, A.H. and Garretsen, G.A. 1977. Inheritance of insensitivity of lettuce to a surplus of exchangeable manganese in steam sterilised soils. *Euphytica*, 26, 47-53.

Epstein, E. and Rains, D.W. 1987. Advances in salt tolerance. *Plant and Soil*, 99, 17-29.

Epstein, E., Norlyn, J.D., Rush, D.W., Kingsbury, R.W., Kelley, D.W., Cunningham, G.A., and Wrona, A.F. 1980. Saline culture of crops: A genetic approach. *Science,* 210, 399-404.

Evans, L.T. 1993. *Crop Evolution, Adaptation and Yield.* Cambridge University Press: Cambridge.

Falconer, D.S. and Mackay, T.F.C. 1996. *Introduction to quantitative genetics.* Longman, London.

FAO, 1998. World Agriculture Information Centre. FAOSTAT (Web site). *www.fao.org/waicent/faostat.htm.*

FAO-UNESCO (Food and agriculture Organisation-United Nations Educational, Scientific and Cultural Organisation), 1974. *Soil Map of the World,* Legend 1:500,000 Paris.

Ferreira, A.R. and Keim, P. 1997. Genetic mapping of soybean [*Glycine max* (L.) Merr.] using random amplified polymorphic DNA (RAPD). *Plant Molec. Biol. Reporter,* 15, 335-354.

Fitzpatrick, R.W., Boucher, S.C., Naidu, R. and Fritsch, E. 1994. Environmental consequences of soil sodicity. *Aust. J. Soil Res.*, 32, 1069-1093.

Flowers, T.J. and Yeo, A.R. 1995. Breeding for salinity resistance in crop plants: where next? *Aust. J. Plant Physiol.*, 22, 875-884.

Flowers, T.J., Garcia, A., Koyama, M. and Yeo, A.R. 1997. Breeding for salt tolerance in crop plants – the role of molecular biology. *Acta Physiologiae Plantarum,* 19, 427-433.

Flowers, T.J. and Yeo, A.R. 1986. Ion relations of plants under drought and salinity. *Aust. J. Plant Physiol.* 13, 75-91.

Flowers, T.J., Troke, P.F. and Yeo, A.R. 1977. The mechanism of salt tolerance in halophytes. *Ann. Rev. Plant Physiol.* 28, 89-121.

Foolad, M.R. 1996a. Response to selection for tolerance during germination in tomato seed derived from P1-174263. *J. Amer. Soc. Hort. Sci.*, 121(6), 1006-1011.

Foolad, M.R. 1996b. Genetic analysis of salt tolerance during vegetative growth in tomato, *Lycopersicon esculentum* Mill. *Plant Breeding*, 115, (4) 245-250.

Foolad, M.R. 1997. Genetic basis of physiological traits related to salt tolerance in tomato, *Lycopersicon esculentum* Mill. *Plant Breeding*, 116, 53-58.

Foolad, M.R. and Chen, F.Q. 1998. RAPD markers associated with salt tolerance in an interspecific cross of tomato (*Lycopersicon esculentum* x *L. pennellii*). *Plant Cell Reports*, 17(4), 306-312.

Foolad, M.R. and Jones, R.A. 1991. Genetic analysis of salt tolerance during germination in *Lycopersicon*. *Theor. Appl. Genet.* 81, 321-326.

Foolad, M.R. and Jones, R.A. 1992. Parent offspring regression estimates of heritability for salt tolerance during germination in tomato. *Crop Sci.*, 32, 439-442.

Foolad, M.R., Arulsekar, S. and Rodriguez, R.L. 1995. Application of polymerase chain reaction (PCR) in plant genome analysis. In: O.L. Gamborg and G.C. Phillips (Eds.). *Fundamental methods for plant cell, tissue and organ*

culture, and laboratory operations. pp. 281-298. Springer, Berlin, Heidelberg, New York, Tokyo.

Foy, C.D. 1973. Manganese and Plants. In: J. Lieben, (Ed.). *Manganese.* Natl. Acad. Sci., Natl. Res. Council Washington D C., pp. 51-76.

Foy, C.D. 1974. Effect of aluminium on plant growth. In: E.W. Carson (Ed.). *The plant growth and its environment.* pp. 601-642. University Press of Virginia, Charlottesville. USA.

Foy, C.D. 1983. Plant adaptation to mineral stress in problem soils. *Iowa J. Res.*, 57, 339-354.

Foy, C.D. 1984. Physiological effects of hydrogen, aluminium, and manganese toxicities in acid soils. In: F. Adams (Ed.). *Soil acidity and liming.* pp. 57-97. Crop Sci. Soc. America. Madison, Wisconsin.

Foy, C.D. 1988. Plant adaptation to acid, aluminium toxic soils. *Commun. Soil Sci. Plant Anal.*, 19, 959-987.

Foy, C.D. 1996. Tolerance of barley cultivars to an acid aluminium toxic subsoil related to mineral element concentrations in their shoots. *J. Plant Nutri.*,19 (10&11), 1361-1380.

Foy, C.D. 1997. Tailoring plants to fit problem soils-progress and problems for future research. In: A.C. Moniz, A.M.C. Furlani, R.E. Schaffert, N.K. Fageria, C.A. Rosolem and H. Cantarella (Eds). *Plant-soil interactions at low pH: sustainable agriculture and forestry production.* Brazilian Soil Science Society. pp. 55-57.

Foy, C.D., Chaney R.L. and White, M.C. 1978. The physiology of metal toxicity in plants. *Ann. Rev. Plant Physiol.*, 29, 511-566.

Foy, C.D., Flemming A.L. and Schwartz, J.W. 1973. Opposite aluminium and manganese tolerances of two wheat varieties. *Agron. J.*, 65, 123-126.

Foy, C.D., Scott B.J. and Fisher, J.A. 1988. Genetics and breeding of plants tolerant to manganese toxicity. In: R.D. Graham, R.J. Hannam and N.C. Uren (Eds.) *Manganese in Soils and Plants*. pp. 261-276. Kluwer Acad. Publishers, Boston, USA.

Fritsch, P., Hanson, M.A., Spore, C.D., Pack, P.E. and Rieseberg, L.H. 1993. Constancy of RAPD primer amplification strength among distantly related taxa of flowering plants. *Plant Molec. Biol. Reporter*, 11(1), 10-20.

Fukutoku,Y. and Yamada, Y. 1982. Accumulation of carbohydrates and proline in water-stressed soybean (*Glycine max* L). *Soil Sci. Plant Nutri.*, 28(1), 147-151.

Galvez, L., Clark, R.B., Klepper, L.A. and Hansen, L. 1991. Organic acid and free proline accumulation and nitrate reductase activity in sorghum (*Sorghum bicolor*) genotypes differing in aluminium tolerance. In: R.J. Wright, V.C. Baligar and R.P. Murrmann. (Eds.). *Plant soil-interactions at low pH*. pp. 859-867. Kluwer Acad. Publishers, Dordrecht, The Netherlands.

Garcia, O. Jr., de Silva, W.J. and Massei, M.A.S. 1979. An efficient method for screening maize inbreds for aluminium tolerance. *Maydica*, 24, 75-82.

Gartside, D.W. and McNeilly, T. 1974a. Genetic studies in heavy metal tolerant plants. I. Genetics of zinc tolerance in *Anthoxanthum odoratum*. *Heredity*, 32, 287-299.

Gartside, D.W. and McNeilly, T. 1974b. Genetic studies in heavy metal tolerant plants. II. Zinc tolerance in *Agrostis tenuis*. *Heredity*, 33, 303-308.

Georges, M. Dietz, A.B., Mishra, A., Nielsen, D., Sargeant, L.S., Sorensen, A., Steele, M.R., Zhao, X., Leipold, H. and Womack, J.E. 1993. Microsatellite mapping of the gene causing weaver disease in cattle will not allow the study of an associated quantitative trait locus. *Proc. Natl. Acad. Sci.* USA, 90, 1058-1062.

Georges, M., Lathrop, M., Hilbert, P., Marcotte, A., Schwers, A., Swillens, S., Vassart, G. and Hanset, R. 1990. On the use of DNA fingerprints for linkage studies in cattle. *Genomics*, 6, 461-474.

Ghassemi, F., Jakeman, A.J. and Nix, H.A. 1995. *Salinisation of land and water resources*. pp. 526. CAB International, Wallingford.

Goring, H. 1982. Stress reaction of plants to unfavourable physical and chemical factors in the environment. *Sov. Agric. Sci.*, 1, 16-18.

Gourley, L.M., Rogers, S.A., Ruiz-Gomes, C. and Clark, R.B. 1990. Genetic aspects of aluminium tolerance in sorghum. *Plant and Soil*, 123, 211-216.

