Screening for zinc efficiency in barley (*Hordeum vulgare* L.)

Yusuf Genc

B. Sc., M. Sc. (Agriculture)

Department of Agronomy

University of Ankara, Ankara, Turkey

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Department of Plant Science

Faculty of Agriculture and Natural Resource Sciences

University of Adelaide, Adelaide, South Australia

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I dedicate this thesis to my late father

Garip Genc

1944-1992

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Declaration

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

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

Yusuf Genc

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Summary

Worldwide, zinc (Zn) deficiency reduces yield and grain quality over many millions hectares, especially in the arid and semi-arid regions where cereals are often grown. Applications of Zn fertilizers are commonly used to correct Zn deficiency, however; Zn fertilizers are costly, are not always effective, often require repeated applications and do not correct Zn deficiency in the subsoil. Long-term alleviation of Zn deficiency can be best achieved by growing genotypes with an improved ability to extract and/or utilize available soil Zn. A Zn-efficient genotype, in an agronomic sense, is a genotype which is able to grow and yield well in soils too deficient for a standard genotype (Graham, 1984). In barley, field screening has been the basis of selecting Zn-efficient genotypes, but field tests are difficult to perform and the results can be influenced by factors other than Zn. The aims of the present study were to develop a reliable method for screening for Zn efficiency as an alternative to the current field-based methods, and to determine the extent of genotypic variation in tolerance to Zn deficiency in barley.

A number of growth room and glasshouse experiments were conducted. The experiments examined the effects of seed Zn content and seed size on early growth, determined a critical concentration for Zn, and compared different selection criteria and developed a soil-based pot system as a selection method. This system was then used to determine the extent of genotypic variation in response to Zn deficiency in 56 barley genotypes, and conduct a preliminary investigation on the genetic control of tolerance to Zn deficiency. The experiments used two soils, Laffer sand and Lancelin sand, both of which are low in available Zn, and are used commonly in Zn nutrition work in Australia.

Two experiments were carried out to determine the effect of seed Zn content on early growth of two genotypes, under low and adequate soil Zn supply using Laffer sand (Chapter 3). In these experiments, seed was hand-sorted to provide uniform seed with different contents of Zn (range: 0.4-5.0 μg/seed) The results showed that plants grown from seed with high Zn content developed less severe deficiency symptoms, and produced greater shoot and root dry matter than those grown from seed with low Zn content, especially when soil Zn supply limited plant growth. High seed Zn content also resulted in high tissue Zn concentrations, and this effect was most evident under Zn deficiency, but only at a seed Zn content ≥ 4.3 μ g/seed. Zn efficiency was affected by

seed Zn content, suggesting that Zn efficiency ranking could differ depending of seed Zn content. Therefore, in routine screening, seed of similar Zn content should be used for sound comparisons among the genotypes where selection is based on dry matter production.

The response to Zn in two Turkish genotypes, Tarm and Hamidiye, known to differ in Zn efficiency was examined in two experiments using Laffer sand (Chapter 4). The results from the first experiment established that the differences found in the field between the two genotypes were expressed in visual symptoms as well as in shoot and root dry matter under controlled conditions. The results also indicated that the two genotypes had similar critical concentrations for Zn (20.0 and 19.7 mg/kg D.W. for Tarm and Hamidiye, respectively), providing evidence that their differences in efficiency depended on differences in their uptake of Zn rather than in their requirement. In the second experiment, however, the degree of Zn deficiency stress was less than that observed in the first experiment. No symptoms of Zn deficiency developed in either genotype and shoot and root growth were greater than in the first experiment. Analyses of soils subsequent to the experiment suggested the different response was due to a higher concentration of Zn in the soil batch used for the second experiment (DTPA-extractable Zn=0.30 mg/kg) compared to that used for the first experiment (DTPA-extractable Zn=0.07 mg/kg). The differential response to Zn between the two batches of soil, which was not anticipated at the time of the experiment, suggested that each batch of the soil should be tested for DTPA-extractable Zn in future experiments to provide some indication of the likely response of plants grown in soil. Given the time constraints in the present study and concern with the variability in Laffer sand, it was decided to try Lancelin sand, which is a widely used alternative to Laffer sand in Western Australia.

In three experiments conducted in a glasshouse (Chapter 5), Lancelin sand was assessed for its potential as a soil medium for screening for tolerance to Zn deficiency (Chapter 5). The first experiment examined the general response to Zn using two genotypes and six levels of Zn. Based on the results of this experiment, 48 genotypes of barley were evaluated for their tolerance to Zn deficiency by growing plants at three levels of Zn fertilization for 28 days. Genotypes differed significantly in the severity of Zn deficiency symptoms and Zn efficiency, but had similar concentrations of Zn in the shoot, especially under Zn deficiency. The differences in Zn efficiency could be partly

attributed to the differences in seed Zn content of the genotypes. The results from the three experiments consistently showed that Lancelin sand was a severely Zn-deficient environment that would be suitable for screening studies, but responses to applied Zn were again affected by differences in seed Zn content.

The severity of Zn deficiency in Lancelin sand appeared to be greater than that in Laffer sand. While both soils have been used extensively for Zn work, there has been no direct comparison between them. Therefore, two experiments were conducted to examine the Zn responses in these soils (Chapter 6). In one experiment, the response to Zn in two sands and their various mixtures (25:75; 50:50; 75:25) were compared. The results established that the proportion of Lancelin sand in the mix markedly affected the expression of visual symptoms of Zn deficiency and shoot and root growth. The greater the proportion of Lancelin sand in the mix, the more severe the deficiency symptoms. Under Zn deficiency, plants grown in Lancelin sand also accumulated higher levels of Fe in the shoot than plants grown in Laffer sand. However, with adequate Zn, Lancelin sand grown plants were still smaller and lower in P than Laffer sand. The hypothesis that at low soil Zn supply, the greater Zn deficiency stress in Lancelin sand was due to the greater Fe content of Lancelin sand, and at adequate soil Zn supply, the lower dry matter in plants grown in Lancelin sand was caused by marginal P deficiency in Lancelin sand, was examined in a second experiment in which Fe and P levels were manipulated in Lancelin and Laffer sands. The results suggested that the high Fe concentration under Zn deficiency in Lancelin sand was an induced nutritional imbalance rather than a direct toxicity, because supplying adequate amounts of Zn to the soil resulted in non-toxic concentrations. Therefore, the possibility of high Fe induced-Zn deficiency could be excluded as the primary cause of greater Zn deficiency stress in Lancelin sand. More severe Zn stress in Lancelin sand, compared with Laffer sand, was attributed to the greater organic matter content of Lancelin sand, and therefore, a greater potential for Zn-organic matter complexes which in turn result in lower plant available Zn. At adequate soil Zn supply, plants grown in Lancelin sand responded to increased P fertilization, indicating that in future screening with Lancelin sand, a greater amount of P than that used in Laffer sand should be applied to prevent marginal deficiencies of P and achieve optimum yields in plants grown in Lancelin sand.

Based on the results of this work, a seedling screening method was developed using small pots containing 400 g of Lancelin sand and growing seedlings for 21 days. This method was used to explore genotypic variation in response to Zn deficiency in 56 barley genotypes (Chapter 7). The results demonstrated that genotypes differed considerably in severity of deficiency symptoms and reduction in dry matter production. The differences observed in this experiment are likely to be inherent and not confounded markedly by the variation in the seed Zn content among the varieties since almost all genotypes had reasonably similar Zn contents in the seed. A significant positive relationship between a visual score based on deficiency symptoms and reduction in shoot dry matter (r=0.80) suggested that visual scores could be used as a parameter for assessing the tolerance of genotypes to Zn deficiency. Deficiency symptoms were better correlated with the Zn content in the shoot (r=-0.78) than the Zn concentration in the shoot (r=-0.46).

Hand sorting of seed is a laborious and time consuming task and is not very suitable for routine screening. The effect of mechanically grading seed, to achieve a sample of uniform seed size, was examined in six genotypes differing in Zn efficiency (Chapter 7). Seeds were sorted into three size classes (mm in diameter): small (2.25-2.5), medium (2.5-2.8), and large (>2.8). Seed Zn content did not change consistently with seed size and so Zn efficiency was not consistently affected by seed size. However, visual symptoms appeared to be consistent across genotypes and independent of seed size. Although seed selection based on seed weight still appears to be the most practical method of achieving similar Zn contents for screening studies, grading seed into different seed sizes did not affect ranking of genotypes based on visual symptoms. This was probably due to a small range for seed Zn content among the three seed sizes within each genotype (e.g. Forrest: 0.8, 1.0, 0.9 µg/seed; Skiff: 0.6, 0.7, 0.9 µg/seed, for small, medium and large seed, respectively).

A comparison of the Zn efficiency based on vegetative growth and grain yield was investigated in 15 genotypes that differed in Zn deficiency symptoms 21 days after sowing (Chapter 7). The results found that relative grain yield correlated better with visual scores (r=0.68) than with relative shoot growth (r=0.58), both of which were measured independently at the seedling stage. The stronger correlation between visual

deficiency symptoms at the seedling stage and grain yield further suggested that deficiency symptoms at the seedling stage could be useful for screening studies.

Earlier screening work using 16 genotypes identified two Australian varieties, Skiff (moderately tolerant) and Forrest (sensitive) as potential parents to develop an F_2 population for genetic analysis of tolerance to Zn deficiency. The parents, and the F_2 population from the Skiff x Forrest cross were grown in Lancelin sand at one level of Zn fertilization (0.02 mg/kg soil) and using visual scores as the selection criterion (Chapter 8). The results indicated that in this population, tolerance to Zn deficiency is controlled by a single gene with no dominance. In conclusion,

- Using visual symptoms, a large number of genotypes can be screened in response to Zn deficiency at the seedling stage; visual symptoms are a good measure of tolerance to Zn deficiency, and straightforward to measure.

- Zn concentration in the tissue is not a useful selection criterion under Zn deficiency, but it is a good indicator of zinc status of barley plant, and the critical zinc concentration of YEBs can be used as a reference point in the assessment of zinc status of barley.

- Seed Zn content can influence seedling growth, therefore, seedling screening should involve the use of seed of similar Zn content. This can be simply achieved by hand sorting or if the seed Zn concentration does not vary much, by sieving seed. Ideally a screening method independent of seed Zn content needs to be developed.

- Responses to applied Zn can vary considerably with soil type and soil batch within a soil type, and there is a good correlation between soil Zn content (DTPA-extractable) and plant response. Therefore, determination of the critical concentration, and the initial Zn content of the soil, are required to predict the likely responses to Zn at the onset of screening studies.

- Expression of visual symptoms of Zn deficiency in seedlings of Skiff seems to be under simple genetic control.

CHAPTER 1

General Introduction

Zinc (Zn) deficiency is a serious nutritional problem over many millions of hectares of the world's cereal growing areas (Graham *et al.*, 1992; Takkar *et al.*, 1993; Cakmak *et al.*, 1996a), reducing both yield and grain quality. In Turkey, yield losses of up to 86% in wheat (Cakmak *et al.*, 1996a) and 50% in barley (Yilmaz *et al.*, 1996) have been recorded. When cereals are grown on Zn-deficient soils, in addition to yield losses, they often produce grain with low Zn content (Graham *et al.*, 1992), which reduces their nutritional value for humans, and reduces the vigour of seedlings when the grain is resown.

Soil and foliar applications of Zn are common agronomic practices used for correcting Zn deficiency, and large increases in grain yield and grain quality of cereals by Zn application have been reported by many workers in Australia (Graham *et al.*, 1992), India (Pathak *et al.*, 1979; Takkar *et al.*, 1983) and Turkey (Cakmak *et al.*, 1996a; Yilmaz *et al.*, 1996). However, Zn fertilizers are costly, are not always effective, often require repeated applications and do not correct Zn deficiency in the subsoil. Subsoil deficiency is best overcome by growing a genotype with a root system more able to mobilize the subsoil Zn (Graham, 1984).

A Zn efficient genotype, in an agronomic sense, is a genotype which is able to grow and yield well in soils too deficient for a standard genotype (Graham, 1984). There is considerable variation in response to Zn deficiency among cereals and genotypes within a given species (Cakmak *et al.*, 1997a; Cakmak *et al.*, 1997b; Graham *et al*, 1992), indicating the potential for selecting and/or breeding genotypes with higher Zn

efficiency. The combined use of Zn fertilizer and genotypes with improved Zn efficiency appears to be a practical approach to solving the problem of Zn deficiency (Graham *et al.*, 1992; Cakmak *et al.*, 1999). The use of Zn-efficient genotypes may not eliminate the need for Zn fertilizer, but it will allow improved yields in situations where Zn fertilizer is not used because of high price or poor availability, or it will improve the use efficiency of Zn fertilizer. Graham and Welch (1996) have listed the benefits of breeding for greater Zn efficiency as:

(i) decreased fertilizer requirements,

(ii) improved seedling vigour,

(iii) correction of yield losses resulting from unrecognized or subclinical deficiencies,

(iv) increased resistance to pathogens, and

(v) enhanced yield and nutritional quality of grain where applicable (e.g. when consumed by humans).

However, so far, progress towards breeding for greater Zn efficiency in barley has been hampered by (i) the lack of rapid and sound selection criteria which would be a good measure of tolerance to Zn deficiency and (ii) an understanding of the genetic control of this tolerance mechanism.

In selecting genotypes with greater Zn efficiency, the plus-and-minus treatments approach of Graham (1984) has been used extensively as a selection method in several crop species such as wheat (Graham *et al.*, 1992; Cakmak *et al.*, 1996a,b) chickpea (Khan *et al.*, 1998a), canola (Grewal *et al.*, 1997) and medics (Streeter, 1998). The method has been used successfully under both field and controlled environment conditions. There has been little work on the tolerance of barley to Zn deficiency, and to

date, field screening has been the basis of selection for tolerance (Takkar *et al.*, 1983; MacNaeidhe and Fleming, 1990; Graham *et al.*, 1992; Yilmaz *et al.*, 1996). However, field screening has a number of limitations which make it difficult to select for genotypes with greater Zn efficiency. These include (i) the high spatial variability of Zn at field sites, (ii) growth limiting factors other than Zn, (e.g. deficiencies or toxicities of other nutrients, diseases, drought etc.), (iii) seasonal variation, and (iv) time and cost in the screening process (Graham, 1984). Therefore, numerous investigators have chosen to do screening in potted soil and several successful examples exist already (Graham *et al.*, 1992; Cakmak *et al.*, 1996a; Grewal *et al.*, 1997; Khan *et al.*, 1998a; Streeter, 1998). This type of screening can be useful in a breeding program because of its ability to screen a large number of genotypes rapidly and economically; however, its potential for barley has yet to be determined. Therefore, the present study was designed to develop a screening method under controlled conditions which could overcome the problems encountered in the field screening and which would allow a large number of genotypes to be screened rapidly and economically. As part of the study, various aspects of Zn nutrition in barley, such as the importance of seed Zn content for early growth, the critical Zn concentration for diagnosing Zn status of plants, and evaluation of genetic differences in response to Zn deficiency under controlled conditions need to be investigated. Using the soil-based screening method developed during this study, a preliminary analysis of the genetic control of tolerance to Zn deficiency was investigated in an F_2 population from contrasting genotypes identified in the present study, namely Skiff (moderately tolerant) and Forrest (sensitive).

CHAPTER 2

Literature Review

2.1 Introduction

This review describes current knowledge of various aspects of zinc (Zn) in plants and soils but with specific reference to the Zn nutrition of barley (*Hordeum vulgare* L.). Zn deficiency, genotypic variation in tolerance to Zn deficiency and the mechanisms of tolerance are discussed. However, specific information on Zn nutrition in barley is scanty, so references to other crops, in particular wheat, will be made when necessary.

2.2 Chemistry of zinc

Knowledge of the chemistry of Zn is of considerable importance in understanding its soil chemistry. In the periodic table "*Zn occupies a position in Group II and Period 4 which enables it to be regarded either as the final member of the transition series otherwise ending at copper, or as the first member of the series of normal elements otherwise beginning with gallium*" (Farnsworth and Kline, 1973). Because the *d* orbitals in the third shell of the zinc atom are never used in bond formation, Zn does not show the variable valency which is one of the characteristics of transition elements (Farnsworth and Kline, 1973). This contributes to the great stability of Zn compounds compared with other transition metals.

In Nature, Zn often occurs in minerals as a divalent ion (Zn^{2+}) (Barak and Helmke, 1993) which forms stable complexes with polarizable ligands such as ammonia, amines and cyanide ion. The benefits of this are seen in analytical procedures for determination

of Zn. An example of the reactions that Zn undergoes is given below. Zn^{2+} hydrolyzes in aqueous solutions to give slightly acid solutions:

$$Zn^{2+} + H_2O \leftrightarrow Zn(OH)^+ + H^+$$

$Zn(OH)_2$ (hydroxide) is precipitated when a base is added to solutions of zinc salts. When more base is added, the hydroxide dissolves to give zincate ion, which is represented variously as: $Zn(OH)_3^-$, $Zn(OH)_4^{2-}$, $HZnO_2^-$ or ZnO_2^{2-}. Zinc has a strong tendency to form stable, complex ions: $Zn(OH)_2$ readily dissolves in aqueous ammonia to form a zinc-ammonium complex ($Zn(NH_3)_4^{2+}$) and the hydroxide also dissolves in cyanide solutions to form a zinc-cyanide complex ($Zn(CN)_4^{2-}$).

Knowledge of the chemistry of Zn in the soil (e.g. reactions of Zn with other nutrients) enables a better understanding of some of the major factors affecting its solubility and, as a result, soil available Zn. For example, it is well known that the presence of nitrates, chloride or sulfate ions in the soil solution favour formation of soluble zinc nitrate $(ZnNO_3)_2$, chloride ($ZnCl_2$) or sulfate ($ZnSO_4$) and consequently increase the amount of soil available Zn (Sauchelli, 1969). In contrast, in the presence of high concentrations of hydroxyl $(OH)^-$ ions, the solubility, and consequently availability, of Zn would be very low due to the formation of the nearly insoluble $Zn(OH)_2$ (Sauchelli, 1969).

2.3 Zinc in soils

The average Zn concentration in the Earth's upper crust is estimated to be 57 mg/kg (Brehler and Wedepohl, 1978), but its concentration in soils ranges from 10 to 300 mg/kg with an average of 70 mg/kg (Swaine, 1955). The concentration of Zn in soils differs with parent material and soil concentration of quartz. Quartz in the soil dilutes soil Zn since it contains very low levels of Zn, ranging from 1.0 mg/kg (Helmke *et al.*,

1977) to <5-8 mg/kg (Brehler and Wedepohl, 1978). In igneous systems, mafic rocks have a greater Zn concentration (100 mg/kg) than felsic rocks (40 mg/kg) (Taylor, 1964), since Zn^{2+} can substitutes for Mg^{2+} and Fe^{2+} in pyroxene. Of the sedimentary rocks, shales (95 mg/kg) have a higher concentration of Zn than limestone (20 mg/kg) and sandstone (16 mg/kg) (Turekian and Wedepohl, 1961). Brehler and Wedepohl (1978) report the concentration of Zn in numerous minerals and rocks.

Most of the Zn in soil forms complexes with clay minerals, organic matter and metal oxides. Therefore, total Zn in the soil is distributed among a number of different forms such as exchangeable, specifically adsorbed, bound to organic matter and penetrated into the particles. Consequently, the amount of Zn bound to the soil components is higher than the amount in soil solution. However, despite its relatively small percentage of the total soil Zn, the available Zn accounts for the majority of the Zn taken up by plants.

2.3.1 Availability of soil zinc to plants

One of the major factors that determines the severity of Zn deficiency is the concentration of Zn in the soil. Only a fraction of the total soil Zn is exchangeable, that is, in a form which is likely to be available to plants. For example, Tiller *et al.* (1972) showed that less than 14% of Zn in acid soils from Australia was isotopically exchangeable, while Vale (1982) found that 0.3 to 0.5% of the total zinc in acid soils from Wisconsin, USA and Portugal was isotopically exchangeable.

Despite its importance to Zn nutrition, estimating the amount of available Zn in the soil is not straightforward. It relies on chemical extraction and there is no single recommended method of extraction. Consequently, estimates of available Zn can vary

depending on extraction method. A number of extractants (e.g. water, neutral salts, weak and strong acids, and chelating agents such as ethylene diamine-tetra-acetic acid (EDTA) and diethylene triamine penta-acetic acid (DTPA) have been designed to predict the amount of soil available Zn (Lindsay and Cox, 1985; Sims and Johnson, 1991). The suitability of different extractants for monitoring soil Zn and predicting plant availability is contentious and has been discussed by a number of investigators. Vittal and Gangar (1975) reported that diothizone and EDTA were better than acids, water, or DTPA in predicting Zn uptake, whereas Osiname *et al.* (1973) found 0.1 N HCl to be the best when combined with pH, organic matter, and silt plus clay. Brown *et al.* (1971) showed that of four extractants tested, DTPA gave the best correlations with plant data.

Ideally, the extractant should reflect both the initial concentration of the nutrient in solution (intensity, I) and the ability of the soil to replenish the soil solution (capacity, Q) (Brennan *et al.*, 1993). An example of this type of extractant can be chelating agents. Viets *et al.* (1973) argued that chelating agents were better extractants since they took into consideration both intensity and capacity factors.

Estimates of critical soil Zn vary with different extractants. For example, the critical soil concentrations of Zn for the extractants DTPA, Mehlich-1 and 0.1 M HCl are quoted as 0.5-1.0, 1.1 and 1.0-5.0 mg Zn/kg soil respectively (Lindsay and Cox, 1985; Cox, 1987). Despite the problems in estimating the amount of available Zn in the soil, the effects of soil and environmental properties on Zn availability have been extensively studied. These show that soil properties such as pH, temperature and moisture, clay minerals,

and organic matter influence the availability of Zn in soils, and they are discussed below.

2.3.1.1 pH

Of the soil properties, pH has the most marked influence on plant availability of Zn. However, it should be noted that it is the pH of the rhizosphere rather than of the bulk soil, which is more important when considering the effects of pH as nutrient dynamics are influenced more by the rhizosphere pH than the bulk soil pH (Tagliavini *et al.*, 1995).

It is well known that availability of Zn decreases as soil pH increases. Marschner (1993) reported that the equilibrium concentration of Zn may decrease up to 45-fold for each unit increase in soil pH over a range 5.5-7.0. Peach (1941) found that when the pH of the soil was increased with $CaCO_3$, the amount of extractable Zn diminished, and at pH 9 virtually all the zinc was fixed. In neutral and alkaline soils where pH is above 7, Zn deficiency is more pronounced due to lower availability of Zn caused by high pH (Rashid, 1993). The lower availability of zinc in such soils is often attributed to the formation of insoluble calcium zincate or zinc salts, such as hydroxide, oxide, carbonate or phosphate.

In contrast, in acid soils, the pH factor becomes less important and thus availability depends on the amount of Zn present in the soil. Viets (1966) observed that availability of natural and supplied Zn in a sandy loam doubled when soil pH was reduced from 7 to 5 by treatment with ammonium sulfate.

As discussed above, Zn deficiency is more likely in alkaline soils due to unavailability of the zinc present in the soil to the plant. Genotypic differences in the ability of plants to withstand such conditions have been reported previously. However, the magnitude of these differences as well as mechanisms responsible for such differences are not yet fully understood.

2.3.1.2 Clays

Clays, negatively charged colloids, attract and hold cations at their surfaces. The quantity of cations held or exchanged, (the cation exchange capacity; CEC), plays a key role in nutrient availability to plants. It is well known that Zn is strongly adsorbed by soil clays (Ellis and Knezek, 1972), but adsorption varies with clay type (e.g. vermiculite, montmorillonite, and kaolinite), pH (DeMumbrum and Jackson 1956; Bingham *et al.*, 1964), concentrations of competing ions, and the nature of ligands present (Farrah and Pickering, 1977).

Elgabaly (1950) who studied Zn adsorption by clays, found that fixation varied from about 10% of the total on bentonite to nearly 50% on kaolinite, and approximately 75% on a vermiculite. In a later study, Reddy and Perkins (1974) investigated Zn adsorption by clays such as bentonite, illite and kaolinite at various pH levels with alternate wetting and drying, and with incubation at moisture saturation. The authors found that, when subjected to successive wetting and drying, bentonite and illite adsorbed significant amounts of Zn, with the total amount increasing with increases in the pH of the clay suspension. In their study, incubation resulted in less adsorption (nearly 50% of the wetting and drying treatment). Regardless of treatment, kaolinite adsorbed relatively small amounts of Zn compared to bentonite and illite. Differences in Zn adsorption by

soil clays have also been reported by other investigators. Singal and Gupta (1977) found that Zn was adsorbed more strongly than sodium on montmorillonite, while Singhal and Kumar (1977) observed a weaker adsorption on bentonite and illite as compared with magnesium.

The presence or absence of other ions may also influence the amount of Zn adsorbed by soil clays. An example of this is adsorption of Zn on montmorillonite in the presence of silicate ions (Tiller, 1967). The possible explanation for this is that in the presence of silicic acid, additional active sites are created on the clay suspensions.

The total amount of Zn specifically adsorbed by clays can also vary with the time of interaction. In some clays, equilibrium is not reached rapidly, and the form of derived adsorption isotherms is consistent with the fact that binding energy may vary with surface coverage.

From the published results, it can be assumed that soils with clays of especially high cation exchange capacity (e.g. montmorillonite) often have higher adsorption capacities, and thus a lower amount of the total Zn as plant available Zn than those with low clay. Therefore, it can be postulated that such soils may be often prone to Zn deficiency.

2.3.1.3 Temperature and moisture

In some cases, Zn deficiencies occur early in the growing season when soil temperatures are relatively low. As the soil warms up toward midseason, Zn deficiency symptoms disappear (Ferres, 1949; Millikan, 1953; Lindsay, 1972). This phenomenon has been attributed to the fact that (i) the root system may be poorly established in low soil temperatures such that the feeding zone is restricted (Lindsay, 1972) and the root system

becomes better established as the soil temperature rises (Viets and Lindsay, 1973), and (ii) low soil temperatures reduce microbial activity which may reduce the release of Zn from organic matter.

Soil water content undoubtedly has an enormous impact on the availability of nutrients but its impact differs with its content in the soil and the crop plant. For example, under submerged conditions of rice cultivation, Zn availability is decreased as a result of reaction of Zn with free sulfide (Mikkelsen and Shiou, 1977) or with sesquioxides (Sajwan and Lindsay, 1988). In tropical regions, Zn deficiency is reported to be the most widespread micronutrient deficiency affecting rice crops (Lopes, 1980). In contrast, in cereal crops grown in dry areas (e.g. wheat and barley), the soil moisture does not seem to have a major direct effect on the availability of Zn (Moraghan and Mascagni, 1991).

2.3.1.4 Organic matter

The interaction of Zn with organic matter has received a great deal of attention since organic constituents in soils are known to play a major part in the chemical solubility of Zn and its availability to plants. Zn availability is often reported to be low in soils of high organic matter contents (Lindsay, 1972) due to increased adsorption of Zn onto organic matter. In the process of Zn adsorption, components of organic matter, humic acid and fulvic acid, are of key importance. The quantitative measure of the affinity of the Zn for humic acid and fulvic acid is termed the stability constant, and its numerical value is useful to predict the solubility and movement of Zn in the soil.

The stability constants between organic constituents and Zn have been reported by numerous investigators using various methods. Randhawa and Broadbent (1965a,b) found that small amounts of Zn were adsorbed in the form of a Zn-humic acid complex,

but the amount was pH dependent. The same authors reported the stability constants of 4.42 at pH 3.5, 6.18 at pH 5.6, and 6.8 at pH 7 indicating that adsorption increases as pH increases. Similarly, Schnitzer and Skinner (1966) found stability constants of 1.73 at pH 3.5 and 2.34 at pH 5.0 for Zn and fulvic-acid. Because of the lower stability constant, the fulvic acid fraction binds Zn less strongly than does the humic acid fraction. This is supported by Himes and Barber (1957) who found the stability constant of 5.6 at pH 7 for a Zn-soil-organic matter complex, indicating that most of the Zn may have been adsorbed in the form of a Zn-humic acid complex.

The formation of Zn-organic matter complexes in soil has practical implications in that soils with high organic matter content are likely to be low in plant available Zn. In fact this has been demonstrated by Jensen and Lamm (1961) who were able to extract almost all of the extra source of Zn with dithizone after organic matter of the surface soil was destroyed. In the presence of organic matter, only 50-75% of the Zn was recovered. In agreement with the findings of Jensen and Lamm (1961), Hodgson *et al.* (1966) reported that 60% of the Zn in soil solution existed as an organic complex. It is, therefore, possible that increases in soil organic matter through improved farming practices may reduce Zn availability.

2.3.2 Transport of zinc to the root surface

Nutrients are transported toward the root surface by two main mechanisms: mass flow, driven by the uptake of water by the plant root, and diffusion, when the nutrient concentration at the plant root surface is not equal to that in rhizosphere. The relative importance of each of these two mechanisms to nutrient uptake depends on the concentration of the nutrient in the water which flows toward the plant root. When the

solution moving through the soil to the root contains a relatively large concentration of a nutrient, mass-flow becomes the dominant mechanism bringing nutrients to the root surface. This is the main mechanism for nutrients such as Ca, Mg, N and S (Barber, 1984). However, in cases where the nutrients occur in low concentration (e.g. P, K, Zn, Cu, Fe and Mn), diffusion becomes the main mechanism for the transport of Zn to the root surface because mass-flow can only carry a small fraction of the nutrients required by the plant root (Barber, 1984). As Zn is a diffusion limited nutrient, the rate of Zn diffusion as well as uptake may be influenced by root growth and surface area, which determine the distance over which Zn will have to travel. Therefore, it can be assumed that a greater root growth and surface area may increase the transport of Zn to the root surface. According to Marschner (1993), for a given amount of DTPA extractable Zn (e.g. 0.5 mg Zn/kg soil), the spatial accessibility of this fraction increases about two-fold by doubling the root surface area.

2.3.3 Uptake, translocation and distribution of zinc

Zinc is taken up predominantly as a divalent cation (Zn^{2+}) as well as monovalent cation ($ZnOH^{+}$). Halvorsen and Lindsay (1977) observed in corn plants that, Zn^{2+} was the only form absorbed and a low concentration of Zn^{2+} was adequate for plant growth. Zn may also be absorbed in several other forms such as complex ions and Zn-organic chelates (Tiffin, 1972; Loneragan, 1975; Weinberg, 1977).

In general, absorbed Zn is translocated from roots to shoots after a very short lag phase, during which time, presumably, root requirements are satisfied. The forms of Zn translocation in both xylem and phloem are still uncertain. However, evidence from examinations of sap contents (Van Goor and Wiersma, 1976; White *et al.*, 1981a,b)

suggests that zinc is translocated as a complexed form (e.g. anionic) in the xylem and phloem. In the xylem, Zn is bound to soluble low molecular weight proteins. Zinc is also found in the form of insoluble complexes such as Zn-phytate. When the chemical compositions of xylem of tomato and soybean stem were analyzed, Zn was found to bind predominantly to citric acid and malic acid (White *et al.*, 1981b). Micronutrient ionic activities and metal complexes in the phloem are different than those of xylem (Welch, 1995). The activity of micronutrient cationic metals (e.g. Fe, Mn, Zn, Cu and Ni) are low due to the high pH and phosphate level of phloem sap. Therefore, it is assumed that these micronutrients must form metal complexes in phloem sap if they are to move freely in the phloem stream (Welch, 1995). In their study with citrus trees, Taylor *et al.* (1988) identified four different anionic complexes of Zn (II) that were similar to the poly (γ-glutamyl-cysteinyl) glycine peptides (e.g. phytochelatins) in size, anionic character, pH, and temperature stability, capacity to bind other metals, and spectrophotometric properties.

Zn translocation in plants varies with plant species, genotypes within the same species, plant age and Zn supply. Cakmak *et al.* (1997a) reported that Zn-fertilized rye and triticale had a greater concentration and content of Zn in the shoot than bread wheat. The Zn concentrations in shoots of rye and triticale under Zn deficient conditions were similar to those of Zn-inefficient wheat genotypes, but this was considered to be caused by a dilution effect, which was the result of greater dry matter production of the rye and triticale.

Within wheat, differences in uptake and root to shoot transport have also been reported by Cakmak *et al.* (1996a) who found that bread wheat had a greater uptake and root to

shoot transport of Zn than durum wheat, and that root to shoot transport capacity of Zn was greater at deficient Zn supply. In another study using wheat genotypes with wide differences in their tolerances to Zn deficiency, Grewal *et al.* (1996) reported differences in accumulation of Zn in the root. They suggested that the higher root Zn content of the Zn-inefficient durum cultivar Durati, compared to Zn-efficient bread wheat cultivar Excalibur could be attributed to differences in internal Zn requirement for root functioning (higher for the cv. Durati) or impaired translocation of Zn from roots to shoots in the cv. Durati.

Peaslee *et al.* (1981) also studied absorption and translocation of ^{65}Zn applied to leaves for two corn cultivars (Pioneer and Conica) differing in Zn utilization. They reported that Pioneer had a greater translocation of ^{65}Zn to its leaves than Conica. They also noted significant differences in distribution of translocated Zn within shoots (e.g. the amount of ^{65}Zn translocated to younger leaves was greater than that to older leaves).

Recently, it has been suggested that higher rates of phytosiderophore release may be involved in increased root-to-shoot transport (Cakmak *et al.*, 1996c). Apart from their role in enhancing solubility and transport of Zn to the root surface, phytosiderophores also have the ability to enhance mobility of Zn within plants (Cakmak *et al.*, 1996b).

Zn distribution in plants can differ with plant species, plant tissues, plant age and Zn supply. In their studies with ^{65}Zn, Singh and Steenberg (1974) observed an even distribution of ^{65}Zn in the main and auxiliary roots but a relatively higher concentration at the root-stem junction. They reported that radioactive and total Zn followed different distribution patterns; ^{65}Zn was higher in nodes than internodes, and in young leaves than old leaves while total Zn was highest in the roots, followed by sheaths and blades. Zn

concentrations are usually higher in growing tissues than in mature tissue. Also an increase in external Zn concentration usually results in an increase in Zn concentration of plant tissues.

2.3.4 Interactions between zinc and other nutrients

Zinc may interact with other nutrients in ways which influence its availability in the soil, or its absoprtion, distribution or utilization in the plant (Loneragan and Webb, 1993). These authors have suggested that the most important interactions of Zn with other nutrients are those with nitrogen (N) and phosphorus (P) fertilizers in soils where both Zn and N or P are limiting the plant growth. Interactions of Zn with P and N, and those with other micronutrients will be discussed separately to a certain extent. For more details of these interactions, readers are referred to the review of Loneragan and Webb (1993).

2.3.4.1 P-Zn interactions

Among all the interactions of Zn with other nutrients, P-Zn interactions have received the greatest attention. However, the nature of interactions still remains controversial. According to Loneragan and Webb (1993), much of the confusion on P-Zn intreactions in the literature has arisen from workers who failed to identify the factor operative in an interaction or who used conditions irrelevant to Zn deficiency. The authors have suggested two categories of P-Zn interactions: P either decreases or does not decrease Zn concentrations in plant shoots (Loneragan and Webb, 1993).

The most important and widespread interaction is where P decreases Zn concentrations. When supplies of P and Zn are marginal or limiting, addition of P dilutes Zn concentrations in the plants to deficient levels by promoting growth (dilution effect)

(Boawn *et al.*, 1954; Loneragan *et al.*, 1979; Singh *et al.*, 1988). Some authors have also stressed that increasing P can induce or enhance Zn deficiency by depressing Zn concentrations beyond that explainable by the dilution effect and concluded that P-induced Zn deficiency may result from depressed Zn uptake by roots or inhibited translocation of Zn from roots to shoots (Rogers and Wu, 1948; Loneragan, 1951; Bingham *et al.*, 1958; Sharma *et al.*, 1968).

In the P-Zn interactions in which P does not decrease Zn concentrations in plant shoots, plant growth is depressed but shoot Zn concentration remains unaffected (Millikan, 1951, 1963; Boawn and Leggett, 1964; Boawn and Brown, 1968; Millikan *et al.*, 1968; Loneragan *et al.*, 1979, 1982; Christiensen and Jackson, 1981). This incident has been attributed to enhanced internal requirement of Zn as a result of increased P within the plant.

There are also reports that Zn deficiency enhances accumulation of P in plant leaves to toxic levels. Webb and Loneragan (1988) reported that Zn deficiency in wheat increased whole plant P concentration, which depressed whole plant dry matter (DM) more than P content in old leaves. This was supported by data of Christiensen and Jackson (1981) who observed that Zn deficiency reduced DM by 44 and 63% while it decreased P content by only 5 and 32% in corn grown at 1 and 3 mM Zn, respectively. Zn deficiency also enhances P transport from roots to shoots (Cakmak and Marschner, 1986). The high concentration of P in shoots is attributed to the depressed retranslocation of P from shoots to roots.

2.3.4.2 N-Zn interactions

N can affect Zn nutrition of plants by promoting plant growth, and to a lesser extent changing the pH of the rhizosphere. Heavy applications of N fertilizer can promote plant growth so much that the requirement for Zn is beyond the available supply. As a result, the concentration of Zn within the plant decreases (dilution effect) below the critical level thereby causing deficiency symptoms to appear. This suggestion is supported by Chaudhry and Loneragan (1970) who showed that nitrogen fertilizers accentuate deficiencies of both Zn and Cu by increasing plant growth to such an extent that absorbed nutrients are diluted to deficient concentrations. Ozanne (1955) suggested N-induced Zn deficiency can also be due to increased retention of absorbed Zn in the roots as protein complexes preventing its translocation from roots to shoots.

N fertilizers can ameliorate or intensify Zn deficiency by affecting adsorption of Zn as a result of acidification. Application of ammonium with $ZnSO_4$ has been reported to be effective in correcting Zn deficiency on soils where $ZnSO_4$ alone was unsatisfactory (Viets *et al.*, 1953).