Greenway, H. and R. Munns, 1980. Mechanism of salt tolerance in non-halophytes. *Ann. Rev. Plant Physiol.* 31, 149-190.

Gregoria, G.B., and Senadhira, D. 1993. Genetic analysis of salinity tolerance in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 86, 333-338.

Grime, J.P. 1979. *Plant strategies and vegetation processes.* John Wiley, Chichester.

Grote, E.M., Ejeta, G. and Rhodes, D. 1994. Inheritance of glycinebetaine deficiency in sorghum. *Crop Sci.*, 34, 1217-1220.

Haiyuan, D., Gengyun, Z., Yan, G., Shaolin, C. and Shouyi, C. 1998. RAPD tagging of salt tolerant genes in rice. *Chinese Sci. Bullt.*, 43(4), 330-332.

Hassneian, A.M. and Azab, M. 1993. Salt tolerance of grain sorghum. In: H. Leith and A. Al-Masoom (Eds.). *Towards the rational use of high salinity tolerant plants.* pp. 153-156. Kluwer Academic Publishers, The Netherlands.

Haug, A. 1984. Molecular aspects of aluminium toxicity. *Crit. Rev. Plant Sci.*, 1, 345-373.

Hearne, C.M., Gosh, S. and Todd, J.A. 1992. Microsatellites for linkage analysis of genetic traits. *Trend. Genet.*, 8, 288-294.

Hennan, D.P., Campbell, L.C. and Carter, O.G. 1981. Inheritance of tolerance to high manganese supply in soybeans. *Crop Sci.*, 21, 626-627.

Hewitt, E.J. 1966. Sand and water culture methods used in the study of plant nutrition. *Commonwealth Agriculture Bureaux. Technical Communication,* No. 22.

Hill, P.R., Ahlrichs J.L. and Ejeta, G. 1989. Rapid evaluation of sorghum for aluminium tolerance. *Plant and Soil,* 114, 85-90.

Hoffman, A.A. and Pearson, G.A. 1991. *Evolutionary Genetics and Environmental Stress.* Oxford Uni. Press, New York.

Horst, W.J. 1983. Factors responsible for genotypic manganese tolerance in cowpea (*Vigna unguiculata*). *Plant and Soil*, 72, 213-218.

Howeler, R.H. and Cadavid, L.F. 1976. Screening of rice cultivars for tolerance to Al toxicity in nutrient solutions as compared with a field screening method. *Agron. J.*, 68, 551-555.

Huff, D.R. 1997. RAPD characterisation of heterogeneous perennial ryegrass cultivars *Crop Sci.*, 37(2), 557-564.

Humphreys, M.O. and Nicholls, M.K. 1984. Relationship between tolerance to heavy metals in *Agrostis capillaris* L. (*A. tenuis* Sibth.) *New Phytol.*, 98, 177-190.

IRRI. 1993. '*IRRI Almanac*.' P. 9. International Rice Research Institute, Manila.

Jackson, T.L. and Reisenauer, H.M. 1984. Crop response to lime in western United States. In: F. adams (Ed.). *Soil acidity and liming*. pp. 333-347. ASA, CSSA, SSSA, Madison, WI, USA.

Jackson, W.A. 1967. Physiological effects of soil acidity. *Agron. Monogr.*, 12, 3-124.

Jinks, J.L. 1981. *The genetic framework of plant breeding*. Phil. Trans. R. Soc. London, 292, 407-419.

Jinks, J.L. 1983. Biometrical genetics of heterosis. In: R. Frankel (Ed.). *Heterosis*. pp. 1-46. Monograph on theoretical and applied genetics 6. Springer-Verlag, Berlin.

Jinks, J.L. and Perkins, J.M. 1970. A general method of detecting additive, dominance and epistatic components of variation. III. F_2 and backcross populations. *Heredity*, 25, 419-429.

Jinks, J.L., Perkins, J.M. and Breese, E.L. 1969. A general method of detecting additive, dominance and epistatic variation of metrical traits. II. Application to inbred lines. *Heredity*, 24, 45-47.

Jinks, J.L., Perkins, J.M. and Pooni, H.S. 1973. The incidence of epistasis in normal and extreme environments. *Heredity*. 31. 263-269.

Jones, H.G. and Jones, M.B. 1989. Some terminology and common mechanisms. In: H.G. Jones, T.J. Flowers and M.B. Jones (Eds). *Plants under stress*. Cambridge University Press: Cambridge.

Kandpal, R.P. and Rao, N.A. 1985. Alterations in the biosynthesis of proteins and nucleic acids in finger millet (*Eleucine coracana*) seedlings during water-stress and the effect of proline on protein-biosynthesis. *Plant Sci.*, 40(2), 73-79.

Kearsey, M.J. 1965. Biometrical analysis of a random mating population: Comparison of five experimental designs. *Heredity*, 20, 205-235.

Kearsey, M.J. 1980. The efficiency of North Carolina Expt. III and the selfing backcrossing series for estimating additive and dominance variation. *Heredity*, 45, 73-82.

Kearsey, M.J. and Jinks, J.L. 1968. A general method of detecting additive, dominance and epistatic variation of metrical traits. 1. Theory. *Heredity*, 23, 403-409.

Kearsey, M.J., Hayward, M.D., Devey, F.D., Arcioni, S., Eggleston, M.P. and Eissa, M.M. 1987. Genetic-analysis of production characters in *Lolium* .1. Triple test cross analysis of spaced plant performance. *Theor. Appl Genet*, 75(1), 66-75.

Kebebew, F. and McNeilly, T. 1995. Variation in response of accessions of minor millets, *Pennisetum americanum* (L.) Leeke (Pearl Millet) and *Eleusine coracana* (L.) Gaertn (Finger Millet), and *Eragrostis tef* (Zucc.) Trotter (Tef), to salinity in early seedling growth. *Plant and Soil*, 175, 311-321.

Kebebew, F. and McNeilly, T. 1996. The genetic basis of variation in salt tolerance in Pearl millet, *Pennisetum americanum* (L.) Leeke. *J. Genet. and Breed.* 50, 129-136.

Kepinski, S.S. 1997. Development of DNA profiling techniques in *Anthoxanthum odoratum*. Ph.D. Thesis, The University of Liverpool. Liverpool, UK.

Kerridge, P.C. and Kronstad W.E. 1968. Evidence of genetic resistance to aluminium toxicity in wheat. (*Triticum aestivum*). *Agron. J.*60, 710-711.

Klein-Lankhorst, R.M., Vermunt, A., Weide, R., Liharska, T. and Zabel, P. 1992. Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). *Theor. Appl. Genet.*, 83, 108-114.

Klimashevskii, E.L. 1983. Identification of plant resistance to soil acidity. *Sov. Agric. Sci.*, 10, 1-5.

Klimashevskii, E.L., Markova, Yu.A. Seregina, M.L., Grodzinskii, D.M. and Kozarenko, T.D. 1970. Specifics of the physiological activity of pea plants

in connection with unequal resistance of different varieties to mobile aluminium. *Sov. Plant Physiol.*, 17, 372-378.

Larcher, W. 1995. *Physiological plant ecology.* 3rd Ed. Springer, London.

Lark, K.G., Chase, K., Adler, F., Mansur, L.M. and Orf, J.H. 1995. Interactions between quantitative trait loci in soybean in which trait variation at one locus is conditional upon specific allele at another. *Proc. Natl. Acad. Sci.* USA, 92, 4656-4660.

Lawrence, M.J. 1984. The genetical analysis of ecological traits; In: B. Shorrocks (Ed.) *Evolutionary ecology.* pp. 27-63. Blackwell Sci. Publ. Oxford, London, Edinburgh.

Liang, G.H., Reddy, C.R. and Dayton, A.D. 1972. Heterosis, inbreeding depression and heritability estimation in a systematic series of grain sorghum genotypes. *Crop Sci.*, 12, 409-411.

Little, R. 1988. Plant soil interaction at low pH. Problem solving – the genetic approach. *Commun. Soil Sci. Plant Anal.*, 19(7-12), 1239-1257.

Maas, E.V. 1993. Plant growth response to salt stress. H. Lieth and A. Al-Masoom (Eds): *Towards the rational use of high salinity tolerant plants.*1. pp. 279-291. Kluwer Academic Publishers, The Netherlands.

Maas, E.V., and Hoffman, G.J. 1977. Crop salt tolerance current assessment. *J. Irrig. and Drain.* Divi. ASCE, 103, 115-134.

Maas, E.V., Hoffman, G.J., Chaba, G.D., Poss, J.A. and Shannon, M.C. 1983. Salt sensitivity of corn at various growth stages. *Irrig. Sci.*, 4, 45-57.