2.3.4.3 Interactions of Zn with other macronutrients

In interactions of Zn with macronutrients, the composition and nature of the environment (e.g. soil or solution culture) plays an important role. Ca, Mg and K generally inhibit Zn uptake by plants from solutions. In their short term studies with wheat seedlings Chaundry and Loneragan (1972 a,b) observed Zn uptake to decrease as concentrations of $Ca(NO_3)_2$ increased from 0 to 40 mM. In contrast to solution culture, in soil it is likely that the effects of these salts on soil pH are more important than their inhibitory effect on uptake. For example, the concentration of Zn in plants increased

when soil was treated with $CaSO_4$ which decreased soil pH from 5.6 to 4.8, but decreased significantly when treated with an equivalent amount of $CaCO_3$ which increased soil pH from 5.7 to 6.6 (Wear, 1956). Similarly K and Mg have been reported to inhibit Zn absorption from solutions in which Ca was low (Chaudhry and Loneragan, 1972a,b). Their inhibitory effects on Zn uptake decreased with increasing Ca concentration to a high level (2.5-10 mM). It is suggested that K and Mg operate through the same mechanism as Ca (Chaudhry and Loneragan, 1972a,b).

2.3.4.4 Zn-micronutrient interactions

Cu-Zn interactions have been studied in various plant species such as wheat (Kausar, 1976; Chaudhry and Loneragan, 1970) and sugarcane (Bowen, 1969). Both Zn and Cu inhibit uptake of each other presumably being absorbed through the same carrier sites (Bowen, 1987). In wheat, Chaudhry and Loneragan (1970) indicated that Zn fertilizers reduced Cu concentrations in plant tops either by effects of Zn on Cu availability in soils or on the uptake process as reported earlier. Similarly, Cu depressed Zn concentrations in wheat mainly by inhibiting Zn uptake and partly by diluting Zn (Chaudhry and Loneragan, 1970). However, in short term water culture studies with excised tissues (Schmid *et al*, 1965; Hawf and Schmid, 1967; Bowen, 1969) and seedlings (Chaudhry and Loneragan, 1972c), Cu did not decrease Zn absorption while Zn depressed Cu uptake. This difference between soil and solution cultures has been attributed to complex formation of Zn and Cu in soils. In solution cultures, both Zn and Cu are present predominantly in divalent ions, Zn^{2+}, Cu^{2+}, whereas in soil, Zn and especially Cu are complexed, (Hodgson *et al.*, 1965, 1966; Geering and Hodgson, 1969). Zn, therefore, is assumed to be more competitive than Cu at absorption sites.

Zn-Fe interactions appear similar to those of Zn-Cu. Olsen (1972) reported that increasing Zn decreased Fe concentration in plants due to competition between Zn^{2+} and Fe^{2+} in uptake and translocation. In a similar way, Fe^{2+} strongly depressed Zn uptake by rice seedlings in a solution of high Fe concentration (100 μM Fe^{2+}, 0.05 $ZnCl_2$) (Chaudhry and Loneragan, 1972c) but did not affect Zn absorption by wheat seedlings when it was present at 10 μM Fe^{2+} in solutions containing 1 or 10 μM Zn and 50 μM $Ca(NO_3)_2$ (Giordano *et al.*, 1974). In addition to the antagonism between Fe and Zn, a greater accumulation of Fe has also been observed in Zn-deficient plants of sugar beet (Rosell and Ulrich, 1964), navy beans (Ambler and Brown, 1969) and corn (Jakson *et al.*, 1967).

In the case of interactions of Zn with boron (B), it has been reported that Zn deficiency results in high boron accumulation. In nutrient solutions of different B concentrations, Graham *et al.* (1987) observed a high rate of B accumulation in one-week-old seedlings of barley when no Zn was supplied. Increased B absorption by these young seedlings appears to be due to the absence of Zn in the external solution rather than the internal concentration because seed Zn reserves were adequate for plant growth of this stage. From these results, they concluded that Zn has a protective role at the external surfaces of root-cell membrane. The findings of Graham *et al.* (1987) confirm those of Welch *et al.* (1982) that despite a high internal concentration of Zn, membrane leakiness occurred when Zn was inadequate in the external solution. Similar to barley, Zn deficiency increased B concentration of wheat while depressing dry matter (Singh *et al.*, 1990).

A relatively high concentration of Cobalt (Co) also inhibits Zn absorption by plants. In wheat, a ten-fold excess of Co depressed Zn absorption by 10 % (Chaudhry and

Loneragan, 1972c). Mn did not affect Zn concentrations of roots and shoots of barley when its concentration and activity differed 10,000-fold from deficiency to toxicity in a nutrient solution containing all nutrients. In the absence of Ca^{2+}, however, a 2,000-fold excess of Mn decreased Zn uptake by 50 % (Giordano *et al.*, 1974). Na^{+} is also reported to suppress uptake of Zn by the same mechanism as, but more weakly than, K^{+} (Chaudhry and Loneragan, 1972a).

2.4 Zinc in plants

2.4.1 Zinc and plant metabolism

The severity of Zn deficiency on plant growth is related to the numerous roles of Zn in plant metabolism. Although some aspects of Zn metabolism still remains controversial, recently knowledge of the role of Zn in some areas has increased greatly. In the summary below, a brief account of areas in which involvement of Zn is well understood is given (see also Brown *et al.*, 1993).

2.4.1.1 Carbohydrate metabolism

The functions of Zn in carbohydrate metabolism are through its involvement in photosynthesis and sugar and starch transformations. In plants suffering from Zn deficiency, rate of net photosynthesis is reduced by 50-70% depending on species and degree of Zn deficiency stress (Brown *et al.*, 1993). A reduction in photosynthesis can result from a number of effects of low Zn, including a decrease in carbonic anhydrase (CA) activity (Sharma *et al.*, 1982) in particular, and other photosynthetic enzymes (Jyung *et al.*, 1972) in general, reduced chlorophyll content and abnormal structure of chloroplast (Shrotri *et al.*, 1978; Thomson and Weiser, 1962; Jyung *et al.*, 1975).

The involvement of Zn in sugar transformations can be demonstrated by its functions in the enzymes, fructose 1,6-bisphosphatase and aldolase. Under Zn deficiency, aldolase activity is drastically depressed and as a result the conversion of fructose 1-6 diphosphate to its components is impaired. Evidence that Zn is involved in sugar transformation is a decrease in the level of sucrose in Zn deficient sugarbeet (Singh and Gangwar, 1974) and maize (Shrotri *et al.*, 1980). The fact that Zn also plays a role in starch metabolism has been confirmed by Jyung *et al.* (1975). They reported that the starch content, activity of starch synthetase, and the number of starch grains were all depressed in leaves of Zn deficient beans. However, whether this effect on starch formation is a primary result of Zn deficiency remains unanswered.

2.4.1.2 Protein metabolism

The essentiality of Zn in protein synthesis is well documented for some plant species (Cakmak *et al.*, 1989; Kitagishi *et al.*, 1987; Kitagishi and Obata, 1986). Due to its role in protein metabolism, deficiency results in decrease in protein synthesis (Cakmak *et al.*, 1989; Kitagishi *et al.*, 1987; Kitagishi and Obata, 1986), which is attributed to a reduction in RNA and deformation and reduction of ribosomes. Cakmak *et al.* (1989) reported that compared to control plants, the concentration of free amino acids in Zn-deficient bean leaves was increased by a factor of 6.5, which was decreased to 5.1, 2.7 and 1.4 after Zn was resupplied to deficient plants for 24 h, 48 h and 72 h respectively.

In addition to its involvement in RNA and DNA, Zn is reported to be essential for at least two chromatic proteins, namely the chromatin TFIIIA protein which is required for transcription (Hanas *et al.*, 1983) and $g^{32}P$ protein which is necessary for replication (Giedroc *et al.*, 1986).

2.4.1.3 Membrane integrity

Zn is required to maintain the structure and integrity of biomembranes in animals (Bettger and O'Dell, 1981; Chvapil, 1973) and this function has also been investigated for plant species (Welch *et al.*, 1982; Cakmak and Marschner, 1988a). Welch *et al.* (1982), using exudation (net flux) from roots as an indicator of root plasma membrane permeability, observed greater leakage of ^{32}P from roots of Zn-deficient wheat plants than from Zn-adequate roots. This result was confirmed by Cakmak and Marschner (1988a). They reported that Zn deficiency enhanced exudation of K^+, amino acids, sugars and phenolics and resupplying Zn to deficient plants decreased this leakage. The increased leakage of organic and inorganic solutes from root cells is attributed to enhanced activity of an $O_2^{\cdot}$-generating NADPH oxidase and depressed Superoxide Dismutase (SOD) activity. The increased level of the toxic $O_2^{\cdot}$ radicals and related oxidants under Zn deficiency are major factors responsible for peroxidation of membrane lipids and an increase in membrane permeability (Cakmak and Marschner, 1988b). From these results, it is clear that Zn is required in maintaining the integrity of biomembranes in plants.

2.4.1.4 Auxin metabolism

Zn is closely involved in auxin metabolism. In Zn-deficient tomato plants, low rates of stem elongation, low auxin activities and low tryptophan contents have been reported (Skoog, 1940). Using the same species, Tsui (1948) not only confirmed the findings of Skoog but found that the decreased indole acetic acid (IAA) levels were caused by a reduced rate of tryptophan synthesis, the precursor for the biosynthesis of IAA. This function of Zn in IAA has been supported by the findings of Salami and Kenefick (1970) who demonstrated that Zn deficiency symptoms in maize could be eliminated by

additions of either Zn or tryptophan to the nutrient medium and concluded that Zn is required for the synthesis of IAA from tryptophan. More recently Cakmak *et al.* (1989) reported that low levels of IAA in Zn deficient plants might be the result of inhibited synthesis or enhanced degradation of IAA, although it should be noted that tryptophan accumulated in the Zn deficient plants.

2.4.1.5 Defence mechanism

The role of Zn in the defense mechanisms of higher plants is controversial. However, the hypothesis that Zn deficiency results in impaired defence has been supported by some experimental results. For example, in pot experiments, Thongbai *et al.* (1993) reported that Zn deficiency increased severity of Rhizoctonia root rot and disease became less severe with increasing Zn. In another investigation with wheat, Sparrow and Graham (1988) demonstrated that 0.06 mg/kg soil of applied Zn decreased the rate of progress of *Fusarium graminearum* through the stele of plants. It is suggested that depression of disease by Zn may be through its function in biomembrane integrity. Zn may prevent leakiness of cell contents such as sugars, amides and amino acids that promote development of pathogenic organisms in the external environment (Welch *et al.*, 1982; Loneregan *et al.*, 1987; Graham *et al.*, 1987). Further research is required to test this hypothesis for barley cultivars.

2.4.1.6 Reproduction

Zn is assumed to have a specific function in flowering and seed formation. It has been reported that under Zn deficiency stress, a delay in microspore development leads to pollen sterility in wheat plants (Sharma *et al.*, 1979). Anthers of Zn-deficient plants are smaller, often empty and completely devoid of pollen grains. Pollen sterility may be

attributed to the derangement in RNA metabolism induced by Zn deficiency in the anthers. Depression in flowering and seed production due to Zn deficiency has also been observed in beans, peas and other plants (Reed, 1941; Hu and Sparks, 1990).

2.4.2. Zinc deficiency in plants

2.4.2.1 Symptoms of Zn deficiency

The classic symptoms of Zn deficiency in barley are stunted growth, shortening of internodes, and chlorosis of young leaves followed by linear chlorotic areas appearing between the margin and the mid-vein (Grundon, 1987). The first symptoms develop on young leaves since Zn is partly mobile in barley. The pale, yellow linear chlorotic areas are usually followed by grey or dark brown necrosis. In some genotypes, the chlorosis advances rapidly until the whole-mid section of the leaf is affected. Consequently, the chlorotic tissue dies and turns pale brown with darker brown lesions, often leaving the base and tip of the leaves green (Grundon, 1987). However, leaf symptoms may not be useful in correcting deficiencies in broad acre crops and pastures since by the time they are apparent a reduction in plant growth by up to 60% may have occurred (Hannam *et al.*, 1994). For successful crop production, therefore, procedures are needed to predict the potential for Zn deficiency prior to the appearance of symptoms or loss in crop production (e.g. plant tissue and soil analyses).

According to Grundon (1987), tiller production in barley is not affected by Zn deficiency, but only a few tillers develop heads when the deficiency is mild. In cases of severe deficiency, the whole plant dies before any heads are produced. As for grain yield, it can be severely reduced even by mild deficiencies.

2.4.2.2 Diagnosing Zn deficiency

Plant tissue analysis

Plant tissue analysis is used as a tool for diagnosing nutrient deficiency or toxicity. The concept of 'critical nutrient concentrations' forms the basis of most methods plant tissue analysis used to assess plant nutrient status (Reuter and Robinson, 1997).

The critical deficiency concentration (CDC) is defined as the concentration where there is a 10% reduction in the yield of the plant (Ulrich and Hills, 1967), a concentration below which a growth stress is likely to occur. CDC's are derived from the growth response curves established in the field, in pots or using solution culture by supplying essential nutrients in adequate quantities and varying nutrient supply over a wide concentration range, from deficiency to toxicity (Figure 2.1). The growth response curves are commonly divided into regions such as deficient (reduced growth and visible deficiency symptoms), marginal (reduced growth and no visible symptoms), adequate (maximum growth and increase in nutrient concentration of the specified plant part), high (nutrient concentration in the specified plant part between the adequate range and toxic or excessive ranges), and excessive (severely reduced quality or excessive vigour) or toxic (reduced growth and visible toxicity symptoms) (Reuter and Robinson, 1997).

CDC values vary with the plant part and plant age (Brennan *et al.*, 1993). The plant parts considered for diagnosing the Zn status of plants are usually whole shoots, young leaves and grain. Sampling procedures and principles of plant analysis will not be discussed here in detail since they have been reviewed elsewhere (Smith, 1986; Jones, 1991).

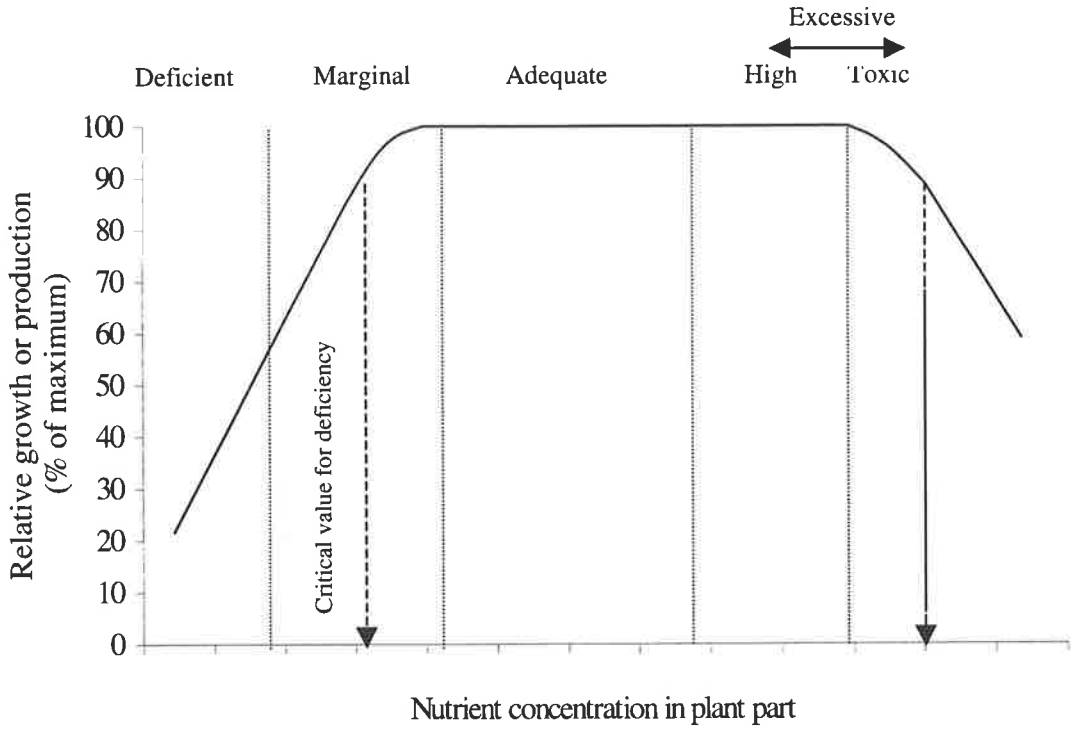


Figure 2.1. Diagrammatic representation of the nutrient status of plants (adapted from Reuter and Robinson, 1997)

In barley, Ward *et al.* (1973) have recorded the adequate range, derived from field experiment and survey data from commercial crops, as 25-100 mg Zn/kg dry wt. (awn visible stage, whole shoot). Jones (1974) has also noted a similar range (20-70 mg Zn/kg dry wt.) of adequacy (pre-heading stage, upper leaf blade). However, the reported values cover a wide range and thus the Zn requirement of barley needs to be defined more appropriately as has been done in wheat (e.g. 16 mg/kg dry wt.) (Dang *et al.*, 1993) and chickpea (e.g. 20-21 mg/kg dry wt) (Khan *et al.*, 1998b).

Soil analysis

Soil analysis is a useful analytical method of predicting Zn status of soils and its availability to the plant. Over the years a large number of methods of assessing plant available soil Zn has been developed. The most common methods are DTPA, Mehlich-

1, and 0.1 M HCI. However, the amount of Zn extracted from the soil differs with the nature of the method, the pH of the solution, kinetics of the reaction, and the mode and time of the extraction (Bansal *et al.*, 1980). The average critical concentrations, below which responses to Zn fertilization are likely to occur, for major extractants are 0.5-1.0 mg Zn/kg for DTPA, 1.1 mg Zn/kg for Mehlich-1 and 1.0-5.0 mg Zn/kg for 0.1 M HCI (Lindsay and Cox, 1985; Cox, 1987).

Recently, there has been a general trend towards using DTPA-extractable Zn and this method has been shown to be a reliable soil test for determining critical levels for various crops (Lindsay and Norvell, 1978; Bansal *et al.*, 1980; Singh and Shukla, 1985; Brennan, 1992), although the critical DTPA levels differ with soil type (Brennan, 1992). Using this method, Lindsay and Norvell (1978) were able to successfully classify a wide range of soils in Colorado (USA) according to their response to Zn application in maize with DTPA-extractable Zn of 0.8 mg/kg soil separating the responsive soils from the non-responsive soils. A recent study in Western Australia examined the relationship between DTPA-extractable Zn and yield response of wheat to Zn fertilization in 42 soils and revealed a significant correlation between the response and DTPA-extractable Zn (Brennan, 1992). In this study, the critical level of DTPA-extractable Zn ranged from 0.12 to 0.27 mg/kg depending on the soil type.

The critical value for a particular extractant can also vary with plant species (Brennan *et al.*, 1993). However, the critical value for barley has not been documented, so, it is important that correlation between a soil test (e.g. DTPA) and plant growth be established in a wide range of soils over which the extractant is useful to assist with management of Zn nutrition of barley.

2.4.3 Role of seed reserves

Low seed nutrient reserves can restrict plant growth in nutrient-deficient conditions (Asher, 1987). For example, many of the cereals produced worldwide are grown on Zn-deficient soils and often produce seed with low Zn concentrations (Graham *et al.*, 1992). When seeds of these cereals with low Zn concentrations are resown in Zn-deficient soils, plants have poor seedling vigour and ultimately low yield at harvest (Rengel and Graham, 1995a,b). Increasing the seed Zn content has been shown to increase yield in these conditions (Rengel and Graham, 1995a,b). Similar responses have been reported with Mn in barley (Longnecker *et al.*, 1991), wheat (Marcar *et al.*, 1986) and lupin (Crosbie *et al.*, 1994) and with P in lupin (Thompson *et al.*, 1992). However, there are no reports in the literature concerning the effect of seed Zn content on either growth or yield of barley.