Magnavaca, R., Gardner, G.O. and Clark, R.B. 1987. Inheritance of aluminium tolerance in maize. In: H.W. Gabelman and B.C. Loughman (Eds.). *Genetic aspects of plant mineral nutrition.* pp. 201-212. Nijihoff, Dordrecht. The Netherlands.

Maiti, R. K., Amaya, L.E.D., Cardona, S.I., Dimas, A.M.O., De La Rosa-Ibarra, M. and Castillo, H.D.L. 1996. Genotypic variability in maize cultivars (*Zea mays* L.) for resistance to drought and salinity. *J. Plant Physiol.*, 148, 741-744.

Mann, C.E., Pollmer, W.G. and Klein, D. 1981. Magnitude and stability over environments of reciprocal-cross differences in maize hybrids and their implications on maize breeding. *Maydica*, 26, 239-252.

Mano, Y. and Takeda, K. 1997. Diallel analysis of salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare* L.). *Breeding Sci.*, 47(3), 203-209.

Marsan, P.A., Egidy, G., Monfredini, G., Di Silvestro, S. and Motto, M. 1993. RAPD markers in maize genetic analysis. *Maydica*, 38, 259-264.

Martin, G.B., Williams, J.G.K. and Tanksely, S.D. 1991. Rapid identification of markers linked to *Pseudomonas* resistance gene in tomato by using random primers and near isogenic lines. *Proc. Natl. Acad. Sci.* USA, 88, 2336-2340.

Martin, J.M. and Hallauer, A.R. 1976. Relation between heterozygous and yield of four types of maize inbred lines. *Egypt J. Genet. Cytol.*, 5, 119-135.

Martinez-Cob, A., Aragues, R. and Royo, A. 1987. Salt tolerance of barley (*Hordeum vulgare* L.) cultivars at the germination stage: Analysis of response functions. *Plant and Soil*, 104, 53-56.

McGrath, J.M., Quiros, C.F., Harada, J.J. and Landry, B.S. 1990. Identification of *Brassica oleracea* monosomic alien chromosome addition lines with molecular markers reveals extensive gene duplication. *Mol. Gen. Genet.*, 223, 198-204.

McNeilly, T. 1990. Selection and breeding for salinity tolerance in crop species: A case for optimism? *Acta Ecologica*, 11 (4), 595-610.

McNeilly, T. 1994. Metal toxicity. In: A.R. Yeo and T.J. Flowers (Eds.). *Monographs on theoretical and applied Genetics*, 21, pp. 145-174. Springer-Verlag, Berlin Heidelberg.

McWilliams, J.R. 1986. The national and international importance of drought and salinity effects on agricultural production. *Aust. J. Plant Physiol.*, 13, 1-13.

Meigs, P. 1968. Deserts of the world. In: W.G. McGinnies, B.J. Goldman, and P. Paylore (Eds.). *An appraisal of Research into Their Physical and Biological Environments*. Uni. Arizona Press, Tucson. USA.

Moeljopawiro, S. and Ikehashi, H. 1981. Inheritance of salt tolerance in rice. *Euphytica*, 30, 291-300.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T.G., Yano, M., Bhatia, C.R. and Sasaki, T. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molec. Breeding*, 3 (2), 87-103.

Moreno, G. 1994. Genetic architecture, genetic behaviour, and character evolution. *Annu. Rev. Ecol. Syst.*, 25, 31-44.

Munns, R. 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant Cell and Environment*, 16, 15-24.

Munthali, M.T., Newbury, H.J. and FordLloyd, B.V. 1996. The detection of somaclonal variants of Beet using RAPD. *Plant Cell Reports*, 15(7), 474-478.

Nelson, L. E. 1983. Tolerance of 20 rice cultivars to excess Al and Mn. *Agron. J.*, 75, 134-138.

Neuffer, M.G. 1994. Growing maize for genetic studies. In: M. Freeling and V. Walbot (Eds). *The maize handbook.* pp. 197-209. Springer-Verlag, New York, Inc.

Neumann, P.M. 1995. Inhibition of root growth by salinity stress: toxicity or an adaptive biophysical response? In: F. Baluska, M. Ciamporova, O. Gasparikova and P.W. Barlow (Eds.). *Structure and function of roots. Developments in plant and soil sciences.* pp. 299-304. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Noble, C.L. and Rogers M.E. 1992. Arguments for the use of physiological criteria for improving the salt tolerance in crops. *Plant and Soil*, 146, 99-107.

Noble, C.L. and Shannon, M.C. 1988. Salt tolerance selection of forage legumes using physiological criteria. In: S.K. Sharma, P.V. Sabe, S.C. Bharagava

and P.K. Agrawal (Eds.). *Proc. Int. Plant Physiol. Cong.*, Soc. Plant Physiol. Biochem. New Delhi, India. pp. 989-994.

Northcote, K.H. and Skene, J.K.M. 1972. Australian soils with saline and sodic properties. *Soils Publication No. 27. CSIRO.*, Australia, Melbourne.

Osborn, T.C., Alexander, D. and Fober, J.F. 1987. Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. *Theor. Appl. Genet.*, 73, 350-356.

Paleg, L.G., Stewart, G.R. and Bradbeer, J.W. 1984. Proline and glycine-betain influence protein salvation. *Plant Physiol.*, 75, 974-978.

Parker, D.R., Kinraide, T.B. and Zelazny, L.W. 1989. On the phytotoxicity of polynuclear hydroxy-aluminium complexes in soil. *Sci. Soc. America J.*, 53(3), 789-796.

Patel, J.D. and Bains, K.S. 1984. Genetics of reciprocal differences for quantitative characters in wheat. *Can. J. Genet. Cytol.*, 26, 615-621.

Paterniani, E. 1990. Maize breeding in tropics. *Crit. Rev. Plant Sci.*, 9(2), 125-154.

Pessarakli, M. 1991. Formation of saline and sodic soils and their reclamation. J. *Env. Sci. Health*, 26 (7), 1303-1320.

Pintro, J., Barloy J. and Fallavier, P. 1996. Aluminium effects on the growth and mineral composition of corn plants cultivated in nutrient solution at low aluminium activity. *J. Plant Nutri.*, 19(5), 729-741.

Plummer, D.T. 1987. *Practical biochemistry.* 3rd Ed. pp. 179-180. McGraw-Hill Book Company.

Poehlman, J.M. and Sleper, D.A. 1995. *Breeding Field Crops*. Iowa State University Press, USA.

Polle, E., Konsak, C.F. and Kittrack, J.A. 1978. Visual detection of aluminium tolerance levels in wheat by haematoxylin staining of seedling roots. *Crop Sci.*, 18, 823-827.

Pooni, H.S. and Jinks, J.L. 1979. Sources and biases of the predictors of the properties of recombinant inbreds produced by single seed descent. *Heredity*, 42, 41-48.

Pooni, H.S., Kumar, I.S.H. and Khush, G.S. 1994. A general method of detecting additive, dominance and epistatic variation of metrical traits. IV. Triple test cross analysis of disomically inherited traits expressed in triploid tissues. *Heredity*, 72, 563-569.

Possingham, J.V. 1956. The effect of mineral nutrition on the contents of free amino acids and amides in tomato plants. *Aust. J. Biol. Sci.*, 9, 539-551.

Qayyum, M.A. and Malik, M.D. 1988. Farm production losses in salt-affected soil. In: *Proc. First Nat. Cong. of Soil Science*. Lahore, Pakistan.

Qureshi, H.A. 1990. Soil salinity situation in Pakistan. In: *Proc. International conference on current developments on salinity and drought tolerance of plants*. AERC, Pakistan.

Rao, A.P. and Fleming, A.A. 1978. Cytoplasmic-genotypic effects in the GT 112 maize inbred with four cytoplasms. *Crop Sci.*, 18, 935-937.

Rao, S.A. 1997. The potential for breeding *Zea mays* L. for saline conditions. Ph.D. Thesis, The University of Liverpool. Liverpool, UK.

Rathert, G. 1984. Sucrose and starch content of plant parts as a possible indicator for salt tolerance of crops. *Aust. J. Plant Physiol.*, 11, 491-495.

Reidy, M.F., Hamilton, W.J. and Acquadro, C.F. 1992. Excess of non-parental bands in offsprings from known primate pedigrees assayed using RAPD-PCR. *Nucleic Acid. Res.*, 20, 918-926.

Reiter, R.S., Williams, J.G.K., Feldmann, K.A., Rafalsky. A., Tingey, S.V. and Scolnik, P.A. 1992. Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. *Proc. Natl. Acad. Sci.* USA, 89, 1477-1481.

Rhue, R.D., Grogan, C.O. and Stockmeyer, E.W. 1978. Genetic control of aluminium tolerance in corn. *Crop Sci.*, 18, 1063-1067.

Ring, S.M., Fisher, P.R., Poile, G.J., Helyar, K.R., Conyers, M.K. and Morris, S.G. 1993. Screening species and cultivars for their tolerance to acid soil. *Plant and Soil*, 155/156, 521-524.

Robinson, H.F., Comstock, R.E. and Harvey, P.H. 1955. Genetic variances in open pollinated varieties of corn. *Genetics*, 40, 45-60.

Rosen, H. 1957. A modified ninhydrin colorimetric analysis of amino acids. *Archives of Biochem. Biophysics*, 67, 10-15.

Royle, N.J., Clarkson, R.E., Wong, Z. and Jeffreys, A.J. 1988. Clustering of hyper variable minisatellites in proterminal regions of human autosomes. *Genomics*, 3, 352-360.

Saadan, H.M. 1991. Inheritance of manganese tolerance in sorghum. Ph.D. Thesis, Mississippi State University, Mississippi State, USA.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higurchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 239, 487-491.

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. *Molecular cloning*: *A laboratory manual* (2nd Ed.). Cold Spring Harbour Laboratory Press. New York. USA.

Saneoka, H., Nagasaka, C., Hahn, D.T., Yang, W., Premachandra, G.S., Joly, R.J. and Rhodes, D. 1995. Salt tolerance of glycinebetaine-deficient and glycinebetaine-containing maize lines. *Plant Physiol.*, 107, 631-638.

Saranga, Y., Zamir, D., Marani, A. and Rudich, J. 1992. Breeding tomatoes for salt tolerance: inheritance of salt tolerance & related traits in inter-specific populations. *Theor. Appl. Genet.* 84, 390-396.

Sartain, J.B. and Kamprath, E.J. 1978. Aluminium tolerance of soybean cultivars based on root elongation in solution culture compared with growth in acid soil. *Agron. J.*, 70, 17-20.

Schachermayr, G., Siedler, H., Gale, M.D., Winzeler, H., Winzeler, M. and Keller, B. 1994. Identification and localisation of molecular markers linked to the LR9 leaf rust resistance gene of wheat. *Theor. Appl. Genet.*, 88(1), 110-115.

Schat, H. and ten Bookum, W.M. 1992. Genetic control of copper tolerance in *Silene vulgaris*. *Heredity*, 68, 219-229.

Schulz, B., Westphal, L. and Wricke, G. 1994. Linkage groups of isozymes, RFLP and RAPD markers in carrot [*Daucus carota* (L.) *Sativus*]. *Euphytica*, 74(1-2), 67-76.

Scott, B. J. and Fisher, J. A. 1989. Selection of genotypes tolerant of aluminium and manganese. In: A.D. Robson (Ed.). *Soil Acidity and Plant Growth*. pp. 167-203. Acad. Press, Sydney, Australia.

Shannon, M.C. 1982. Genetics of salt tolerance: New challenges. In: A. San Pietro (Ed.). *Biosaline Research*. pp. 277-282. Plenum Press, New York.

Shannon, M.C. 1985. Principles and strategies in breeding for salt tolerance. *Plant and Soil*, 89, 227-241.

Shuman, L.M. and Wilson, D.O. 1994. Influence of phosphate and hydroxyl ions on aluminium toxicity in soybean and wheat. *J. Plant Nutri.*, 17(2&3), 345-365.

Silva, J.C. and Hallauer, A.R. 1975. Estimation of epistatic variance in Iowa stiff stalk synthetic maize. *The Journal of Heredity*, 66, 290-296.

Singh, B.B., Singh, U.P., Rai, B. and Singh, R.M. 1986. Triple test cross analysis in F_2 population of four promising crosses of field pea. *Plant Breeding*, 97, 357-363.

Singh, N.T. 1992. Dry land salinity in the Indo-Pakistan sub-continent. In: H.E. Derange (Ed.). *Degradation and Restoration of Arid Lands*. pp. 179-248. Texas Technical University, Lubbock, USA.

Singh, S. and Singh, R.B. 1976. Triple test cross analysis in two wheat crosses. *Heredity*, 37, 173-177.

Singh, T.N., Aspinall, D. and Paleg, L.G. 1972. Proline accumulation and varietal adaptability to drought in barley: A potential metabolic measure of drought resistance. *Nature*, 236, 88-90.

Singh, Y.P., Khairwal, I.S. and Singh, S. 1991. Genetic analysis for physiological traits in pearl millet. *Crop Improv.*, 18(2), 132-134.

Sivaramakrishnan, S., Patell, V.Z., Flowers, T.J. and Peacock, J.M. 1988. Proline accumulation and nitrate reductase activity in contrasting sorghum lines during mid-season drought stress. *Physiol. Plant*, 74, 418-426.

Smith, E.L., Carver, B.F., Hunger, R.M., Sherwood, J.L., Ward, R.G. and Jordan, B.G. 1997. Registration of OK91P648 an acid-soil tolerant wheat germplasm. *Crop Sci.*, 37(1), 297-297.

Song, K.M., Osborn, T.C. and Williams, P.H. 1988. Brassica taxonomy based on nuclear restriction length polymorphisms (RFLPs). 1. Genome evolution of diploid and amphidiploid species. *Theor. Appl. Genet.*, 75, 784-794.

SPSS, 1994. Repeated Measure Analysis of Variance. pp. 107-143. In: *SPSS Advanced Statistics*. Release 6.1.

Steppuhn, H., Wall, K., Rasiah, V. and Jame, Y.M. 1996. Response functions for grain yield from spring-sown wheat grown in saline rooting media. *Canadian Agricultural Engineering*, 38 (4), 249-256.

Strogonov, B.P. 1973. Structure and function of plant cells in saline habitats. In: A. Mercado and B. Gollek (Eds.). *New trends in the study of salt tolerance*. (Translated from Russian), John Wiley and Sons, New York.

Stuber, C.W., Lincoln, S.E., Wolff, D.W., Helentjaris, T. and Lander, E.S. 1992. Identification of genetic-factors contributing to heterosis in a hybrid from 2 elite maize inbred lines using molecular markers. *Genetics*, 132(3), 823-839.

Subbaraman, N. and Sree Rangasamy, S.R. 1989. Triple test cross analysis in rice. *Euphytica*, 42, 35-40.

Szabolcs, I. 1987. The global problems of salt-affected soils. *Acta Agonomica Hungarica*, 36, 159-172.

Szabolcs. I. 1993. Soils and salinisation. In: M. Pessarakli (Ed), *Handbook of Plant and Crop Stress*. pp. 3-11. Marcel, Dekker, Inc, New York.

Tanji, K.K. 1990. Nature and extent of agricultural salinity. In: K.K. Tanji (Ed.) *Agriculture Assessment and Management*. pp, 1-17. Am. Soc. Civ. Engs. New York.

Taylor, G.J. 1991. Current views of the aluminium stress response; the physiological basis of tolerance. *Current Topics in Plant Biochem. Physiol.*, 10, 57-93.

Taylor, G.J. 1995. Overcoming barriers to understanding the cellular basis of aluminium resistance. *Plant and Soil*, 171, 89-103.

Tingey, S.V. and del Tufo, J.P. 1993. Genetic-analysis with random amplified polymorphic DNA markers. *Plant Physiol.*, 101(2), 349-352.

Troll, W. and Lindsley, J. 1955. A photometric method for the determination of proline. *J. Biol. Chem.*, 255, 655-660.

UNEP, 1992. '*World Atlas of Desertification.*' Edward Arnold: London.

Urrea-Gomez, R., Ceballos, H., Pandey, S., Bahia Filho A.F.C. and Leon, L.A. 1996. A greenhouse screening technique for acid soil tolerance in maize. *Agron. J.*, 88, 806-812.

Van Assche, F. and Clijsters, H.A. 1990. Effects of metals on enzyme-activity in plants. *Plant Cell Environ.*,13(3), 195-20.

van Genuchten, M.T. 1983. *Analysis of crop salt tolerance data*: Model description and user manual USDA-ARS, ASSL, Riverside California. Research Report # 120, 1-50.

van Genuchten, M.T. and Hoffman, G.J. 1984. Analysis of crop salt tolerance data. In: I. Shainberg and J. Shalevet (Eds.). *Soil salinity under irrigation.* pp. 258-271. Springer Verlag, Berlin, Germany.

Vander Pluym, H.S.A., Paterson, B. and Holm, H.M. 1981. Degradation by salinisation. In: *Proc. 18th Alberta Soil Sci. Workshop.* Edmonton. pp. 9-40.