2.5 Breeding for zinc efficiency

Successful breeding for Zn efficiency requires certain criteria be satisfied. These criteria can be listed as: (i) genotypic variation for the character (Graham, 1984), (ii) screening methodologies for assessment of genotypic variation for the character (Graham, 1984; Chaney *et al.*, 1989), (iii) sound and economic reasons for pursuing a breeding solution to the nutrient limitation (Graham, 1984) and (iv) understanding of genetic control of Zn efficiency. These criteria are discussed in relation to Zn efficiency in barley. Zn efficiency is defined as the ability of a genotype to grow and yield well in soils too deficient for a standard genotype (Graham, 1984).

2.5.1 Genotypic variation in sensitivity to Zn deficiency

Genotypic variation in sensitivity to Zn deficiency for various plant species has been recognized for many years (Clark 1978; Graham *et al.*, 1992; Hartwig *et al.*, 1991; Mahadevappa *et al.*, 1981; Rao *et al.*, 1977; Majumder *et al.*, 1990). Cereals are often reported to be less sensitive, while other crops such as potatoes, tomatoes, sugar beet and lucerne are moderately sensitive, and maize, hops, flax, and field beans are sensitive (Viets *et al.*, 1954). Despite the fact that cereals are classified as insensitive, in recent years, considerable genotypic variation within cereals has also been reported: rye is known to be the most insensitive to Zn deficiency and durum wheats the least (Graham, 1984; Cakmak *et al.*, 1997a). Graham (1984) ranked sensitivity to Zn deficiency as rye<triticale<bread wheat<durum wheat. In another investigation (Cakmak *et al.*, 1998) in which sensitivity was based on visible deficiency symptoms, rye and triticale were found to be remarkably insensitive to Zn deficiency while durum wheat and oats were found to be very susceptible. The order of sensitivity was suggested as rye<triticale< barley <bread wheat < oats < durum wheat.

There has been relatively little work on sensitivity to Zn deficiency in barley. However, in experiments conducted in Turkey, barley appears to be more tolerant to Zn deficiency than wheat (M. Kalayci, pers. comm.). In a field experiment conducted in Turkey, when Zn was applied to the soil at rates of 0.0, 5.0, 10 and 15 kg Zn/ha, wheat and barley showed markedly different responses to Zn application: wheat yields were increased from 1550 to 2200 kg/ha with an application of 15 kg Zn/ha (Zn efficiency 70%), but in barley, the yields increased from 2660 to 2940 kg/ha (Zn efficiency 90%). Efficiency rates for 5 and 10 kg Zn/ha were 87 and 86% in barley and 73 and 73% in wheat,

respectively. An application of 5 kg Zn/ha gave the highest grain yield and further Zn fertilization did not enhance grain yield of either wheat or barley.

Takkar *et al.* (1983) examined Zn responses of six barley genotypes in India and reported that all genotypes were tolerant to Zn deficiency. Of the genotypes tested, PL 56 produced the highest yield under severe Zn deficiency stress and its yield was almost the same as or appreciably higher than the yield of any of the other varieties under no Zn deficiency stress. In contrast, in their study with ten barley genotypes in India, Pathak *et al.* (1979) found that at low soil Zn, the depression in yield of barley ranged from 8.6 to 54.8%. This indicates that there is considerable variability among barley genotypes in response to Zn deficiency. In another study with ten barley cultivars tested in three locations in Turkey (Eskisehir, Konya and Comakli) yields were increased from 15% (Eskisehir) to 102% (Comakli) when Zn was applied at the rate of 23 kg Zn/ha (Yilmaz *et al.*, 1996). Of the cultivars tested, Hamidiye, Erginel 90 and Cumhuriyet were sensitive whereas Tokak 157/37, Obruk 86, Tarm 92 and Yesevi were insensitive to Zn deficiency. Overall, Hamidiye was the most sensitive while Tarm was the least sensitive cultivar (Yilmaz *et al.*, 1996). However, in their subsequent experiment in the following year, the same authors observed less response to Zn application (A.Yilmaz, pers. comm.).

Graham *et al.* (1992) also observed significant responses to Zn fertilization among barley genotypes grown in a field trial conducted at Lameroo, South Australia, in 1988. However, in the following years, they found smaller responses to Zn fertilization and difficulty in measuring differences in efficiency similar to those measured in 1988 (R. D. Graham, pers. comm.). The spatial and seasonal variation observed in Turkey and

South Australia is due mainly to environmental factors such as variability in climatic conditions and soil profile over sites and years, and it makes it difficult to assess reliably the extent of genotypic variation in Zn efficiency in the field.

2.5.2 Screening methodologies for assessment of genotypic variation in response to Zn deficiency

Genotypic variation in sensitivity to nutrient deficiencies has been mainly assessed by three methods: field testing, soil culture and solution culture. The principles of these methods are discussed in general below.

2.5.2.1 Field testing

Evaluation of genotypic variation in tolerance to a nutrient deficiency is commonly carried out in the field where the soil is deficient enough in the limiting nutrient to seriously reduce the yield of some lines (Graham, 1984). Field tests are often conducted without any nutrient treatments when there are a large number of genotypes to be tested, genotypes being located within an array of plots of a standard genotype to provide a covariate on heterogeneity of the site (Graham, 1984). This type of field test assesses genotypes in terms of absolute yields and it is performed under the assumption that differences in general adaptation and yield potential are small compared with differences in tolerance to the nutrient deficiency. If not, another treatment with an adequate amount of nutrient is introduced, which defines yield potential. In this approach, genotypes are evaluated by the ratio of yield with no applied nutrient to the yield with nutrient applied, which is termed nutrient efficiency (Graham, 1984). For example,

$$\text{Zn efficiency } (\%) = 100*(GY-/GY+),$$

where GY- is the yield with no Zn applied and GY+ is the yield with Zn applied. Graham and Rengel (1993) have reported that such normalization eliminates much of the background genetic effect but does not exclude nutrient x genotype x environment effects which can occasionally be important. This plus and minus fertilizer paired plot approach has found wide acceptance in assessing genotypic variation in tolerance to Zn deficiency in cereals (Graham *et al.*, 1992; Yilmaz *et al.*, 1996; Cakmak *et al.*, 1996a).

Graham (1991) has further suggested that a genotype can be better characterized by a yield response curve to increasing rates of fertilizer than the first two approaches reported here. This multi-level testing approach by Graham (1991) is supported by the fact that several loci may be involved in tolerance to Zn deficiency, each operating at a different level of stress, as has been shown in tolerance of wheat to boron toxicity (Paul, 1990). However, using this multi-level testing approach, only a small number of genotypes can be handled in field experiments because of the increased experimental area and cost associated with increasing number of Zn fertilizer levels.

In field tests, tolerance to Zn deficiency is often measured in terms of grain yield, which is the valued product of cereals. However, some authors have raised concerns over the use of yield as a selection index (Graham, 1984; Blum, 1988). Blum (1988) indicated that yield as a selection index is not justified, where the inheritance of tolerance is simple, where deficiency symptoms are readily recognizable or when an efficient chemical or biochemical test is available. Graham (1984) has also suggested that grain yield, as a selection index, is complex and almost everything in the genome ultimately contributes to final grain yield. Thus grain yield as a selection index should be supported

by other observations of growth and/or deficiency symptoms at seedling and mid-season stages.

The other shortcomings of field testing is the high spatial variability of the limiting nutrient in field sites, growth limiting factors other than Zn (drought, diseases, other nutrient deficiencies and/or toxicities) and seasonal variations.

2.5.2.2 Soil culture

Soil cultures are generally used to examine efficiency characteristics operating externally, at the soil-root interface, such as mobilization of unavailable nutrients from rhizosphere and root system geometry (Graham, 1984). Soil cultures are simpler, faster and less expensive than field work, and generally may overcome difficulties associated with field experimentation or may supplement field work. They can also be useful in accurate testing after populations are reduced to a manageable size or in assessing large number of segregants from crosses of nutrient efficient x nutrient inefficient parents (Graham, 1991).

Soil cultures have been used extensively by many workers in assessing efficiencies of Cu (Graham, 1978), Zn (Graham *et al.*, 1992; Graham and Rengel, 1995a; Cakmak *et al.*, 1996a; Grewal *et al.*, 1997), and Mn (Huang, 1996). Tolerance to a nutrient deficiency is measured in seedlings using parameters such as deficiency symptoms, plant growth and chemical and biochemical tests in plants (Blum, 1988). It is desirable that results of soil cultures have a high correlation with plant response in field.

2.5.2.3 Solution culture

Solution cultures are often used to study efficiency factors operating internally, from the root surface to the plant interior, such as phytosiderophore release-assisted uptake, absorption rate, translocation and efficiency of nutrient utilization. In some cases, they can be used to select efficiency factors operating at the root-soil interface. Rengel and Graham (1996) demonstrated that chelate-buffered nutrient solution technique ranked wheat genotypes in the same order as field experiments and concluded that it could be used reliably to distinguish between Zn-efficient and Zn-inefficient wheat genotypes. It remains to be seen whether it can be applied to barley.

2.5.3 Selection criteria for tolerance to Zn deficiency

Another significant aspect of screening for tolerance to Zn deficiency is that there should be sound selection criteria which would reliably rank genotypes for their tolerance to Zn deficiency. Zn deficiency has been shown to depress early vegetative growth in many crop species such as wheat (Rengel and Graham, 1995a; Cakmak *et al*, 1996a), rapeseed (Grewal *et al*, 1997) chickpea (Khan *et al.*, 1998a) and medics (Streeter, 1998). There is a possibility that depression in growth, a well known phenomenon, and in severe cases, distinct Zn deficiency symptoms (see section 2.5.1), can be used as selection criteria provided that they correlate well with the yield responses. Cakmak *et al.* (1996a) have demonstrated that the appearance and severity of Zn deficiency symptoms in shoots is a better criterion than Zn concentration or dry matter production in distinguishing wheat genotypes in their tolerance to Zn deficiency. From the evidence elsewhere, it is likely that symptom expression, a single and simple selection criterion, can also be a useful screening tool if it is correlated with yield responses in barley. However, this relationship in barley is yet to be shown.

In summary, to date screening for Zn efficiency in barley has been mainly based on field testing (Graham *et al*, 1992; Yilmaz *et al*, 1996). However, field experiments are rather expensive and do not always produce consistent results for many years across many sites (R.D.Graham, A.Yilmaz, pers. comm.). Screening in soil culture under controlled conditions can be an alternative to field testing but its potential has not been evaluated yet and still awaits to be explored, as has been done in crops such as wheat (Graham et al., 1992), rapeseed (Grewal et al., 1997), and chickpea (Khan *et al.*, 1998a). Webb *et al.* (1993) have pointed out that the chelate-buffer nutrient solution system has great potential for studies of micronutrient nutrition of plants but its potential for screening for Zn efficiency in barley is yet to be realized.

The lack of rapid and reliable screening procedure which is capable of screening a large number of genotypes in a short time still continues to be an obstacle to breeding for Zn efficiency in barley. At the present moment, development of this type of procedure still remains an urgent need.

2.5.4 Sound and economic reasons for pursuing a breeding solution to Zn deficiency

Despite the fact that Zn deficiency can be corrected through use of Zn fertilizers, there are certain situations such as subclinical Zn deficiency in which Zn fertilization can not cost-effectively overcome Zn deficiency (Graham and Rengel, 1993). In these circumstances, there is a need to breed for Zn efficiency in crop plants. There are several other benefits of breeding genotypes with higher Zn efficiency such as improvement in the nutritional value of grain (e.g. higher Zn content in the grain), less soil degradation due to reduction in use of fertilizers and greater resistance to diseases.

2.5.5 Understanding of genetic control of tolerance to Zn deficiency

The numbers of genes involved in tolerance to Zn deficiency and their heritability influence the success and speed of incorporation of tolerance into desired lines. Our knowledge of the genetic control of Zn tolerance is poor as compared to other nutrients. For example, studies of tolerance to Fe deficiency in soybeans (Weiss, 1943), Mg and B deficiency in celery (Pope and Munger, 1953a,b), B deficiency in tomato (Wall and Andrus, 1962), Cu and Mn deficiency in rye (Graham, 1984) and Mn deficiency in barley (McCarthy *et al.*, 1988) have suggested that single major genes with minor genes are involved in tolerance to deficiency of these micronutrients.

In contrast, genetic studies in rice (Mahadevappa *et al.*, 1981), *Agrostis tenuis* (Gartside and McNeily, 1974) and soybean (Hartwig *et al.*, 1991) suggested the presence of a multigenic control of tolerance to Zn deficiency, showing continuous variation and transgressive segregation in F_2 generation. A recent report indicated that tolerance to Zn deficiency in rice is mostly additive, and to a lesser extent dominant (Majumder, 1990). Likewise, from the study of addition lines of rye, it is likely that several loci are involved in tolerance to Zn deficiency (Graham, 1984). More recently, Cakmak *et al.* (1997c) have confirmed the conclusion of Graham (1984) by demonstrating that rye chromosomes 1R and 7R are largely responsible for the higher tolerance to Zn deficiency of rye. A possible multigenic control of tolerance to Zn deficiency has been further supported by the findings of Schlegel *et al.* (1998) that the chromosomes L1, L2 and L3 of *Agropyron intermedium* and chromosomes V2 and V7 of *Haynaldia villosa* may carry genes conferring tolerance to Zn deficiency in these species. Although there is some evidence that tolerance to Zn deficiency in other cereals is likely to be under polygenic control, the nature of the genetic control in barley is not known.

2.6 Mechanisms of tolerance to Zn deficiency

Our knowledge of the mechanisms of tolerance to Zn deficiency is currently incomplete. However, recent studies have suggested that there may a number of possible mechanisms operating at various levels of plant organization (molecular, physiological, structural and developmental) in different species (Rengel and Graham, 1993). Some mechanisms may operate in soil environments (differences in root geometry as well as in capacity to sustain mycorrhizal infection), while others (differential uptake and shoot to root translocation, better utilization and differences in ability to produce phytosiderophores) may be evident in both soil and nutrient solution environments. Recently, work has increased to understand the mechanisms of tolerance with a greater emphasis on the role of phytosiderophore release, root morphology and microbial activity, and their involvement in tolerance mechanism(s) has been discussed below.

2.6.1 Release of phytosiderophores

Phytosiderophores (PS), non-proteinogenic amino acids, are released from roots of graminaceous specious under both Fe and Zn deficiencies (Cakmak *et al.*, 1994; Zhang *et al.*, 1989). PS can mobilize not only Fe but also Zn from sparingly soluble pools and adsorption sites, both in the rhizosphere and in plants (Treeby *et al.*, 1989; Zhang *et al.*, 1991; Cakmak *et al.*, 1996a). There is evidence that enhanced synthesis and release of PS may be involved in Zn efficiency of crop species. Cakmak *et al.* (1996c) demonstrated that the Zn-efficient bread wheat cultivars, Gerek and Kirac, released markedly higher amounts of PS than the Zn-inefficient durum wheat cultivars, Kiziltan and Kunduru. This result has confirmed the earlier findings of Cakmak *et al.* (1994) that greater amounts of PS were released from the roots of Aroona, a Zn-efficient bread wheat, than from the roots of Durati, a Zn-inefficient durum wheat under Zn deficiency.

They further reported that the PSs released under Zn stress were the same as those released under Fe stress (3-hydroximugineic (HMA) and 2'-deoxymugineic acid (DMA)). Cakmak *et al.* (1996d) also reported that enhanced release of PS occurred in wild grasses grown under Zn deficiency and the dominating PS in Zn-deficient Agropyron and Hordeum was 3-epi-hydroxymugineic acid (epi-HMA) and was 3-hydroxymugineic acid (HMA) in Secale.

In contrast to the earlier results by Cakmak *et al.* (1994, 1996c) in which higher sensitivity of durum wheats to Zn deficiency, compared with bread wheats, was attributed to their low capacity to synthesize and release of PS, it has been reported that the most Zn-efficient and Zn-inefficient bread wheat genotypes did not differ in their capacity to release phytosiderophores from roots (Erenoglu *et al.*, 1996): under Zn deficiency, Zn-inefficient genotype SBVD-22, had the highest rate of PS and also Zn-inefficient genotypes, BDME-10 and SBVD1-21 released as high as the most Zn-efficient genotype Dagdas-94, indicating that PS release does not always relate well with differences in tolerance to Zn deficiency (Erenoglu *et al.*, 1996). Moreover, PS release under Zn deficiency reported by Zhang *et al.* (1989, 1991) were lower than those observed under Fe deficiency.

In barley, PS release has been shown to be a specific response to Fe deficiency and not to be significantly induced by deficiencies of other nutrients such as Cu and Mn (Gries *et al.*, 1995). Enhanced release of PS under Zn deficiency has been observed only in severely deficient plants and even then the rates are very low and not comparable with those under Fe stress (Gries *et al.*, 1995). However, these results are based on one

genotype and require confirmation on a broader scale of genotypes before the role of PS release in tolerance of barley genotypes can be speculated.

From the results reported in the literature, it appears that the role of PS release in tolerance is not clear-cut. Therefore, further studies are needed for a better clarification of the role of PS release in tolerance to Zn deficiency of crop species.

2.6.2 Root morphology

The root is the main nutrient uptake organ and hence its growth is likely to affect uptake and transport of nutrients in plants, especially when grown in soil. Greater absorbing surface area as a result of enhanced root growth reduces the distance that diffusion limited ions, such as Zn and Cu, are required to travel in soil solution to the root absorption sites (Marschner, 1993). For example, in a Zn-deficient soil, Dong *et al.* (1995) studied root morphology of wheat genotypes differing in tolerance to Zn efficiency and reported that the Zn-efficient genotype Excalibur, has the ability to develop longer and thinner roots than the less Zn-efficient genotype Gatcher, and Zn-inefficient genotype Durati. This character would enable plants to extract more of the slowly-diffusible Zn ions from a given soil volume. Graham (1991) has also suggested breeding cereal genotypes with root systems capable of greater mobilization of Zn from soils of low Zn availability as an environmentally-friendly approach which can decrease fertilizer requirements.

Root morphology (length and diameter) varies among plant species (Hackett, 1968; Itoh and Barber, 1983; Fitter, 1991; Schwarz *et al.*, 1991) and different patterns of root development may not only affect nutrient uptake but may also play an important role in differential sensitivity to nutrient deficiency (e.g. Zn) (Dong *et al.*, 1995). Longer and

thinner roots may be one of the contributing factors associated with Zn-efficient genotypes but not a specific mechanism of tolerance to Zn since the longer and thinner roots will also be of benefit to uptake of other nutrients such as Cu, Mn and Fe and tolerances of these nutrients are likely to be controlled by different genes independent of each other (Graham and Rengel, 1993). In addition, more than one mechanism is often responsible for tolerance to Zn deficiency in one genotype (Graham and Rengel, 1993). In contrast to wheat, no reports on the relationship between root morphology and tolerance to Zn deficiency have been found for barley. Therefore, research to relate root morphology to tolerance to Zn deficiency for this crop is needed.