Venekamp, J.H. 1989. Regulation of cytosol acidity in plants under conditions of drought. *Physiol. Plantarum*, 76(1), 112-117.

von Uexkull, H.R. and Mutert, E. 1995. Global extent, development and economic impact of acid soils. *Plant and Soil*, 171, 1-15.

Weber, B., Riess, O., Hutchinson, G., Collins, C., Lin, B.Y., Kowbel, D., Andrew, S., Schappert, K. and Hayden, M.R. 1991. Genomic organisation and complete sequence of the human gene encoding the beta-subunit of cGMP phosphodiesterase and its location to 4p16.3. *Nucleic Acid. Res.*, 19, 6263-6268.

Weber, J.L, and May, P.E. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain-reaction. *American J. Human Genet.*, 44(3), 388- 396.

Welsh, J. and McClennad, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acid. Res.*, 18, 7213-7218.

Welsh, J., Honeycutt, R.J., McClennad, M. and Sobral, B.W.S. 1991. Parentage determination in maize hybrids using arbitrary primed polymerase chain reaction (AP-PCR). *Theor. Appl. Genet.*, 82, 473-476.

Wheeler, D.M., 1995. Relative aluminium tolerance of ten species of Graminae. *J. Plant Nutri.*, 18(11), 2305-2312.

Wigan, L.G. 1944. Balance and potence in natural populations. *J. Genetics*, 46, 150-160.

Wild, H. and Bradshaw, A.D. 1977. The evolutionary effects of metalliferous and other anomalous soils in south central Africa. *Evolution*, 31, 282-293.

Wilde, J., Waugh, R. and Powell, W. 1992. Genetic fingerprinting of *Theobroma* clones using randomly amplified ploymorphic DNA markers. *Theor. Appl. Genet.*, 83, 871-877.

Wilkinson, R.E. and Duncan, R.R. 1994. Differences in sorghum cultivar root response to concomitant excess hydrogen and manganese ion applications. *J. Plant Nutri.*, 17(2&3), 501-509.

Williams, J.G.K., Kubelic, A.R., Livak, K.J., Rafalsky, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid. Res.*, 18, 6531-6535.

Wolf, D.P. and Hallauer, A.R. 1997. Triple test cross analysis to detect epistasis in maize. *Crop Sci.*, 37, 763-770.

Woolhouse, H.W. 1983. Toxicity and tolerance in the response of plants to metals. In: O.L. Lange, P.S. Noble, C.B. Osman and H. Ziegler (Eds.). *Physiological plant ecology. III. Response to the chemical and biological environment.* pp. 254. Springer Verlag, Berlin, Germany..

Wright, R.J., Baligar, V.C., Ritchey, K.D. and Wright, S.F. 1989. Influence of soil solution aluminium on root elongation of wheat seedlings. *Plant and Soil*, 113, 294-298.

Wyn Jones, R.G. 1981. Salt tolerance. In: C.B. Johnson (Ed.). *Physiological Processes Limiting Plant Productivity.* pp, 271-291. Butterworth, London.

Yaron, A. and Naider, F. 1993. Proline-dependent structural and biological properties of peptides and proteins. *Cri. Rev. Biochem. Molec. Biol.*, 28(1), 31-81.

Yeo, A.R. 1994. Physiological criteria in screening and breeding. In: A.R. Yeo and T.J. Flowers (Eds.). *Monographs on Theoretical and Applied Genetics*, 21, pp. 37-59. Springer Verlag, Berlin, Germany.

Yeo, A.R. and Flowers, T.J. 1990. Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. *Theor. Appl. Genet.*, 79: 377-384.

Zaifnejad, M., Clark, R.B. and Sullivan, C.Y. 1997. Aluminium and water stress effects on growth and proline of sorghum. *J. Plant Physiol.*, 150, 338-344.

Zaither, H.Z. and Mahfouz, B. 1993. Salinity effects on root and shoot characteristics of common and teary beans evaluated under hydroponics and sand culture. *J. Plant Nutri.* 18 (8) 1569-1592.

Zeigler, R.S., Pandey, S., Miles, J., Gourley, L.M. and Sarkarung, S. 1995. Advances in the selection and breeding of acid-tolerant plants: Rice, maize, sorghum and tropical forages. In: R.A. Date (Ed.). *Plant soil interactions at low pH.* pp. 391-406. Kluwer Acad. Publishers. The Netherlands.

Appendices

Appendix 1.1. Type, source and origins of maize accessions used in the study.

S.N	Acc. No./Name	Type	Source	Origin
1	Bozm 0095	Land race	CIFA (Bolivia)	Cochabamba (Bolivia)
2	Bozm 0170	Land race	CIFA (Bolivia)	Chuquisaca (Bolivia)
3	Bozm 0253	Land race	CIFA (Bolivia)	Tariji (Bolivia)
4	Bozm 0715	Land race	CIFA (Bolivia)	Santa Cruz (Bolivia)
5	Bozm 0883	Land race	CIFA (Bolivia)	Cochabamba (Bolivia)
6	Bozm 0944	Land race	CIFA (Bolivia)	Tariji (Bolivia)
7	Bozm 0999	Land race	CIFA (Bolivia)	Santa Cruz (Bolivia)
8	Bozm 1014	Land race	CIFA (Bolivia)	Cochabamba (Bolivia)
9	Bozm 1052	Land race	CIFA (Bolivia)	Beni (Bolivia)
10	Bozm 1057	Land race	CIFA (Bolivia)	Tariji (Bolivia)
11	Bozm 1294	Land race	CIFA (Bolivia)	Cochabamba (Bolivia)
12	Bozm 1330	Land race	CIFA (Bolivia)	Cochabamba (Bolivia)
13	Bozm 1335	Land race	CIFA (Bolivia)	Cochabamba (Bolivia)
14	Bozm 1337	Land race	CIFA (Bolivia)	Cochabamba (Bolivia)
15	Bozm 1345	Land race	CIFA (Bolivia)	Cochabamba (Bolivia)
16	Bozm 1376	Land race	CIFA (Bolivia)	Cochabamba (Bolivia)
17	Bozm 1413	Land race	CIFA (Bolivia)	Tariji (Bolivia)
18	Bozm 1416	Land race	CIFA (Bolivia)	Tariji (Bolivia)
19	Bozm 1457	Land race	CIFA (Bolivia)	Potosi (Bolivia)
20	Bozm 1483	Land race	CIFA (Bolivia)	Potosi (Bolivia)
21	Bozm 1510	Land race	CIFA (Bolivia)	Cochabamba(Bolivia)
22	Bozm 1511	Land race	CIFA (Bolivia)	Cochabamba(Bolivia)
23	Bozm 1532	Land race	CIFA (Bolivia)	Cochabamba(Bolivia)
24	Bozm 1533	Land race	CIFA (Bolivia)	Cochabamba(Bolivia)
25	Bozm 1535	Land race	CIFA (Bolivia)	Cochabamba(Bolivia)
26	Bozm 1536	Land race	CIFA (Bolivia)	Cochabamba(Bolivia)
27	Chzm 01001	Land race	INIA, La Platina (Chile)	Northern Chile
28	Chzm 01007	Land race	INIA, La Platina (Chile)	Camarones (Chile)
29	Chzm 01008	Land race	INIA, La Platina (Chile)	Camarones (Chile)
30	Chzm 01009	Land race	INIA, La Platina (Chile)	Northern Chile
31	Chzm 03004	Land race	INIA, La Platina (Chile)	Conay (Chile)
32	Chzm 08038	Land race	INIA, La Platina (Chile)	SaltoDelLaja (Chile)
33	Chzm 13002	Land race	INIA, La Platina (Chile)	Chacabuco (Chile)
34	C 88	Land race	CIMMYT (Mexico)	Mexico
35	C 89	Land race	CIMMYT (Mexico)	Mexico
36	C 235	Land race	CIMMYT (Mexico)	Mexico
37	C 10881	Land race	CIMMYT (Mexico)	CIMMYT (Mexico)
38	C 10932	Land race	CIMMYT (Mexico)	CIMMYT (Mexico)
39	C 11025	Land race	CIMMYT (Mexico)	CIMMYT (Mexico)
40	C 11026	Land race	CIMMYT (Mexico)	CIMMYT (Mexico)
41	C 12299	Land race	CIMMYT (Mexico)	Northern Chile
42	C 12338	Land race	CIMMYT (Mexico)	Northern Chile
43	C 12353	Land race	CIMMYT (Mexico)	Northern Chile
44	Sultan	Variety	Ayub Agri. Res. Institute	Pakistan
45	Sadaf	Variety	Ayub Agri. Res. Institute	Pakistan
46	Golden	Variety	Ayub Agri. Res. Institute	Pakistan
47	Akbar	Variety	Ayub Agri. Res. Institute	Pakistan
48	Aghoghi	Variety	Ayub Agri. Res. Institute	Pakistan
49	Agati 72	Variety	Ayub Agri. Res. Institute	Pakistan
50	Agati 94	Variety	Ayub Agri. Res. Institute	Pakistan