2.6.3 Microbial activity

Soil biological activity also has an effect on Zn availability and uptake through the composition of organic Zn compounds or the formation of Zn chelates. Of all the soil microorganisms, vesicular-arbiscular mycorrhizal (VAM) colonization has been reported to increase Zn uptake and shoot contents in dry matter in maize and wheat grown on soils with low Zn content despite an increase in shoot biomass (Swamvinathan and Verma, 1979; Faber *et al.*, 1990). The association of VAM with plant roots not only increases the uptake of P but also enhances uptake rates of Zn and Cu. The fungal roots extending from the plant root into the soil penetrate greater distances than do root hairs. This enables plants with infected roots to better explore the soil and consequently increase the uptake of nutrients such as P and Zn. It has been reported that mycorrhizal plants of green gram (*Vigna radiata*) (Sharma and Srivastava, 1991), maize (Faber *et al.*, 1990; Kothari *et al.*, 1990; Sharma *et al.*, 1992), and pigeon pea (*Cajanus cajan*) (Wellings *et al.*, 1991) have higher tissue Zn concentrations than non-mycorrhizal plants. It remains to be seen whether this relationship exists in barley.

2.7 Conclusion

Zn deficiency is a widespread nutritional problem affecting many millions of hectares of the world's cereal growing areas. Improving the tolerance of cereals to Zn deficiency has the potential to raise the productivity in these areas. However, progress toward improving Zn efficiency of barley has been hampered by the lack of a rapid and reliable screening procedure and understanding of genetic control of tolerance to Zn deficiency.

So far, field screening has been the main method of assessment of genotypic variation in tolerance to Zn deficiency. However, field-based methods have a number of inherent problems such as presence of growth limiting factors other than Zn (e.g. drought, diseases and other nutrient deficiencies and toxicities), site and seasonal variation. Moreover, the number of genotypes tested in field trials is often small due to considerable time and cost associated with field screening. In addition, plant breeding needs a rapid and reliable screening method that is able to screen a large number of genotypes. Therefore, the present study was carried out to develop a soil-based pot screening method for assessing barley genotypes in tolerance to Zn deficiency. A range of experiments was performed with the following objectives:

a) to develop a screening method which overcomes shortcomings of the field screening and is able to screen large number of genotypes in tolerance to Zn deficiency at the early growth stage,
b) to identify selection criteria for tolerance to Zn deficiency and to determine the critical Zn concentration for diagnostic purposes
c) to quantify the effect of seed Zn content on the early growth of barley and its relation to the current screening method which is based on seedlings,

d) to determine the extent of genotypic variation in tolerance to Zn deficiency in germplasm available, and to establish the relationship between vegetative and grain measures of tolerance to Zn deficiency, and

e) to gain insight into genetic control of tolerance to Zn deficiency.

CHAPTER 3

Effect of seed zinc content on early growth of barley under low and adequate soil zinc supply

3.1 Introduction

Seed nutrient reserves play an important role during early growth, especially when seeds are sown in soils deficient in a given nutrient. Worldwide, millions of hectares of soils on which cereals are grown are zinc (Zn) deficient (Cakmak *et al.*, 1996a; Cakmak *et al.*, 1997a; Graham *et al.*, 1992; Takkar and Walker, 1993). Cereals grown under deficient conditions generally produce seed with low Zn concentration and content (Graham *et al.*, 1992) and when these are resown in a Zn-deficient soil, plants have poor seedling vigour and ultimately low yield at harvest (Rengel and Graham, 1995a,b). Increasing the seed Zn content has been effective in increasing yield in these conditions (Rengel and Graham, 1995a,b). Similar responses have been reported with Mn in barley (Longnecker *et al.*, 1991), wheat (Marcar *et al.*, 1986) and lupin (Crosbie *et al.*, 1994) and with P in lupin (Thomson *et al.*, 1992).

Considerable effort is going into identifying Zn-efficient genotypes to improve productivity on Zn-deficient soils. One useful index of nutrient efficiency is the ratio of the growth of plants under deficient and adequate nutrient supply and is an indicator of the tolerance of the genotype to low supplies of a nutrient. The beneficial effects of seed Zn on the growth and yield of wheat (Rengel and Graham 1995a,b) suggest that the value of Zn efficiency may be sensitive to seed Zn content, and therefore using seed of different Zn contents may affect the interpretation of screening studies. Based on their work with manganese, Marcar *et al.* (1987) argued that seeds with similar nutrient contents are

required to make sound comparisons between genotypes in routine screening, but there has been little critical appraisal of the effect of seed nutrient content on the ability to distinguish between efficient and inefficient genotypes. In particular, it is not known to what extent seed Zn content contributes to the overall growth of barley, especially in Zn-deficient soils. To establish reliable guidelines for screening for Zn efficiency, the effect of seed Zn reserves on the response to applied Zn needs to be evaluated. In the present study, the effect of seed Zn content on the early growth of barley and its relation to Zn efficiency were investigated.

3.2 Materials and methods

Two experiments were conducted to examine the effect of seed Zn content on early growth of two barley genotypes (*Hordeum vulgare* cvv. Amagi Nijo and Tantangara: NSW WB 198) with putative differences in Zn efficiency. Amagi Nijo was considered to be less Zn-efficient than Tantangara, based on the results of a preliminary screening study. In both experiments, a Zn-deficient siliceous sandy soil (DTPA-extractable Zn=0.06 mg/kg) was used (Rengel and Graham, 1995a,b). Following the addition of calcium carbonate (0.5% w/w) and basal nutrients (Rengel and Graham, 1995a), an equivalent of 1 kg of air-dry soil was packed into a 600 ml cardboard carton (7x7x17.5 cm) lined with a plastic bag.

In Experiment 1, seeds of Amagi Nijo and Tantangara with very low and low Zn contents (Table 3.1) were grown at five soil Zn levels: 0, 0.4, 0.2, 0.8 and 3.2 mg Zn/kg soil (designated as Zn_0, $Zn_{0.04}$, $Zn_{0.2}$, $Zn_{0.8}$ and $Zn_{3.2}$). The seed of both genotypes was obtained from a field experiment in which plants were grown with or without applied Zn. The seed used was hand sorted to a uniform size (43 ± 2 mg) for each genotype.

In Experiment 2, the same genotypes were used, but seed with a wider range of Zn contents (Table 3.1) was grown. There were three levels of soil applied Zn (0, 0.04, 0.8 mg/kg Zn soil, designated as Zn_0, $Zn_{0.04}$ and $Zn_{0.8}$). Seeds for this experiment were obtained from a glasshouse experiment in which both genotypes were grown in Laffer sand at four different Zn fertilization rates (0.2, 0.8,3.2 and 12.8 mg/kg soil). Seed of the two genotypes was graded to uniform size (50 ± 5 mg).

Table 3.1. Concentration (mg/kg D.W.) and content (µg/seed) of Zn in seeds of barley genotypes used in Experiments 1 and 2. Standard errors are based on three replicates.

Seed Zn treatment	Zn concentration		Zn content	
	Tantangara	Amagi Nijo	Tantangara	Amagi Nijo
		*Experiment 1**		
Very low	10.5	10.5	0.4	0.4
Low	19.5	18.1	0.7	0.7
		Experiment 2		
Low	14.2 ± 0.5	13.3 ± 0.1	0.7 ± 0	0.7 ± 0
Medium	27.1 ± 0.6	27.6 ± 0.2	1.3 ± 0	1.4 ± 0
High	54.2 ± 0.6	51.4 ± 2.8	3.0 ± 0	2.7 ± 0.2
Very high	96.5 ± 4.2	107.0 ± 7.9	4.3 ± 0.2	5.0 ± 0.4

* Due to limited seed, only one replicate containing 10 seeds was analyzed for elemental composition.

In both experiments, plants were grown in a growth chamber at 20°/15 °C day/night temperature, 14 h photoperiod and 300 µmol/m^2/s light intensity at plant height. The treatments were factorially combined in a completely randomized block design with three replicates. Four pre-germinated seeds of each genotype were sown into each pot and thinned to two plants after emergence. Pots were watered daily to weight with double deionized (DD) water (18 Mohms/cm resistivity) to maintain soil water content at 12% (w/w), which corresponds to the field capacity moisture content.

Plants were harvested at tillering (Feekes scale (FS) 3.0, Large, 1954), when severe Zn deficiency symptoms became apparent in plants grown with no applied Zn. This occurred 14 days after sowing in Experiment 1 and 26 days after sowing in Experiment 2. At harvest, soil was washed from the roots under tap water. Shoots and roots were dipped into deionized water followed by a quick rinse in double deionized water (DD). Shoots, youngest expanded leaf blades (YEBs) and roots were then separated, dried at 65 °C for 48 h, and weighed to estimate dry matter production. The dried plant material was digested in 70% (v/v) HNO_3 and nutrient concentrations measured by an Inductively Coupled Plasma (ICP) Emission Spectrometer (Zarcinas *et al.*, 1987). Shoot and root Zn contents were calculated by multiplying shoot and root dry weights with their corresponding Zn concentrations.

Genotypic responses to Zn are generally assessed in terms of Zn efficiency. To examine how the value of Zn efficiency may be influenced by seed Zn content, Zn efficiency was calculated as the ratio of shoot dry matter of plants grown at low Zn supply (Zn_0 or $Zn_{0.04}$) to that at adequate Zn supply ($Zn_{3.2}$ (Experiment 1) or $Zn_{0.8}$ (Experiment 2)) for each seed Zn content separately.

Results were analyzed by the GENSTAT 5 statistical package (GENSTAT, 1988), and pairwise comparisons of means were made using Tukey's Honestly Significant Difference (HSD) at α=0.05, (Steel and Torrie, 1960). To overcome the problem of non-homogeneity of variances, the data for tissue Zn concentration and Zn content were transformed to logarithms prior to analysis of variance.

3.3 Results

3.3.1 Experiment 1

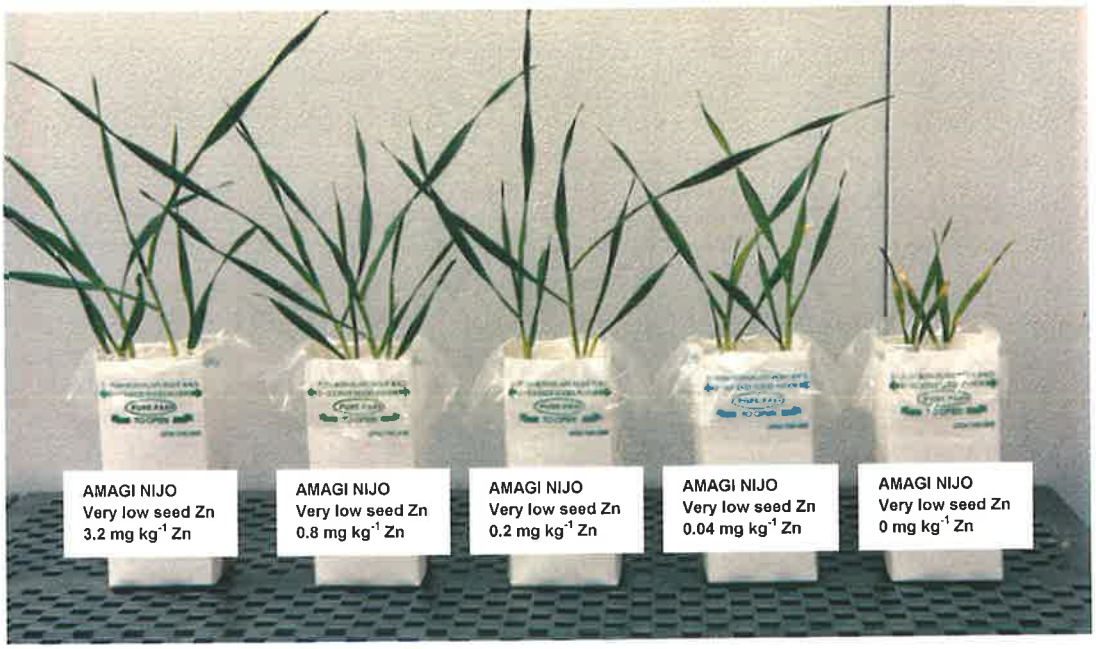
3.3.1.1 Visual symptoms

Typical Zn deficiency symptoms were reduced shoot growth and pale yellow, linear chlorotic areas followed by a grey and dark brown necrosis. Symptoms appeared on the old and middle leaves of seedlings of Amagi Nijo grown from seed with very low-Zn at the two-leaf stage (one week old). By the end of the experiment, deficiency symptoms at Zn_0 were more pronounced in plants of Amagi Nijo grown from very low-Zn seed than those grown from low-Zn seed (Plates 3.1 and 3.2). The severity of deficiency symptoms decreased with increasing seed Zn content and Zn fertilization. At this stage, Tantangara exhibited only reduced shoot growth, when soil Zn addition was $\leq$ 0.8 mg/kg. This reduction in shoot growth was more evident in plants grown from very low-Zn seed than those grown from low-Zn seed.

3.3.1.2 Shoot and root dry matter

Shoot and root dry matter were increased significantly by Zn fertilization but the response differed with genotype. Amagi Nijo had higher shoot and root yields than Tantangara when Zn was added to the soil (Figure 3.1). Higher seed Zn content tended to increase dry matter yield of shoots and roots, especially at low soil-applied Zn, but differences were not significant at any Zn levels. There was no significant interaction between genotype and seed Zn for either shoot or root dry matter yield. Higher seed Zn content also increased Zn efficiency of both genotypes, but perhaps more importantly, the difference between genotypes narrowed as seed Zn content increased (Table 3.2).

a)



b)

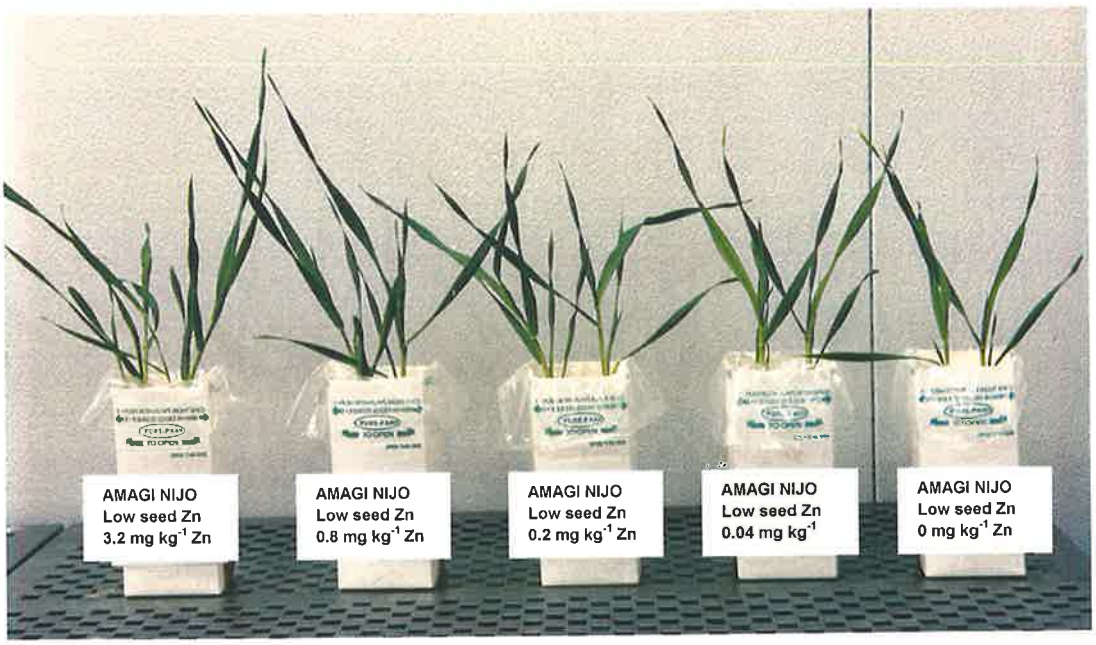


Plate 3.1. The effects of seed Zn content (a, very low seed Zn; b, low seed Zn) and Zn fertilization on expression of Zn deficiency symptoms in barley genotype Amagi Nijo at 14 DAS in Experiment 1.

a)

b)

Plate 3.2. The effects of seed Zn content (a, very low seed Zn; b, low seed Zn) and Zn fertilization on expression of Zn deficiency symptoms in barley genotype Tantangara (NSW WB 198) at 14 DAS in Experiment 1.

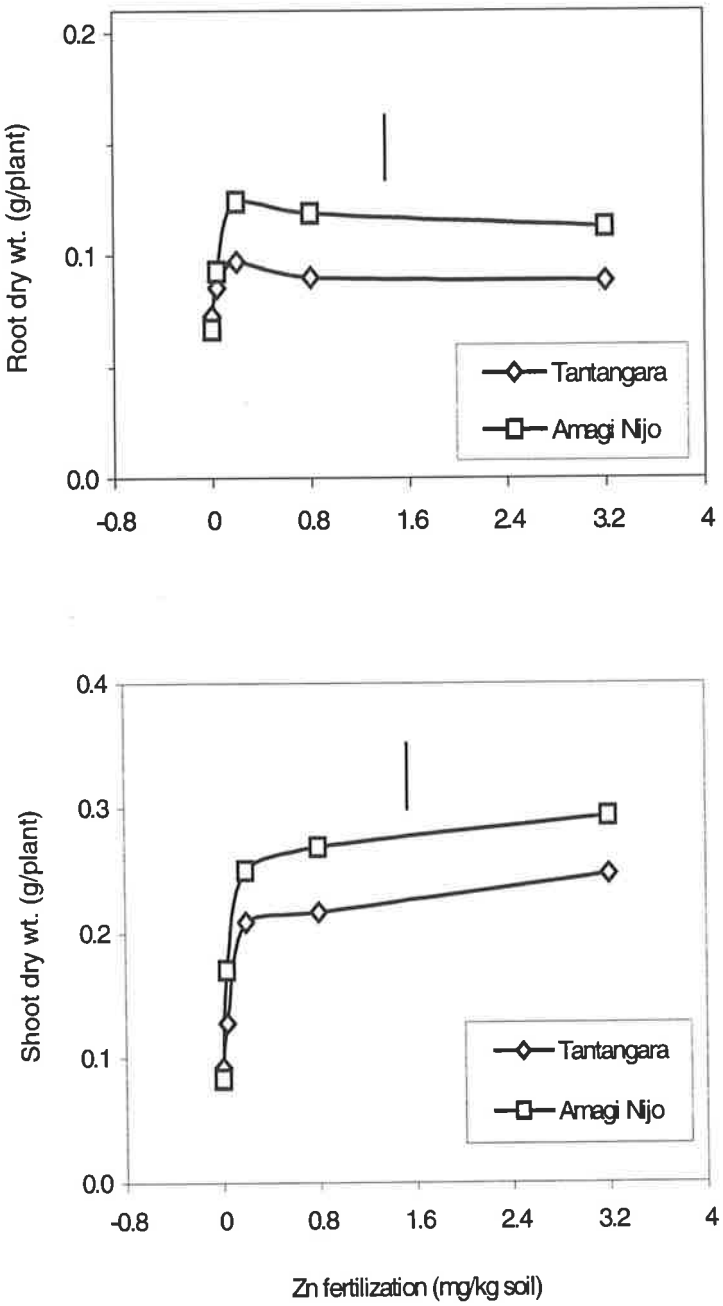


Figure 3.1. Effect of Zn fertilization on shoot and root dry matter of barley genotypes in Experiment 1. The vertical bars represent Tukey's $HSD_{0.05}$ values for Genotype x Zn fertilization interaction. Data were averaged over two seed Zn contents because no interaction involving seed Zn effect was significant.

Table 3.2. Effects of seed Zn content and Zn fertilization (mg/kg soil) on Zn efficiency * of barley genotypes at tillering (14 days after sowing) in Experiment 1.

	Seed Zn content			
	Very low		Low	
Zn fertilization	Tantangara	Amagi Nijo	Tantangara	Amagi Nijo
0	35	22	39	36
0.04	50	61	53	58
0.2	85	87	84	88
0.8	86	89	89	99
3.2	100	100	100	100

* Zn efficiency was calculated as the ratio of shoot dry matter yield at each Zn level to that at $Zn_{3.2}$ where maximum yields were achieved, expressed as percentage.

3.3.1.3 Zn concentration and content of shoots and roots

There was a continuous increase in Zn concentration of shoots of both genotypes with increasing Zn fertilization (Table 3.3). Irrespective of seed Zn content, Tantangara generally had a higher shoot Zn concentration than Amagi Nijo except when grown at Zn_0 where the two genotypes had similar Zn concentrations (Table 3.3). At each level of Zn fertilization, however, there was no significant difference in tissue Zn concentration between plants grown from very low and low-Zn seed contents. Shoot Zn content was also increased significantly by Zn fertilization. In contrast to Zn concentration in shoots, genotypes did not differ significantly in shoot Zn content (Appendix 3.1). Overall, plants grown from seed of high Zn contents had a higher shoot Zn content than those grown from seed with very low Zn content.

Zn concentration in roots was also increased by Zn fertilization and significant genotypic differences occurred at the highest Zn level, $Zn_{3.2}$: Tantangara had greater Zn concentrations than Amagi Nijo (Table 3.3). There was no significant effect of seed Zn or

a significant interaction of Zn fertilization x Seed Zn for either genotype. Zn content in roots displayed a similar trend to Zn concentration in roots (Appendix 3.2).

Table 3.3. Effect of Zn fertilization (mg/kg soil) on Zn concentrations (mg/kg D.W.) in shoots and roots of barley genotypes at tillering (14 days after sowing) in Experiment 1.

	Zn concentration in shoots		Zn concentration in roots	
Zn fertilization	Tantangara	Amagi Nijo	Tantangara	Amagi Nijo
0	6.0 (1.78)[a]	6.0 (1.78)	18.4 (2.90)	15.9 (2.76)
0.04	10.7 (2.37)	8.4 (2.13)	15.8 (2.76)	14.8 (2.69)
0.2	24.6 (3.20)	22.9 (3.09)	26.6 (3.28)	24.2 (3.17)
0.8	60.0 (4.09)	53.0 (3.97)	97.2 (4.57)	85.1 (4.43)
3.2	153.8 (5.04)	125.7 (4.83)	625.5 (6.44)	386.9 (5.95)
Tukey's $HSD_{0.05}$ [b]				
Genotype x Zn fertilization	(0.12)		(0.27)	

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

[b] The $HSD_{0.05}$ values are applicable to log-transformed data.

3.3.1.4 Zn concentration in youngest expanded blades (YEBs)

Zn concentration in YEBs also increased progressively as a result of Zn fertilization but the increase varied with genotype. The trend was similar to that observed for Zn concentration in shoots; Tantangara had higher concentrations than Amagi Nijo in the presence of soil-applied Zn at levels $\geq Zn_{0.04}$, (Table 3.4). At each level of Zn fertilization, however, there was no significant difference in Zn concentration of YEBs between plants grown from low- and very low-Zn seed for either genotype.

Table 3.4. Effects of Zn fertilization (mg/kg soil) on Zn concentration (mg/kg D.W.) in YEBs of barley genotypes at tillering (14 days after transplanting) in Experiment 1.

	Zn concentration in YEBs	
Zn fertilization	Tantangara	Amagi Nijo
0	5.6 (1.71)[a]	5.0 (1.61)
0.04	9.6 (2.26)	6.3 (1.84)
0.2	24.3 (3.19)	20.4 (3.02)
0.8	59.8 (4.09)	49.6 (3.90)
3.2	149.5 (5.00)	121.5 (4.80)
Tukey's $HSD_{0.05}$[b]		
Genotype x Zn fertilization	(0.14)	

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.
[b] The $HSD_{0.05}$ value is applicable to log-transformed data.

3.3.2 Experiment 2

3.3.2.1 Visual symptoms

The response to low Zn supply was similar to that observed in Experiment 1, but the first symptoms of Zn deficiency occurred about one week later. Deficiency symptoms appeared after two weeks on the old and middle leaves of Amagi Nijo plants grown from low-Zn seed at nil Zn fertilization. At this time, Tantangara exhibited reduced shoot growth rather than the chlorotic lesions observed in Amagi Nijo, especially when grown from low-Zn seed at Zn_0.

By harvest, symptoms of severe Zn deficiency had developed in Amagi Nijo grown from low-Zn seed at Zn_0 (Plates 3.3 and 3.4). Severity of the deficiency symptoms decreased with increasing seed Zn content and Zn fertilization, and symptoms were not visible in plants grown from seeds with very high seed Zn at Zn_0 and $Zn_{0.04}$ and all seed Zn levels at $Zn_{0.8}$. In contrast to Amagi Nijo, Tantangara exhibited only stunted growth when grown from low-Zn seed at Zn_0.

a)

b)

c)

Plate 3.3. The effects of seed Zn content (low, medium, high and very high) and Zn fertilization (a, b, c; 0, 0.04, 0.8 mg/kg, respectively) on expression of Zn deficiency symptoms in barley genotype Amagi Nijo at 26 DAS in Experiment 2.

a)

b)

c)

Plate 3.4. The effects of seed Zn content (low, medium, high and very high) and Zn fertilization (a, b, c; 0, 0.04, 0.8 mg/kg, respectively) on expression of Zn deficiency symptoms in barley genotype Tantangara (NSW WB 198) at 26 DAS in Experiment 2.

3.3.2.2 Shoot and root dry matter

Both shoot and root dry matter increased significantly with increasing seed Zn content but the response diminished as the level of soil-applied Zn increased (Figure 3.2). This was most apparent with root dry matter. Even with the Zn applied to the soil, shoot growth of seedlings from low-Zn seed was still less than growth of seedlings derived from high-Zn seed. Amagi Nijo produced more shoot and root dry matter than Tantangara when soil-applied Zn was greater than $Zn_{0.04}$ (Appendix 3.3). Amagi Nijo also had greater shoot and root dry matter than Tantangara when grown from high-, and very high-Zn seed (Appendix 3.4).

Seed Zn content significantly influenced Zn efficiency of the two genotypes but the effect differed depending on soil Zn supply. Based on the ratio of shoot dry matter at Zn_0 to that at $Zn_{0.8}$, Zn efficiency ranged from 42% to 71% for Tantangara and from 33% to 63 % for Amagi Nijo depending on the seed Zn content (Table 3.5). Zn efficiency values for the two genotypes tended to increase when Zn was applied at the rate of 0.04 mg/kg soil (e.g. $Zn_{0.04}/Zn_{0.8}$), and the new range in efficiency was recorded as 60-83% for Tantangara and 61-75% for Amagi Nijo, respectively.

At comparable seed Zn contents, Tantangara had higher Zn efficiency than Amagi Nijo and the largest genetic difference in Zn efficiency occurred when plants were grown from low-, and medium-Zn seed with no soil-applied Zn (Table 3.5). The difference in Zn efficiency between the two genotypes tended to decrease with increasing seed Zn content and Zn fertilization. Moreover, when genotypes were not compared at similar seed Zn treatments, the relative efficiencies of the two genotypes could change.

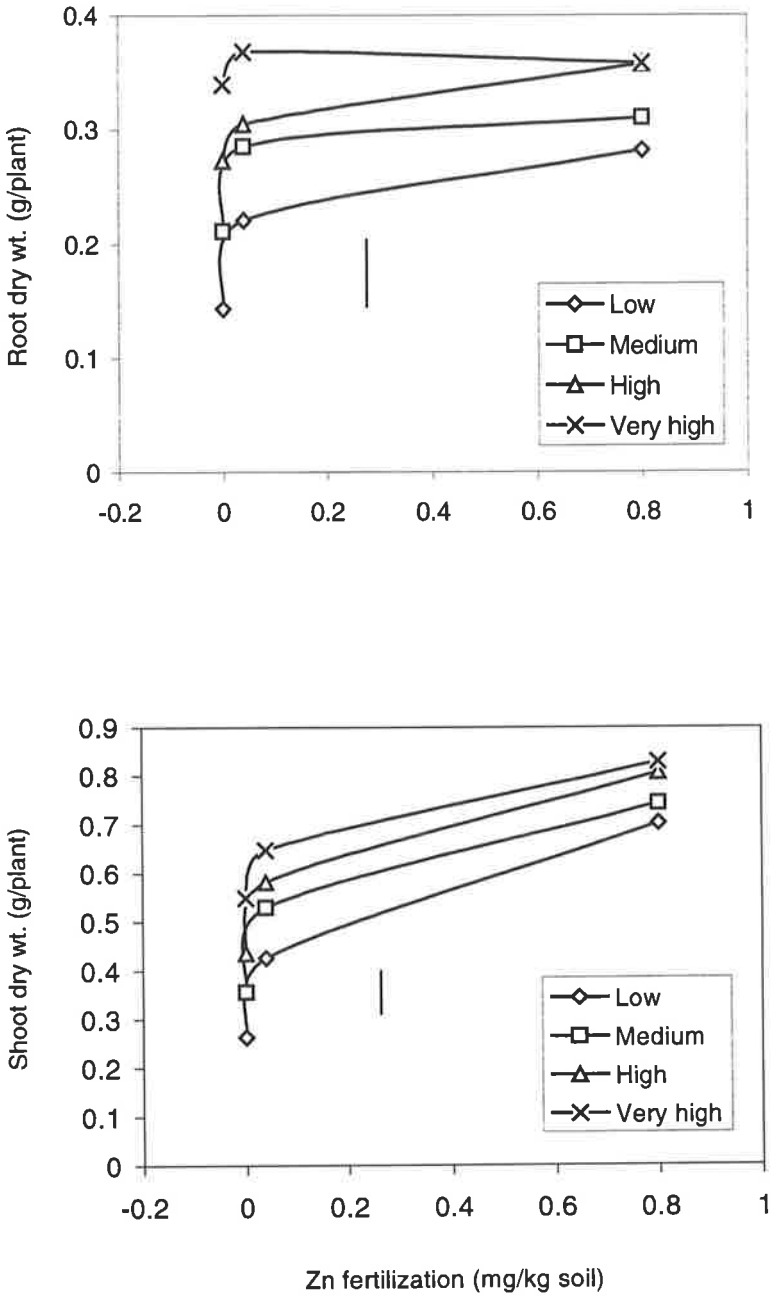


Figure 3.2. Effects of seed Zn content and Zn fertilization on shoot and root growth of barley in Experiment 2. The data were averaged over the two genotypes because the interaction Genotype x Seed Zn x Zn fertilization was not significant. Vertical bars represent Tukey's $HSD_{0.05}$ values for the interaction Seed Zn x Zn fertilization.

Table 3.5. Effects of seed Zn content and Zn fertilization (mg/kg soil) on Zn efficiency * of barley genotypes at late tillering, 26 days after sowing, in Experiment 2.

Genotype	Zn fertilization	Seed Zn content Low	Medium	High	Very high
Tantangara	0	42	54	58	71
	0.04	60	74	80	83
	0.8	100	100	100	100
Amagi Nijo	0	33	43	51	63
	0.04	61	69	67	75
	0.8	100	100	100	100

* Zn efficiency is calculated as the ratio of shoot dry matter yield at each Zn level to maximum dry matter yield at highest Zn level ($Zn_{0.8}$), expressed as percentage.

3.3.2.3 Zn concentration and content of shoots and roots

Higher seed Zn content increased Zn concentration of shoots, but the response differed with genotype: Tantangara had a greater Zn concentration than Amagi Nijo when grown from seed with medium-, high-, and very high-Zn content (Table 3.6a). The effect of seed Zn content on Zn concentration in the shoots was most evident under Zn deficiency, $\leq Zn_{0.04}$; however, only plants grown from seed with very high Zn content had a greater Zn concentration in the shoots than plants grown from seed with low-, medium-, and high-Zn content (Table 3.6b). Soil Zn application also increased Zn concentration in the shoots and there was a significant interaction with genotype. Tantangara had a greater Zn concentration in the shoots than Amagi Nijo when grown at levels $\geq Zn_{0.04}$ (Table 3.6c).

Zn content in shoots followed a different pattern: at low Zn supply, $Zn_{0.04}$, Tantangara had a higher Zn content than Amagi Nijo whereas at adequate Zn supply, Amagi Nijo had a greater Zn content than Tantangara (Appendix 3.5). Seed Zn content only had a small effect on Zn content in shoots: Amagi Nijo had a slightly higher Zn content than Tantangara at high-Zn seed level (Appendix 3.6). Irrespective of genotype, an increase in

seed Zn content resulted in an increase in Zn content of shoots but the increase was most evident at low Zn supply (Appendix 3.7).

Table 3.6. Effects of genotype, seed Zn content and Zn fertilization (mg/kg soil) on shoot Zn concentration (mg/kg D.W.) in barley at late tillering (26 days after sowing) in Experiment 2.

(a) Genotype x Seed Zn

	Seed Zn content			
Genotype	Low	Medium	High	Very high
Tantangara	16.6 (2.35)[a]	18.0 (2.40)	19.0 (2.47)	20.7 (2.67)
Amagi Nijo	16.6 (2.35)	16.1 (2.28)	16.6 (2.32)	16.6 (2.42)
Tukey's $HSD_{0.05}$[b]		(0.11)		

(b) Seed Zn x Zn fertilization

	Seed Zn content			
Zn fertilization	Low	Medium	High	Very high
0	4.9 (1.58)	5.0 (1.60)	5.1 (1.63)	6.4 (1.85)
0.04	6.1 (1.80)	5.7 (1.73)	6.3 (1.84)	7.8 (2.04)
0.8	39.4 (3.67)	39.8 (3.68)	41.3 (3.72)	42.2 (3.74)
Tukey's $HSD_{0.05}$[b]		(0.14)		

(c) Genotype x Zn fertilization

	Genotype	
Zn fertilization	Tantangara	Amagi Nijo
0	5.7 (1.68)	5.2 (1.65)
0.04	7.4 (1.97)	5.7 (1.73)
0.8	43.2 (3.76)	38.1 (3.65)
Tukey's $HSD_{0.05}$[b]	(0.09)	

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.
[b] The $HSD_{0.05}$ values are applicable to log-transformed data.

Zn concentrations in roots were also influenced by Zn fertilization and genotype. Tantangara had greater Zn concentrations than Amagi Nijo in the presence of soil-applied Zn, $\geq Zn_{0.04}$, (Table 3.7). A differential response to seed Zn occurred between the two genotypes only when soil Zn supply was adequate, $Zn_{0.8}$: Tantangara had greater Zn values than Amagi Nijo when grown from medium-, high-, and very high-Zn seed (Table

3.7). In the case of Zn content in roots, Tantangara had a slightly greater Zn content than Amagi Nijo only when grown from low-Zn seed at Zn_0 (Appendix 3.8).

Table 3.7. Effects of Zn fertilization (mg/kg soil) and seed Zn content on Zn concentration in roots (mg/kg D.W.) of barley genotypes at late tillering (26 days after sowing) in Experiment 2.

Genotype	Zn fert.	Zn concentration in roots Seed Zn content Low	Medium	High	Very high
Tantangara	0	10.5 (2.34)[a]	10.9 (2.39)	10.6 (2.36)	13.5 (2.60)
	0.04	11.4 (2.43)	12.2 (2.50)	12.6 (2.53)	14.8 (2.69)
	0.8	50.3 (3.92)	53.7 (3.98)	56.4 (4.03)	57.8 (4.06)
Amagi Nijo	0	9.9 (2.29)	10.9 (2.38)	11.6 (2.44)	12.0 (2.48)
	0.04	13.8 (2.62)	10.4 (2.34)	10.0 (2.30)	12.6 (2.52)
	0.8	43.1 (3.76)	43.3 (3.77)	40.5 (3.70)	39.1 (3.67)
Tukey's $HSD_{0.05}$[b]					
Genotype x Seed Zn x Zn fertilization			(0.32)		

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.
[b] The $HSD_{0.05}$ values are applicable to log-transformed data.

3.3.2.4 Zn concentration in youngest expanded leaves

The Zn concentration in YEBs also increased with increasing Zn fertilization but the increase differed with genotype and seed Zn (Table 3.8). When no Zn was applied to the soil, Tantangara grown from medium-, high-, and very high-Zn seed had significantly higher Zn concentrations than Amagi Nijo. However, at $Zn_{0.04}$, irrespective of genotype, plants grown at all seed Zn levels had Zn values in YEBs below 8 mg Zn/kg D.W., which are much lower than the reported adequate range of 15-70 mg Zn/kg D.W. (Weir and Cresswell, 1994). In contrast, at adequate Zn supply, $Zn_{0.8}$, Zn concentrations in YEBs did not vary significantly, and irrespective of genotype and seed Zn, all the Zn values were within the reported adequate range (Table 3.8).

Table 3.8. Effects of Zn fertilization (mg/kg soil) and seed Zn content on Zn concentration (mg/kg D.W.) in YEBs of barley genotypes at late tillering (26 days after sowing) in Experiment 2.

		Zn concentration in YEBs Seed Zn content			
Genotype	Zn fert.	Low	Medium	High	Very high
Tantangara	0	4.6 (1.51)[a]	4.5 (1.49)	4.7 (1.55)	6.6 (1.88)
	0.04	5.4 (1.68)	6.1 (1.80)	6.3 (1.83)	7.6 (2.02)
	0.8	37.7 (3.63)	39.8 (3.68)	45.6 (3.82)	43.4 (3.77)
Amagi Nijo	0	5.0 (1.59)	3.6 (1.27)	5.2 (1.64)	5.0 (1.60)
	0.04	4.4 (1.47)	3.9 (1.36)	4.3 (1.46)	5.4 (1.68)
	0.8	40.1 (3.69)	37.3 (3.62)	38.2 (3.64)	36.0 (3.58)
Tukey's $HSD_{0.05}$[b]					
Genotype x Zn fertilization x Seed			(0.28)		

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

[b] The $HSD_{0.05}$ values are applicable to log-transformed data.

3.4 Discussion

The results from both experiments demonstrated that severity of Zn deficiency symptoms decreased with increasing seed Zn content and symptoms were not visible in plants grown from very high-Zn seed. The fact that symptoms of Zn deficiency developed earlier and were more pronounced in Experiment 1 than in Experiment 2, can be attributed to the differences in seed Zn content between the two experiments. The very low-Zn seed treatment in Experiment 1 had nearly 40% less Zn than the low-Zn seed treatment in Experiment 2 (Table 3.1). This relationship between seed Zn content and deficiency symptoms suggests that seed Zn content needs to be taken into account in screening studies where visual scores are used as a parameter in assessing genotypic variation in tolerance to Zn deficiency.