(Appendix 1.1 continued)

S.N	Acc. No./Name	Type	Source	Origin
51	EV 6085	Variety	Ayub Agri. Res. Institute	Pakistan
52	Iz 26	Variety	Ayub Agri. Res. Institute	Pakistan
53	Iz 40	Variety	Ayub Agri. Res. Institute	Pakistan
54	Iz 46	Variety	Ayub Agri. Res. Institute	Pakistan
55	Iz 80	Variety	Ayub Agri. Res. Institute	Pakistan
56	Iz 87	Variety	Ayub Agri. Res. Institute	Pakistan
57	Iz 7103	Variety	Ayub Agri. Res. Institute	Pakistan
58	SYP 31	Variety	Ayub Agri. Res. Institute	Pakistan
59	Lg 20:80	Variety	D.E.E.B	France
60	Pyramid	Variety	N.I.A.B (Cambridge)	England
61	Labrador	Variety	N.I.A.B (Cambridge)	England
62	Conquest	F_1 Hybrid	D.E.E.B	England
63	Reward	F_1 Hybrid	D.E.E.B	England
64	Sundance	F_1 Hybrid	D.E.E.B	England
65	Champ	F_1 Hybrid	D.E.E.B	England
66	Zeo 1	Inbred line	ZENECA	England
67	Zeo 6	Inbred line	ZENECA	England
68	G 800	Land race	IPK (Germany)	Georgia
69	Zea 642	Land race	IPK (Germany)	Bulgaria
70	Zea 671	Land race	IPK (Germany)	Unknown
71	Zea 699	Land race	IPK (Germany)	Slovakia
72	Zea 769	Land race	IPK (Germany)	South Italy
73	Zea 1006	Land race	IPK (Germany)	Libya
74	Zea 1072	Land race	IPK (Germany)	South Italy
75	PI 213714	Land race	NCRPIS (USA)	Arizona
76	PI 451716	Land race	NCRPIS (USA)	Arizona
77	PI 503567	Land race	NCRPIS (USA)	Arizona
78	PI 503568	Land race	NCRPIS (USA)	Arizona
79	PI 508270	Land race	NCRPIS (USA)	Arizona
80	PI 561620	Land race	NCRPIS (USA)	Cameroon
81	PI 583749	Land race	NCRPIS (USA)	Colombia
82	PI 583750	Land race	NCRPIS (USA)	Colombia
83	PI 583751	Land race	NCRPIS (USA)	Colombia
84	PI 583752	Land race	NCRPIS (USA)	Colombia
85	PI 583909	Land race	NCRPIS (USA)	Brazil
86	PI 583910	Land race	NCRPIS (USA)	Brazil
87	PI 583911	Land race	NCRPIS (USA)	Brazil
88	PI 583912	Land race	NCRPIS (USA)	Brazil
89	PI 583913	Land race	NCRPIS (USA)	Brazil
90	PI 583914	Land race	NCRPIS (USA)	Brazil
91	PI 583915	Land race	NCRPIS (USA)	Brazil
92	PI 583916	Land race	NCRPIS (USA)	Brazil
93	PI 583917	Land race	NCRPIS (USA)	Brazil
94	PI 583918	Land race	NCRPIS (USA)	Brazil
95	PI 584439	Land race	NCRPIS (USA)	Colombia
96	PI 584440	Land race	NCRPIS (USA)	Colombia

Appendix 1.2. Composition of Rorison solution.

Nutrient source	Concentration	
	1/2 strength	1/10 strength
Major elements		
$Ca(NO_3)_2\,4H_2O$	0.99 mM	0.2 mM
K_2HPO_4	0.50 mM	0.1 mM
Mg $SO_4\,7H_2O$	0.50 mM	0.1 mM
Fe EDTA	0.02 mM	0.003 mM
KCl	0.84 mM	0.17 mM
Trace elements		
$MnSO_4\,4H_2O$	4.50 µM	0.90 µM
H_3BO_3	23.1 µM	4.63 µM
$(NH_4)_6\,Mo_7O_{24}\,4H_2O$	0.07 µM	0.01 µM
$ZnSO_4$	0.77 µM	0.15 µM
$CuSO_4\,5H_2O$	0.78 µM	0.16 µM

Appendix 2.1. Absolute root length and C0 (NaCl concentration at which root length reaches zero) of 10-day-old seedlings 0f 72 maize accessions grown at 4 concentrations of NaCl.

Accessions	Control	60 mM	80 mM	150 mM	C0
Zea 642	10.52	7.11	5.72	3.74	202.71
Zea 671	9.09	5.16	3.94	3.09	172.74
Zea 699	13.40	7.92	6.33	4.10	177.69
Zea 769	13.05	7.19	5.93	3.88	170.46
Zea 1006	8.50	6.75	6.19	3.06	220.02
Zea 1072	14.11	6.42	4.81	2.71	144.13
G 800	10.61	7.83	6.09	3.62	215.32
Pyramid	14.72	8.63	4.65	5.31	167.99
Labrador	12.47	11.67	6.95	3.98	198.18
Lg 20.80	15.32	12.05	11.75	7.01	270.56
C 88	16.60	12.82	9.50	4.03	191.55
C 89	18.96	12.92	11.23	6.00	205.70
C 235	19.50	11.71	6.64	3.88	155.36
C 10881	14.04	8.28	6.02	3.35	165.71
C 10932	14.24	9.89	5.08	3.44	168.77
C 11025	16.37	10.98	8.35	3.98	183.22
C 11026	18.40	15.94	11.17	4.64	186.68
C 12299	17.75	9.96	9.82	4.89	179.47
C 12338	10.32	8.60	7.98	4.96	271.15
C 12353	13.86	10.78	8.44	3.75	198.00
Sadaf	13.25	6.28	3.99	2.41	113.59
Golden	15.23	9.83	6.28	2.60	163.70
Sultan	11.93	7.29	6.02	2.00	168.93
Aghoghi	15.98	9.26	7.87	4.39	175.40
Agati 72	17.00	8.99	8.72	3.28	164.28
Agati 94	11.75	8.25	5.80	3.32	190.57
Akber	15.40	5.10	4.60	3.11	133.75
EV 6085	15.05	8.92	6.79	4.13	172.44
IZ 26	11.67	7.25	5.16	3.03	172.51
IZ 40	12.23	11.10	6.59	3.73	196.39
IZ 46	11.99	7.00	6.78	3.21	183.56
IZ 80	10.96	8.16	5.66	3.88	207.96
IZ 87	11.33	8.61	6.66	3.87	219.93
IZ 7103	14.34	9.74	9.62	5.64	230.38
SYP 31	14.00	11.65	10.30	3.59	188.83
Conquest	8.28	5.65	5.13	4.70	256.54
Reward	14.83	6.55	4.29	3.55	142.88
Sundance	10.29	7.24	7.01	6.04	282.05
Champ	13.88	12.25	10.52	5.17	210.95
Bozm 0095	13.02	12.27	8.71	2.89	176.32

(Appendix 2.1 continued)

Accessions	Control	60 mM	80 mM	150 mM	C0
Bozm 0715	11.90	10.08	6.45	2.18	172.89
Bozm 0944	10.41	8.40	7.41	2.59	188.09
Bozm 0999	12.67	12.23	5.43	3.69	188.06
Bozm 1014	10.63	8.36	5.95	1.97	176.21
Bozm 1052	13.20	10.65	7.59	3.08	185.50
Bozm 1057	10.80	9.91	6.56	2.46	178.19
Bozm 1335	13.84	11.59	7.94	3.48	188.84
Bozm 1337	15.04	10.07	9.34	2.57	180.24
Bozm 1345	12.81	10.28	8.95	2.56	179.34
Bozm 1376	8.00	5.67	4.20	3.50	216.82
Bozm 1416	14.85	12.92	10.15	4.58	198.68
Bozm 1457	15.89	10.42	10.78	0.50	157.00
Bozm 1483	10.74	8.50	7.57	3.46	209.79
Bozm 1510	18.86	16.59	9.88	3.75	174.89
Bozm 1532	13.97	13.00	10.06	4.12	191.67
Bozm 1533	14.15	10.40	9.22	3.90	203.77
Bozm 1536	13.67	12.48	6.59	4.92	214.13
Chzm 01001	13.83	9.98	8.39	3.36	195.06
Chzm 01008	17.42	8.82	7.98	4.79	163.83
Chzm 01009	14.69	12.62	10.04	4.50	200.48
Chzm 03004	12.30	9.33	5.65	2.75	184.81
Chzm 13002	11.75	11.00	5.24	2.57	174.72
PI 213714	13.60	11.35	10.49	1.60	165.68
PI 451716	15.09	14.51	10.97	3.52	178.58
PI 503567	16.80	14.05	12.96	6.75	232.09
PI 503568	16.26	14.67	12.32	5.63	206.31
PI 508270	17.63	15.29	14.43	2.88	171.62
PI 561620	14.44	12.83	8.92	3.18	179.24
PI 583749	15.42	14.01	10.18	2.65	170.39
PI 583750	16.90	16.08	11.73	2.51	165.98
PI 583751	18.28	15.08	9.19	2.64	167.54
PI 583752	18.36	12.73	10.37	2.33	170.14

Appendix 3.1. Family means of absolute and relative root length of 84 F_1 hybrids of maize in 7x12 North Carolina Design II crosses in two NaCl concentrations.

Family	Female	Male	Absolute root length (cm)		Relative root length (%)
			control	80 mM NaCl	80 mM NaCl
1	Zea 1006	Golden	13.84	12.64	91.34
2	Zea 1006	SYP 31	18.51	15.12	82.16
3	Zea 1006	Zea 769	14.45	13.52	95.02
4	Zea 1006	C 12338	16.38	11.84	72.39
5	Zea 1006	Bozm 1335	14.90	08.81	62.64
6	Zea 1006	Bozm 1337	15.09	11.07	73.35
7	Zea 1006	Bozm 1345	11.87	11.30	95.25
8	Zea 1006	Bozm 1416	17.06	13.21	77.88
9	Zea 1006	Bozm 1483	11.42	10.58	93.14
10	Zea 1006	Bozm 1532	16.25	13.76	84.68
11	Zea 1006	G 800	13.77	12.49	90.41
12	Zea 1006	EV 6085	15.57	12.83	82.64
13	Reward	Golden	15.80	14.12	90.33
14	Reward	SYP 31	14.59	12.33	85.44
15	Reward	Zea 769	12.01	10.56	88.07
16	Reward	C 12338	11.61	09.26	79.60
17	Reward	Bozm 1335	14.20	12.76	89.98
18	Reward	Bozm 1337	12.75	10.44	82.94
19	Reward	Bozm 1345	15.47	12.55	81.04
20	Reward	Bozm 1416	10.68	09.61	93.05
21	Reward	Bozm 1483	09.76	09.19	93.83
22	Reward	Bozm 1532	15.39	07.52	48.88
23	Reward	G 800	12.66	10.68	83.72
24	Reward	EV 6085	13.65	13.01	95.35
25	Zea 1006 X Reward	Golden	17.57	13.39	76.29
26	Zea 1006 X Reward	SYP 31	18.19	14.83	82.83
27	Zea 1006 X Reward	Zea 769	14.05	12.39	88.83
28	Zea 1006 X Reward	C 12338	19.03	11.87	62.15
29	Zea 1006 X Reward	Bozm 1335	14.40	13.16	96.63
30	Zea 1006 X Reward	Bozm 1337	15.27	12.05	83.21
31	Zea 1006 X Reward	Bozm 1345	16.32	12.05	75.69
32	Zea 1006 X Reward	Bozm 1416	15.05	11.57	77.33
33	Zea 1006 X Reward	Bozm 1483	15.75	10.73	69.12
34	Zea 1006 X Reward	Bozm 1532	14.56	12.26	85.13
35	Zea 1006 X Reward	G 800	16.52	11.94	74.44
36	Zea 1006 X Reward	EV 6085	17.95	10.87	60.68
37	Champ	Golden	23.26	17.08	73.92
38	Champ	SYP 31	19.04	15.24	79.93
39	Champ	Zea 769	13.78	12.32	93.96
40	Champ	C 12338	18.62	14.58	78.62
41	Champ	Bozm 1335	18.65	13.46	72.21
42	Champ	Bozm 1337	17.08	15.69	92.05
43	Champ	Bozm 1345	16.43	11.58	70.24
44	Champ	Bozm 1416	13.84	13.05	94.28
45	Champ	Bozm 1483	14.27	11.73	82.99

(Appendix 3.1 continued)

Family	Female	Male	Absolute root length (cm)		Relative root length (%)
			Control	80 mM NaCl	80 mM NaCl
43	Champ	Bozm 1345	16.43	11.58	70.24
44	Champ	Bozm 1416	13.84	13.05	94.28
45	Champ	Bozm 1483	14.27	11.73	82.99
46	Champ	Bozm 1532	15.70	15.13	96.44
47	Champ	G 800	17.12	16.50	97.83
48	Champ	EV 6085	14.78	13.38	90.52
49	Sundance	Golden	17.62	16.38	93.67
50	Sundance	SYP 31	21.39	16.70	78.79
51	Sundance	Zea 769	15.25	12.48	82.03
52	Sundance	C 12338	19.65	14.99	76.34
53	Sundance	Bozm 1335	16.94	15.61	96.23
54	Sundance	Bozm 1337	16.41	13.91	85.77
55	Sundance	Bozm 1345	15.41	13.00	85.56
56	Sundance	Bozm 1416	15.84	15.79	99.70
57	Sundance	Bozm 1483	14.07	11.86	89.71
58	Sundance	Bozm 1532	22.28	14.87	67.06
59	Sundance	G 800	17.46	15.51	89.02
60	Sundance	EV 6085	20.12	14.57	72.51
61	Lg 20.80	Golden	16.47	14.73	94.59
62	Lg 20.80	SYP 31	17.06	15.65	93.68
63	Lg 20.80	Zea 769	14.43	14.03	97.34
64	Lg 20.80	C 12338	16.73	12.14	73.67
65	Lg 20.80	Bozm 1335	14.59	12.35	92.12
66	Lg 20.80	Bozm 1337	16.96	12.05	73.03
67	Lg 20.80	Bozm 1345	16.22	12.90	82.09
68	Lg 20.80	Bozm 1416	15.72	09.83	63.56
69	Lg 20.80	Bozm 1483	12.45	11.16	89.00
70	Lg 20.80	Bozm 1532	14.03	13.25	95.12
71	Lg 20.80	G 800	17.20	13.45	78.35
72	Lg 20.80	EV 6085	16.61	11.59	72.63
73	Akber	Golden	16.55	10.05	61.36
74	Akber	SYP 31	17.40	13.46	79.40
75	Akber	Zea 769	14.61	14.06	97.11
76	Akber	C 12338	14.84	13.09	89.62
77	Akber	Bozm 1335	16.93	13.43	80.55
78	Akber	Bozm 1337	14.92	12.07	80.99
79	Akber	Bozm 1345	15.03	10.70	74.58
80	Akber	Bozm 1416	15.46	10.84	73.12
81	Akber	Bozm 1483	16.39	07.53	46.14
82	Akber	Bozm 1532	15.31	08.28	50.04
83	Akber	G 800	16.08	14.40	89.96
84	Akber	EV 6085	17.99	11.74	65.89

Appendix 4.1. Absolute and relative root length, and visual rating of 10-day-old seedlings 0f 72 maize accessions grown at Al and Mn concentrations.

Accessions	Absolute root length (cm)		Relative root length (%)	Visual rating
	Control	0.11 mM Al	0.11 mM Al	2.0 mM Mn
Bozm 0095	13.63	5.11	37.49	5.43
Bozm 0170	13.76	7.34	53.34	4.82
Bozm 0253	14.10	5.64	40.00	5.46
Bozm 0715	16.67	7.78	46.67	2.85
Bozm 0883	16.36	7.43	45.42	4.71
Bozm 0944	12.90	5.50	42.64	4.90
Bozm 0999	13.95	7.63	54.70	5.21
Bozm 1014	14.05	6.53	46.48	4.75
Bozm 1052	15.52	7.79	50.19	5.72
Bozm 1057	15.38	7.67	49.87	4.48
Bozm 1294	14.57	7.49	51.41	5.56
Bozm 1330	15.06	8.77	58.23	4.47
Bozm 1335	11.28	8.98	79.61	4.94
Bozm 1337	11.75	9.05	77.02	4.86
Bozm 1345	13.46	5.80	43.09	5.00
Bozm 1376	12.05	7.71	63.98	4.99
Bozm 1413	15.67	6.72	42.88	5.13
Bozm 1416	16.14	6.75	41.82	5.64
Bozm 1457	13.16	6.12	46.50	4.19
Bozm 1483	15.92	7.98	50.13	5.33
Bozm 1510	13.02	8.91	68.43	5.10
Bozm 1511	14.80	8.40	56.76	5.37
Bozm 1532	14.56	6.74	46.29	5.58
Bozm 1533	14.89	5.14	34.52	3.25
Bozm 1535	12.83	5.46	42.56	4.75
Bozm 1536	11.57	9.03	78.05	4.81
Chzm 01001	12.25	6.49	52.98	3.59
Chzm 01007	8.42	3.86	45.84	5.00
Chzm 01008	12.19	8.78	72.03	3.33
Chzm 01009	13.67	5.44	39.80	2.28
Chzm 03004	11.12	3.13	28.15	6.00
Chzm 08038	11.00	3.97	36.09	4.33
Chzm 13002	09.10	1.80	19.78	4.50
C 88	15.23	7.23	47.47	4.12
C 89	13.91	6.64	47.74	5.57
C 12338	09.89	3.49	35.29	4.05
Conquest	12.01	5.63	46.88	4.36
Reward	09.92	2.47	24.90	4.97