Visual differences in Zn deficiency symptoms between the two genotypes were greater than differences in tissue Zn concentrations. The differential expression of deficiency

symptoms between the two genotypes, despite their similar shoot and YEB Zn concentrations (Tables 3.3, 3.4, 3.5 and 3.7), suggests that tissue Zn concentration, especially under Zn deficient conditions, may not always indicate a tolerance mechanism. Similarly, the concentration of Zn has not been closely associated with severity of Zn deficiency symptoms in cotton (Cakmak and Marschner, 1987) or in wheat and rye (Cakmak *et al.*, 1997b). Only a proportion of the total Zn in leaf tissue may be physiologically active and much of it may be bound to cell walls (Schmid *et al.*, 1965; Youngdahl *et al.*, 1977). Therefore, differences in severity of Zn deficiency symptoms between genotypes in this study might reflect differences in the pools of physiologically active Zn (e.g. the ratio of physiologically active Zn to total Zn may be greater in Tantangara than in Amagi Nijo). If this is the case, processes that depend on the concentration of physiologically active Zn may be more reliable indicators of Zn tolerance to deficiency than total Zn concentration (Cakmak and Marschner, 1987).

That leaf symptoms were visible only in very severe cases of Zn deficiency (e.g. 60-70% reduction in shoot dry matter in Amagi Nijo) may have implications for Zn management in broad acre crops and pastures. Relying only on leaf symptoms to diagnose Zn deficiency will result in substantial reductions in growth before remedial application of Zn can occur. For example, Zn application in severe cases may not be fully effective in correcting the deficiency, especially when Zn deficiency occurs late in the growing season. This suggestion is in accordance with the conclusion of Hannam *et al.* (1994) that Zn deficiency caused reduction in plant growth by up to 60% without exhibiting any visible symptoms. For successful crop production, therefore, procedures are needed to predict the potential for Zn deficiency prior to development of symptoms or loss in crop production. In their study with wheat, Riley *et al.* (1992) reported that Zn deficiency was

unlikely to occur if Zn concentrations in YEBs were 16.5 mg/kg at the early tillering stage and 7.0 mg/kg at the kernel ripe stage. Corresponding values for barley are yet to be determined.

The effect of seed Zn on Zn concentration of plants differed with soil Zn supply. There was a tendency that at low soil Zn supply, plants grown from very high-Zn seed had significantly greater Zn concentrations than those grown from low-, medium- and high-Zn seed. In contrast to findings in the present study, Yilmaz *et al.*, (1998) reported that the effect of seed Zn content on Zn concentrations in wheat plants was not significant, which was attributed to the dilution effect caused by enhanced dry matter production. This disagreement may arise from the differences in experimental conditions between the experiments. For example, very high-Zn seed in our study was about three-fold greater than the high-Zn seed in the study of Yilmaz *et al.* (1998). Given the significant seed Zn x Zn fertilization interaction, which was driven by the very high-Zn seed in the present study, it is possible that Yilmaz *et al.* (1998) may have found similar results had they used a seed Zn level similar to the very high-Zn seed in the present study and measured Zn concentrations at the same growth stage. It is also possible that high Zn concentrations from very high-Zn seed in the present study may have decreased to similar levels resulting from a dilution effect triggered by an enhanced growth, had plants been grown for a longer period of time than that in the present study. This assumption is supported by the results in the present study: Zn concentrations of plants grown from a seed Zn content common to both experiments (low-Zn seed) were higher at tillering in Experiment 1 than at late tillering in Experiment 2 (Tables 3.3 and 3.6). This lower concentration of Zn at late tillering in Experiment 2 than at tillering in Experiment 1 can be attributed to the

dilution effect as a result of greater shoot dry matter at late tillering in Experiment 2 than at tillering in Experiment 1.

There was a significant increase in dry matter yield as a result of higher seed Zn content but highest dry matter production was achieved from a combination of high-Zn seed and adequate Zn fertilization. This result clearly shows that increased seed Zn content alone can not sustain plant growth and replace the need for Zn fertilization. This result is further supported by the fact that at low Zn supply, even the plants grown from very high-Zn seed had Zn concentrations in YEBs much below the reported adequate range of 15-70 mg Zn/kg D.W. for barley (Weir and Cresswell, 1994) and consequently, there was a large response to Zn fertilization in the present study. Similar results were obtained with Mn in barley (Longnecker *et al.*, 1991), and Zn in wheat (Yilmaz *et al.*, 1998).

The greater differences in growth between plants grown from different seed Zn contents in Experiment 2 as compared to Experiment 1 can be attributed mainly to the wider range in seed Zn content. The larger effect of seed Zn on growth when soil Zn supply was $\leq Zn_{0.04}$ could be due to the fact that seed Zn becomes the predominant source of Zn for growth under Zn-deficient conditions. Soil Zn fertilization at $Zn_{0.8}$ generally reduced the differences in shoot and root growth stemming from the difference in the seed Zn content seen at low Zn supply (Figure 3.2). These results are in agreement with results on improved vegetative growth of wheat when sown in Zn-deficient soil (Rengel and Graham, 1995a).

The present results demonstrate that seed Zn content greatly influence estimates of Zn efficiency. Depending on seed Zn content, the relative efficiency of genotypes may change (Table 3.5), consequently, reliable comparisons of Zn efficiency will only be obtained if

seed of comparable Zn content is used. This finding is in accordance with the previously reported results on the effect of seed Mn content on yield and the ranking for Mn efficiency in barley (Uren *et al.*, 1988). It would therefore be advisable to use seed with similar Zn contents in screening for Zn efficiency based on vegetative growth. This practice would help ensure that sound comparisons in Zn efficiency are made between genotypes. However, producing seed with similar Zn content for a large number of genotypes would be a difficult task given that genotypes differ in their ability to load Zn into their seeds (White *et al.*, 1981; Longnecker and Robson, 1993). Thus there is a need to consider other means of screening for Zn efficiency, independent of seed Zn content (e.g. biochemical/molecular markers).

3.5 Conclusion

Higher seed Zn content improves vegetative growth of barley especially in Zn-deficient conditions. The benefits to early vigour of sowing seed with high Zn content can even be seen in Zn-sufficient conditions. The results, however, emphasize that sowing seed with high Zn content alone can not fully overcome acute Zn deficiency as is demonstrated by the larger response to soil-applied Zn than to seed Zn in the present study. The results also suggest that Zn efficiency differs considerably with seed Zn content and the level of Zn-deficiency stress. This has implications for developing screening methods to select genotypes with higher Zn efficiency. Further research is required to determine whether growth responses to high Zn content in the seed persist to final grain yield as has been shown for other nutrients.

CHAPTER 4

Screening for tolerance to zinc deficiency in Laffer sand under controlled conditions and determining a critical tissue zinc concentration

4.1 Introduction

In barley, genotypic variation in response to Zn deficiency has been reported and attempts to exploit this in a breeding program were begun (Graham *et al.*, 1992). However, the number of genotypes examined as potential parents was small and there has not been a large scale screening of progeny. Improving zinc efficiency in barley requires a rapid and reliable screening technique that determines accurately varietal response of genotypes to Zn deficiency. A screening method based on seedlings would be useful because of the ability to screen large numbers of genotypes and to quickly discard poor genotypes. Field screening for Zn efficiency in barley has been the basis for selecting improved genotypes (Takkar *et al.*, 1983; MacNaeidhe and Fleming, 1990; Graham *et al.*, 1992; Yilmaz *et al.*, 1996), but field-based methods have a number of experimental problems which hamper progress towards producing Zn-efficient cultivars. These include existence of other growth limiting factors (e.g., drought, diseases, other nutrient deficiencies and toxicities), soil variability and seasonal variation. In addition, there is considerable time and cost associated with field screening. A more reliable technique needs to be developed to screen barley genotypes for Zn efficiency.

A selection method based on seedlings, conducted under controlled conditions, has been used extensively in screening for Zn tolerance to deficiency in wheat (Graham *et al*, 1992), rapeseed (Grewal *et al.*, 1997) and chickpea (Khan *et al.*, 1998a). This approach may also be useful for screening for Zn efficiency in barley if the results are reliable and

correlate well with the results of field experiments. If so, there is a possibility that the differential responses among genotypes can provide some insight into the mechanisms of tolerance to Zn deficiency. More importantly, characteristics of efficient genotypes may ultimately be useful as criteria for selecting tolerance to Zn deficiency in barley.

The internal Zn requirement of plant species and genotypes within species differs greatly (Reuter and Robson, 1997), which is reflected in the range of critical concentrations of Zn. The requirement for a nutrient is generally defined in terms of a critical concentration which is derived from growth response curves over a wide range of Zn fertilization rates (see section 2.4.2.2). The concept of critical concentrations have been useful over the years for diagnosing the early stages of Zn deficiency, which enables it to be corrected during the growing season. However, a critical level for barley has not been fully defined and there has been no work to examine whether barley genotypes differ in their requirement of Zn.

To redress these gaps in our understanding of zinc nutrition in barley, two experiments were conducted. In Experiment 1, two barley genotypes differing in tolerance to Zn deficiency in the field were tested under controlled conditions to (i) compare their performances over a wide range of Zn fertilization rates, (ii) identify criteria for tolerance to Zn deficiency, (iii) determine a critical deficiency concentration of Zn in tissue. Experiment 2 was conducted to determine the extent of genotypic variation in response to Zn deficiency in germplasm available using selection criteria identified in Experiment 1.

4.2 Materials and methods

4.2.1 Experiment 1: Identifying selection criteria for tolerance to zinc deficiency and defining a critical deficiency concentration of zinc at the early growth stage in barley

A Zn-deficient siliceous sand (DTPA-extractable Zn=0.07 mg/kg soil) collected near Tintinara, South Australia, was washed three times with deionized water, dried and passed through a 2-mm sieve. Calcium carbonate powder was added (0.5 % w/w) and mixed thoroughly in dry soil before the following nutrients were applied in solution (mg/kg dry soil): NH_4NO_3, 350; K_2HPO_4, 150; K_2SO_4, 120; $MgSO_4.7H_20$, 90; $CuSO_4.5H_20$, 10; $MnSO_4.4H_20$, 7; H_3B0_3,1; $CoSO_4.7H_20$, 1; $FeSO_4.7H_20$, 0.15. Zinc treatments were applied as $ZnSO_4.7H_2O$ at the following rates: 0.0, 0.04, 0.08, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, and 6.4 mg/kg dry sand. One kg of soil was placed in polyethylene-lined cylindrical PVC pots with approximate dimensions of 6.5 x 30 cm (diameter x depth).

The barley genotypes used in this experiment, Zn-efficient Tarm and Zn-inefficient Hamidiye, were winter barleys and selected from 10 genotypes tested in field trials at three locations in Turkey (Yilmaz *et al.*, 1996). Zn concentrations in the seed of these genotypes were 8.0 and 7.9 mg/kg dry weight (D.W.) for the two genotypes, respectively. These values were obtained by analyzing unreplicated sample of 10 seeds per genotype, due to limited availability of seed for this study.

Seeds of the two genotypes were imbibed and then vernalized at 5 °C for 4 weeks prior to sowing. Four pre-germinated seeds of the two genotypes were sown into each pot and thinned to two plants after emergence. Pots were randomized and watered daily to a water content of 12 % (w/w). Plants were grown in a growth room at 20/15 °C day/night temperature, 14/10 h light/dark period and 300 µmol m^2/s light intensity at plant height.

Plants were harvested at the tillering stage (FS 5.0; 26 days after sowing (DAS); Large, 1954). Sand was washed from the roots under running tap water, and both shoots and roots were rinsed in deionized water followed by double deionized (DD) water (18 Mohms resistivity). Plants were separated into shoots, roots and youngest emerged leaf blade (YEB), dried at 60 °C for 48 h in a forced draft-oven, and weighed. The dry plant material was digested in 70% HNO_3 (v/v) and analyzed for nutrient concentration by ICP as described earlier (Chapter 3). Total nutrient contents were obtained by multiplying root and shoot Zn concentrations with root and shoot dry weights. Zinc uptake efficiency was calculated as the proportion of soil-applied Zn that accumulated in shoots. Zn efficiency was calculated as the ratio of shoot dry matter at an individual soil Zn level to that at adequate soil Zn level, where maximum shoot growth is achieved, and expressed as percentage.

A modified Mitscherlich model was used to establish the critical level for Zn (Ware *et al.*, 1982):

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

where y is the plant yield at tissue concentration x, and α,β and γ are parameters to be estimated from the observed data. The critical concentration associated with 90% maximum yield was derived from this equation by letting $y/\beta=0.9$:

$$x= \ln(0.1/\gamma)/\alpha \qquad (2)$$

The experiment was set up in a completely randomized block design with three replicates and 10 Zn fertilization rates. Results were analyzed by GENSTAT statistical package (GENSTAT 1988); Tukey's Honestly Significant Differences (HSD) at $\alpha=0.05$ was used in pairwise comparisons of means as described by (Steel and Torrie, 1960). To overcome

the problem of non-homogeneity of variances, the data for tissue Zn concentration and Zn content were log-transformed before being subjected to analysis of variance.

4.2.2 Experiment 2: Screening for tolerance to Zn deficiency in barley using Laffer sand as a screening medium

The effectiveness of Laffer sand as a screening medium was tested with 20 barley genotypes grown with three rates of Zn fertilization, 0, 0.04 and 0.8 mg Zn/kg soil in a completely randomized block design with three replicates. These rates were chosen based on the response curve derived from a wide range of Zn fertilization rates in Experiment 1. The rates were considered as severely deficient, deficient and adequate respectively.

The soil for this experiment came from a different batch to that used in Experiment 1, but apart from this, soil preparation and basal nutrient solutions were the same as in Experiment 1. It was assumed that it had a similar DTPA-extractable Zn to that used in the first experiment since the soil batches were collected from the same natural/virgin site but at different times. However, the soils proved to be quite different in Zn responsiveness; therefore, after the completion of the experiment, the soil was analyzed for DTPA-extractable Zn and other properties as in Experiment 1 (Table 4.1). In this experiment, the same amount of soil (1 kg) as Experiment 1 was used but plants were grown in cardboard milk cartons (7x7x17.5 cm) lined with plastic bags rather than polyethylene-lined PVC pipes simply because of accessibility and cost associated in the process.

Seeds of the barley genotypes (Table 4.2), were obtained from field plots in South Australia and Turkey which received no applied Zn. The seed was prepared and sown

Table 4. 1. DTPA-extractable Zn and properties of soils used in Experiment 1 and Experiment 2.

Properties	Experiment 1	Experiment 2
pH (water)	6.7	6.5
Organic carbon (%)	0.08	0.09
Nitrate-Nitrogen (mg/kg)	1	1
Phosphorus (mg/kg)	2	2
Potassium (mg/kg)	35	37
DTPA-extractable (mg/kg)		
Zn	0.07	0.30
Mn	0.32	0.23
Fe	<1	<1
B	0.30	0.50
Cu	0.04	0.05

Table 4.2. Seed weight (mg/seed), concentration (mg/kg D.W.) and content (µg/seed) of Zn in the seeds of barley genotypes used in Experiment 2. Standard errors are based on three replicates containing 10 seeds.

Genotype	Weight	Zn concentration	Zn content
Yesevi	48	7.4*	0.36*
Hamidiye	46	7.9*	0.36*
Tarm	46	8.0*	0.37*
Erginel	46	10.1*	0.46*
CI3576	41	10.7 ± 0.4	0.44 ± 0.02
WI-2868	48	10.9 ± 1.2	0.53 ± 0.06
WI-2597	47	11.6 ± 0.6	0.55 ± 0.03
Yagan	50	11.0 ± 0.2	0.55 ± 0.01
Clipper	47	12.2 ± 0.6	0.57 ± 0.03
Schooner	45	12.9 ± 0.5	0.58 ± 0.02
Chebec	46	12.8 ± 0.1	0.59 ± 0.01
Harrington	45	13.6 ± 0.3	0.62 ± 0.01
Galleon	47	13.5 ± 0.5	0.63 ± 0.02
Forrest	50	13.0 ± 0.3	0.65 ± 0.02
Amagi Nijo	45	14.7 ± 0.4	0.65 ± 0.04
Tantangara	47	13.9 ± 0.8	0.66 ± 0.04
Skiff	45	15.0 ± 0.3	0.68 ± 0.01
WI-2875	46	15.2 ± 0.8	0.70 ± 0.04
Haruna Nijo	48	16.6 ± 1.3	0.80 ± 0.06
Sahara	29	41.6 ± 2.5	1.21 ± 0.07

* Data were obtained by analyzing 10 seeds per genotype due to limited seed.

using the same methods used in Experiment 1. To verify the results of Experiment 1, Tarm and Hamidiye were included. The conditions for plant growth were the same as in Experiment 1. Plants were harvested at the same growth stage as Experiment 1 (FS 5.0; Large, 1954), YEBs, shoots and roots were separated, dried, and weighed as before. Plant samples (YEBs, shoots and roots) of the genotypes common to the two experiments were analyzed for elemental composition by ICP.

Zn efficiency was calculated as the ratio of the shoot dry matter at low soil Zn supply (0 or 0.04 mg Zn/kg) to that at adequate soil Zn supply (0.8 mg Zn/kg), and expressed as percentage.

4.3 Results

4.3.1 Experiment 1

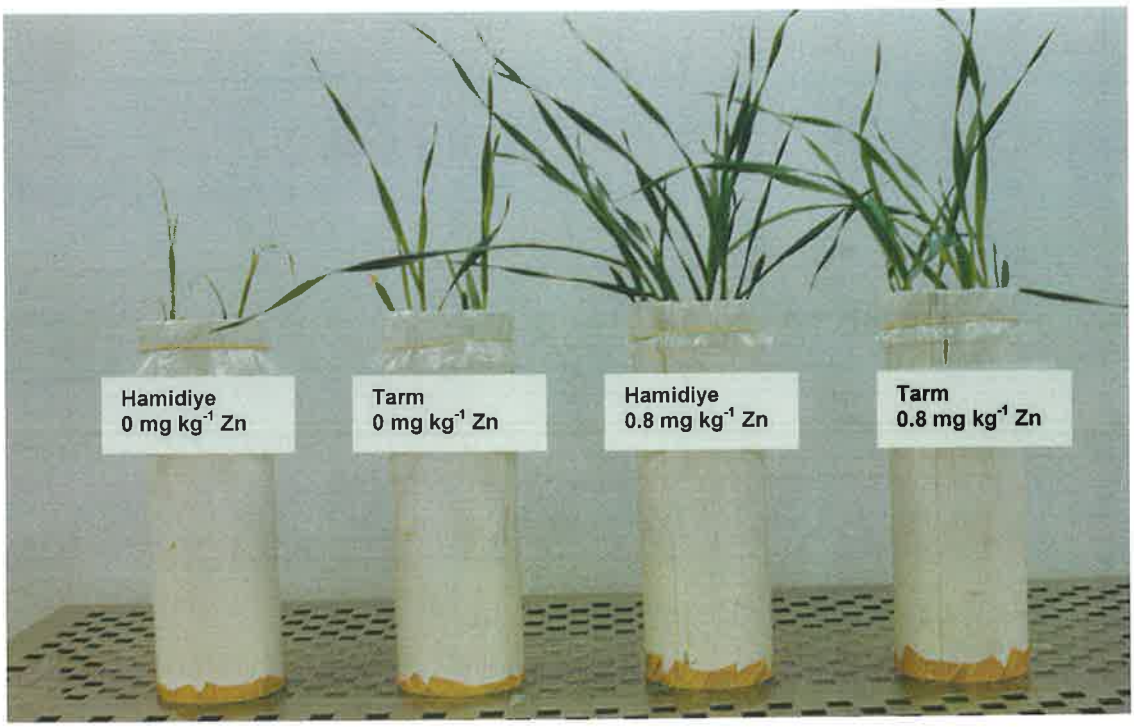
4.3.1.1 Visual symptoms

Visual Zn deficiency symptoms such as inhibition of shoot elongation and development of chlorotic areas on leaves appeared first in Zn-inefficient Hamidiye at the two-leaf stage and in Zn-efficient Tarm at the four-leaf stage. By harvest at tillering stage, Hamidiye had developed severe Zn deficiency symptoms when Zn fertilization was <0.08 mg/kg, while deficiency symptoms in Tarm were slightly visible only at 0 mg Zn/kg (see Plate 4.1a for symptom expression at 0 and 0.8 mg Zn/kg in both genotypes).