(Appendix 4.1 continued)

Accessions	Absolute root length (cm)		Relative root length (%)	Visual rating
	Control	0.11 mM Al	0.11 mM Al	2.0 mM Mn
Sundance	16.69	5.11	30.62	5.30
Champ	15.93	4.66	29.25	2.68
G 800	13.62	7.24	53.16	4.00
Zea 642	12.11	8.80	72.67	3.30
Zea 671	09.27	5.46	58.90	3.66
Zea 699	13.53	8.11	59.94	6.27
Zea 769	13.30	10.91	82.03	2.66
Zea 1006	13.44	4.09	50.54	5.23
Zea 1006xReward	10.11	5.11	53.14	5.47
Zea 1072	10.35	5.50	30.43	5.85
Lg 20.80	17.34	3.53	20.36	2.95
Pyramid	12.60	7.96	63.17	3.00
Labrador	14.57	5.55	38.09	4.00
Aghoghi	13.36	8.36	62.57	5.41
Agati 72	13.87	6.76	48.74	5.38
Agati 94	13.59	5.87	43.19	5.28
Akber	09.30	1.90	20.43	4.60
EV 6085	11.57	5.54	47.88	5.97
Golden	14.19	4.01	28.26	7.00
Sadaf	14.79	5.87	39.69	5.58
Sultan	14.23	3.15	22.14	5.00
SYP-31	14.01	5.16	36.83	5.80
PI 583909	13.13	6.42	48.90	5.52
PI 583910	11.97	4.67	39.01	5.63
PI 583911	11.68	6.58	56.33	5.74
PI 583912	10.97	4.51	41.12	5.36
PI 583913	11.98	4.93	41.15	5.22
PI 583914	10.88	3.88	35.66	3.95
PI 583915	12.62	3.23	25.59	5.69
PI 583916	11.58	3.35	28.93	5.55
PI 583917	11.82	3.80	32.15	5.52
PI 583918	11.85	4.16	35.10	4.64
PI 584439	11.60	5.34	46.03	3.86
PI 584440	13.10	4.31	32.90	4.15

Appendix 5.1. Family means of absolute and relative root length, and leaf chlorosis and/or necrosis of 65 F_1 hybrids of maize in 5x13 North Carolina Design II mating system.

Family	Female	Male	Absolute root length (cm)		Relative root length (%)	Visual ratings
			control	0.11 mM (Al)	0.11 mM (Al)	1.0 mM (Mn)
1	Zea 1006	Golden	15.52	15.00	96.69	3.29
2	Zea 1006	SYP 31	18.95	15.47	81.65	3.83
3	Zea 1006	Zea 769	16.37	12.56	76.73	6.00
4	Zea 1006	C 12338	12.48	10.54	84.43	5.56
5	Zea 1006	Bozm 1335	11.01	14.08	127.94	4.42
6	Zea 1006	Bozm 1337	12.86	13.56	105.43	5.16
7	Zea 1006	Bozm 1345	13.50	12.87	95.29	4.00
8	Zea 1006	Bozm 1416	16.62	11.81	71.10	5.47
9	Zea 1006	Bozm 1438	10.73	8.93	83.29	4.43
10	Zea 1006	Bozm 1532	14.68	10.40	70.84	3.90
11	Zea 1006	G 800	16.75	13.05	77.93	3.72
12	Zea 1006	EV 6085	15.23	12.52	82.24	4.14
13	Zea 1006	Bozm 1533	15.01	13.93	92.78	2.42
14	Zea 1006 X Reward	Golden	17.01	11.58	68.08	3.11
15	Zea 1006 X Reward	SYP 31	20.31	10.16	50.00	3.10
16	Zea 1006 X Reward	Zea 769	14.59	10.64	72.91	4.20
17	Zea 1006 X Reward	C 12338	20.42	12.59	61.67	4.63
18	Zea 1006 X Reward	Bozm 1335	17.58	11.56	65.76	3.64
19	Zea 1006 X Reward	Bozm 1337	14.99	10.35	69.06	3.04
20	Zea 1006 X Reward	Bozm 1345	17.13	13.56	79.18	5.27
21	Zea 1006 X Reward	Bozm 1416	16.32	9.65	59.09	4.00
22	Zea 1006 X Reward	Bozm 1438	12.45	10.12	81.27	3.04
23	Zea 1006 X Reward	Bozm 1532	15.21	10.38	68.20	3.86
24	Zea 1006 X Reward	G 800	17.85	9.71	54.40	4.83
25	Zea 1006 X Reward	EV 6085	18.24	7.00	38.39	2.88
26	Zea 1006 X Reward	Bozm 1533	15.93	12.19	76.50	4.81
27	Sundance	Golden	19.03	13.67	71.85	2.96
28	Sundance	SYP 31	20.53	13.15	64.05	3.00
29	Sundance	Zea 769	14.59	12.68	86.86	4.25
30	Sundance	C 12338	14.80	12.03	81.25	4.00
31	Sundance	Bozm 1335	16.29	14.92	91.64	4.17
32	Sundance	Bozm 1337	17.14	13.71	80.03	4.81
33	Sundance	Bozm 1345	16.23	13.99	86.19	5.92
34	Sundance	Bozm 1416	16.65	14.50	87.08	6.00
35	Sundance	Bozm 1438	16.63	12.98	78.02	4.78
36	Sundance	Bozm 1532	14.98	10.88	72.64	3.71
37	Sundance	G 800	17.18	13.69	79.64	4.84
38	Sundance	EV 6085	19.25	10.24	53.17	3.44
39	Sundance	Bozm 1533	17.31	10.42	60.17	4.00
40	Lg 20.80	Golden	22.51	11.97	53.18	5.52

(Appendix 5.1 continued)

Family	Female	Male	Absolute root length (cm)		Relative root length (%)	Visual ratings
			control	0.11 mM (Al)	0.11 mM (Al)	1.0 mM (Mn)
41	Lg 20.80	SYP 31	18.77	9.53	50.75	4.34
42	Lg 20.80	Zea 769	11.62	8.24	70.92	4.75
43	Lg 20.80	C 12338	18.51	8.63	46.61	5.00
44	Lg 20.80	Bozm 1335	15.24	5.99	39.31	4.13
45	Lg 20.80	Bozm 1337	18.61	5.15	27.66	5.00
46	Lg 20.80	Bozm 1345	19.32	12.46	64.49	5.71
47	Lg 20.80	Bozm 1416	20.67	7.54	36.48	5.75
48	Lg 20.80	Bozm 1438	18.81	9.32	49.52	4.10
49	Lg 20.80	Bozm 1532	20.54	5.50	26.77	5.00
50	Lg 20.80	G 800	21.61	9.39	43.46	4.50
51	Lg 20.80	EV 6085	20.26	7.65	37.75	4.48
52	Lg 20.80	Bozm 1533	19.37	8.67	44.78	3.00
53	Akber	Golden	19.04	8.19	42.98	4.89
54	Akber	SYP 31	17.38	9.32	53.64	4.39
55	Akber	Zea 769	14.53	8.36	57.56	4.00
56	Akber	C 12338	17.11	7.17	41.90	5.70
57	Akber	Bozm 1335	16.55	7.47	45.17	4.73
58	Akber	Bozm 1337	17.01	8.72	51.26	5.60
59	Akber	Bozm 1345	17.23	6.74	39.11	5.85
60	Akber	Bozm 1416	14.14	8.67	61.27	6.29
61	Akber	Bozm 1438	13.52	5.49	40.63	5.00
62	Akber	Bozm 1532	19.37	6.90	35.65	4.75
63	Akber	G 800	17.26	5.64	32.69	5.67
64	Akber	EV 6085	19.08	6.88	36.06	4.43
65	Akber	Bozm 1533	21.09	9.37	44.45	5.23