4.3.1.2 Shoot and root dry matter

Both shoot and root dry matter were significantly increased by Zn fertilization but the response to Zn fertilization of two genotypes followed different patterns. Tarm had significantly greater shoot and root dry matter than Hamidiye when Zn supply was ≤ 0.04 and ≤ 0.1 mg/kg soil, and both genotypes achieved similar yields when Zn supply was

a)



b)

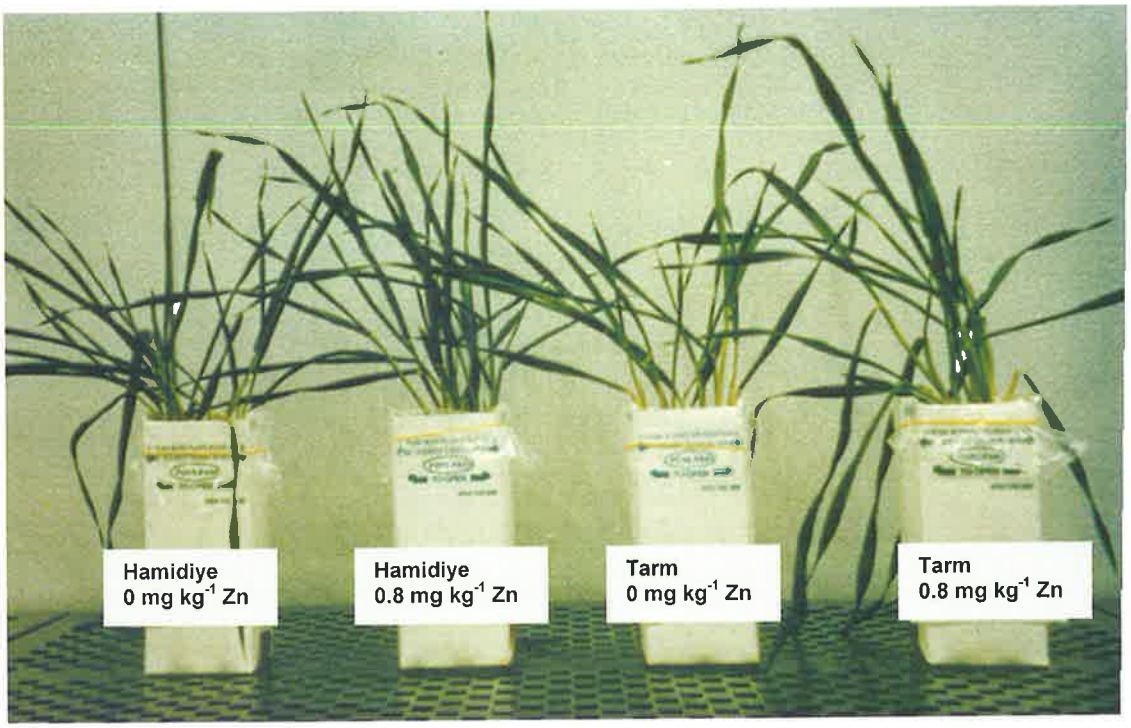


Plate 4.1. Differential response of two barley genotypes to Zn fertilization between the two batches of Laffer sand used in Experiment 1 (a) and Experiment 2 (b) at 26 DAS.

increased beyond these levels. Both genotypes achieved their maximum at 0.8 mg Zn/kg (Figure 4.1).

Zn efficiency varied with soil Zn levels and genotype: Tarm had a greater Zn efficiency than Hamidiye when Zn supply was <0.2 mg/kg soil, and above this level, the two genotypes had similar values of Zn efficiency (Figure 4.2).

4.3.1.3 Root:shoot dry weight ratio

The root:shoot dry weight ratio of both genotypes tended to increase as the soil Zn level fell, indicating higher sensitivity of shoot growth to Zn deficiency than root growth (Table 4.3). The ratio for Tarm was higher than that for Hamidiye when Zn supply was below 0.08 mg/kg with the exception of 0.04 mg/kg at which the two genotypes had similar ratio.

4.3.1.4 Zinc concentration and content of shoots and roots

Both concentration and content of Zn in the shoots increased progressively with increasing Zn fertilization (Table 4.4). Plants achieved high concentrations of Zn when fertilized at 3.2 mg/kg or 6.4 mg/kg. The genotype x Zn fertilization interaction for Zn concentration was not significant. In contrast, significant genotypic differences in Zn content occurred between the two genotypes when supplied with inadequate Zn: Tarm accumulated more Zn than Hamidiye at 0 and 0.04 mg/kg. The percentage of the Zn accumulation in shoots per unit of applied Zn (Zn uptake efficiency) was found to be greater in Tarm than in Hamidiye only at the lowest level of applied Zn; at all other levels there were no significant differences (Table 4.5).

Roots showed a similar trend to that of shoots in that both concentration and content of Zn increased continuously as a result of Zn fertilization (Table 4.6). In contrast to differences seen in shoot content, Hamidiye had greater Zn concentration at 0 mg/kg and Zn content at 3.2 mg/kg in the roots (Table 4.6).

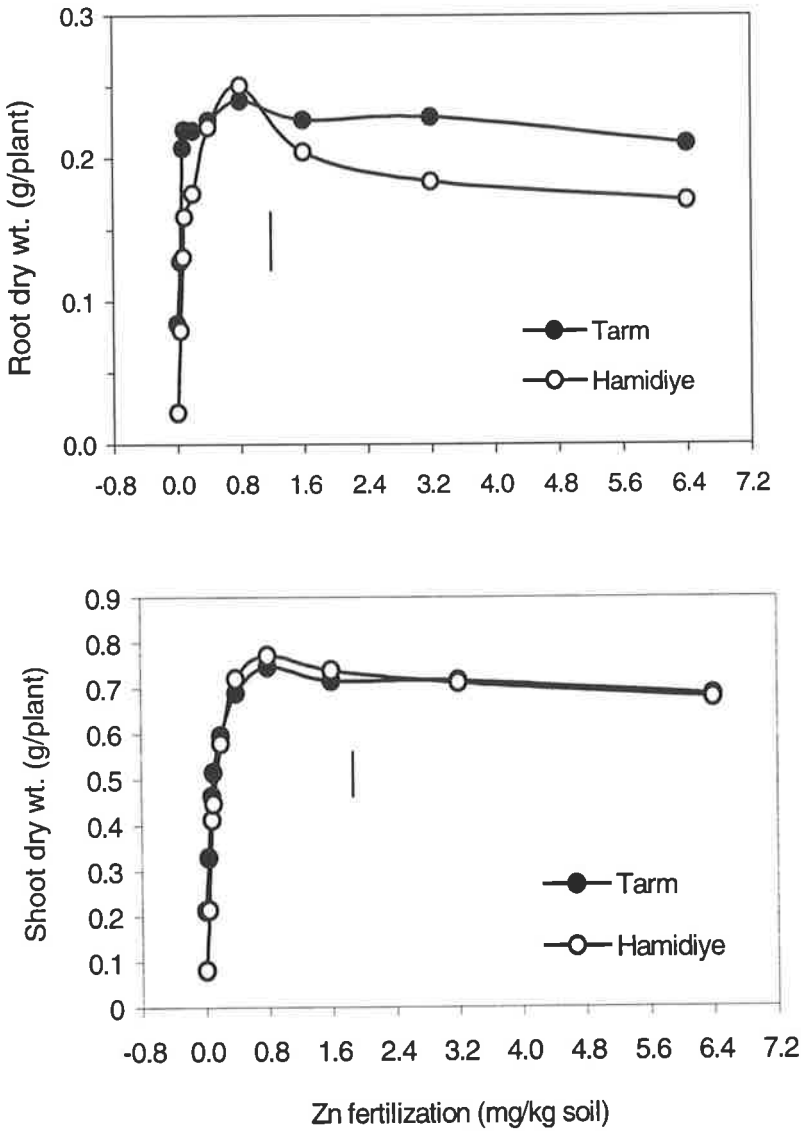


Figure 4.1. Effects of Zn fertilization on root and shoot growth of two barley genotypes at 26 DAS in Experiment 1. Vertical bars represent Tukey's $HSD_{0.05}$ values for genotype x Zn fertilization interaction.

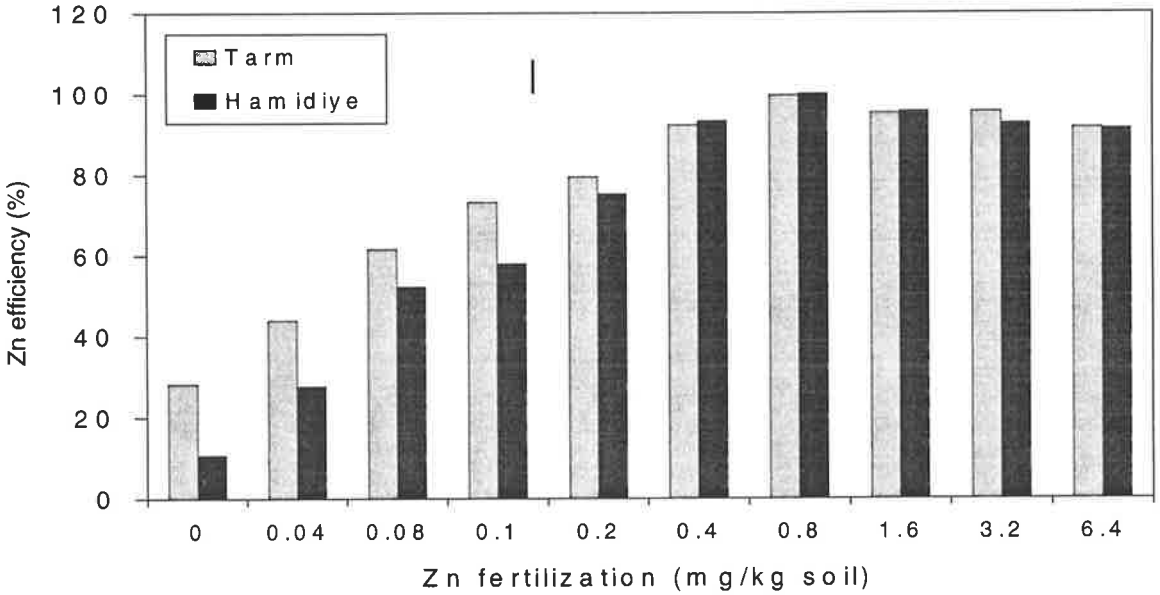


Figure 4.2. Zn efficiency of two barley genotypes over a range of Zn fertilization rates in Experiment 1. Plants were harvested 26 DAS. The vertical bar represents Tukey's $HSD_{0.05}$ value for the genotype effect.

Table 4.3. Root:shoot dry weight ratio of two barley genotypes as a result of Zn fertilization (mg/kg soil) at 26 DAS in Experiment 1.

Zn fertilization	Root:shoot D.W.ratio	
	Tarm	Hamidiye
0	0.40	0.27
0.04	0.39	0.37
0.08	0.45	0.31
0.1	0.43	0.36
0.2	0.36	0.30
0.4	0.33	0.31
0.8	0.32	0.32
1.6	0.31	0.28
3.2	0.32	0.26
6.4	0.31	0.25
Tukey's $HSD_{0.05}$		
Genotype x Zn fertilization	0.07	

Table 4.4. Effects of Zn fertilization (mg/kg soil) on Zn concentration (mg/kg D.W.) and content (µg/plant) in shoots of two barley genotypes after 26 DAS in Experiment 1.

Zn fert.	Zn concentration			Zn content		
	Tarm	Hamidiye	Mean	Tarm	Hamidiye	Mean
0	7.2	6.9	7.0 (1.94)[a]	1.5 (0.92)	0.6 (0.44)	1.0 (0.68)
0.04	8.1	6.5	7.3 (1.98)	2.7 (1.29)	1.4 (0.87)	2.0 (1.08)
0.08	10.5	10.5	10.5 (2.35)	4.9 (1.77)	4.3 (1.67)	4.6 (1.72)
0.1	12.5	12.0	12.3 (2.50)	6.5 (2.00)	5.4 (1.85)	5.9 (1.93)
0.2	21.0	17.1	19.1 (2.94)	12.5 (2.61)	9.9 (2.39)	11.2 (2.50)
0.4	33.6	29.7	31.6 (3.45)	23.2 (3.19)	21.4 (3.11)	22.3 (3.15)
0.8	54.4	45.9	50.2 (3.91)	40.7 (3.73)	35.4 (3.59)	38.1 (3.66)
1.6	84.8	75.3	80.0 (4.38)	60.7 (4.12)	55.6 (4.04)	58.2 (4.08)
3.2	128.5	124.2	126.4 (4.84)	92.1 (4.53)	88.7 (4.49)	90.4 (4.51)
6.4	280.6	259.3	267.0 (5.60)	192.6 (5.26)	176.0 (5.18)	184.3 (5.22)
Mean	64.1 (3.44)	58.7 (3.34)		43.8 (2.94)	39.9 (2.76)	
Tukey's $HSD_{0.05}$[b]						
Genotype (G)		(0.05)			(0.04)	
Zn fertilization (Zn)		(0.17)			(0.15)	
G x Zn		ns			(0.24)	

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

[b] The HSD values in parentheses are applicable to log-transformed data.

ns; non-significant.

Table 4.5. Zn uptake efficiency (proportion of soil-applied Zn accumulated in shoots, %) of two barley genotypes after 26 DAS in Experiment 1.

Zn fertilization (mg/kg soil)	Tarm	Hamidiye
0.04	6.63	3.57
0.08	6.13	5.40
0.1	6.43	5.33
0.2	6.30	4.97
0.4	5.80	5.33
0.8	5.07	4.40
1.6	3.80	3.50
3.2	2.90	2.77
6.4	3.00	2.00
Tukey's $HSD_{0.05}$		
G x Zn		1.52

Table 4.6. Effects of Zn fertilization (mg/kg soil) on Zn concentration (mg/kg D.W.) and content (µg/plant) in roots of two barley genotypes at 26 DAS in Experiment 1.

Zn fert.	Zn concentration			Zn content		
	Tarm	Hamidiye	Mean	Tarm	Hamidiye	Mean
0	22.2 (3.09)[a]	39.7 (3.69)	31.0 (3.39)	1.9 (1.05)	0.9 (0.63)	1.4 (0.84)
0.04	18.7 (2.88)	26.7 (3.28)	22.7 (3.08)	2.3 (1.19)	2.1 (1.13)	2.2 (1.16)
0.08	24.6 (3.20)	26.2 (3.27)	25.4 (3.23)	5.1 (1.81)	3.4 (1.48)	4.3 (1.65)
0.1	15.8 (2.75)	15.2 (2.72)	15.5 (2.74)	3.5 (1.49)	2.4 (1.22)	2.9 (1.36)
0.2	13.9 (2.63)	19.4 (2.97)	16.6 (2.80)	3.0 (1.40)	3.4 (1.48)	3.2 (1.44)
0.4	34.9 (3.49)	27.0 (3.25)	30.9 (3.37)	7.8 (2.13)	5.9 (1.91)	6.9 (2.02)
0.8	44.2 (3.75)	47.7 (3.86)	46.6 (3.80)	10.5 (2.42)	11.9 (2.56)	11.2 (2.49)
1.6	71.4 (4.27)	72.9 (4.28)	72.2 (4.27)	16.2 (2.84)	14.7 (2.75)	15.5 (2.80)
3.2	117.0 (4.76)	246.0 (5.50)	181.5 (5.13)	26.7 (3.32)	45.4 (3.83)	36.1 (3.57)
6.4	674.6 (6.51)	1048.0 (6.95)	861.4 (6.73)	141.7 (4.96)	178.4 (5.19)	160.0 (5.07)
Mean	103.7 (3.7)	156.9 (4.0)		21.9 (2.26)	26.9 (2.22)	
Tukey's $HSD_{0.05}$[b]						
Genotype (G)		(0.10)			ns	
Zn fert. (Zn)		(0.40)			(0.28)	
G x Zn		(0.60)			(0.46)	

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

[b] The HSD values in parentheses are applicable to log-transformed data.

ns; non-significant.

4.3.1.5 Zinc concentration in YEBs and critical concentration

An increase in Zn fertilization resulted in an increase in Zn concentration of YEBs but differences between the two genotypes were not significant (Table 4.7). The critical concentration of Zn in the YEBs was estimated to be 20.0 and 19.7 mg Zn/kg D.W. for Tarm and Hamidiye, respectively (Table 4.8). Similar critical values (20-24 mg Zn/kg D.W.) were obtained from hand-drawn curves (Figure 4.3).

Table 4.7. Effects of Zn fertilization (mg/kg soil) on Zn concentration (mg/kg D.W.) in the youngest expanded leaf blades of two barley genotypes at 26 DAS in Experiment 1.

Zn fertilization	Zn concentration		
	Tarm	Hamidiye	Mean
0	5.7 (1.73)[a]	6.0 (1.78)	5.8 (1.75)
0.04	6.3 (1.84)	5.6 (1.71)	6.0 (1.78)
0.08	9.6 (2.25)	8.6 (2.14)	9.1 (2.20)
0.1	12.3 (2.51)	9.9 (2.28)	11.1 (2.40)
0.2	19.7 (2.98)	15.5 (2.74)	17.6 (2.86)
0.4	23.7 (3.16)	24.6 (3.20)	24.1 (3.18)
0.8	47.2 (3.85)	38.9 (3.66)	43.1 (3.76)
1.6	83.0 (4.42)	75.3 (4.32)	79.1 (4.37)
3.2	123.2 (4.81)	133.6 (4.89)	128.4 (4.85)
6.4	228.1 (5.43)	240.8 (5.48)	234.5 (5.45)
Mean	56.8 (3.30)	55.9 (3.22)	
Tukey's $HSD_{0.05}$[b]			
Genotype		(0.05)	
Zn fertilization		(0.19)	
G x Zn		ns	

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.
[b] The HSD values in parentheses are applicable to log-transformed data.
ns; non-significant.

Table 4.8. Estimated parameters and critical deficiency concentrations by the modified Mitscherlich growth model.

Parameters	Values	
	Tarm	Hamidiye
Gamma	1.288	1.723
Alfa	0.128	0.145
Adjusted R^2	0.99[a]	0.99[a]
Critical deficiency concentrations		
Tarm	20.0	
Hamidiye	19.7	

Adjusted R^2 is defined as 1.0 minus the ratio of the residual sum of squares to the total sum of squares.
The equation is $y=-\ln(0.1/\gamma)/\alpha$.

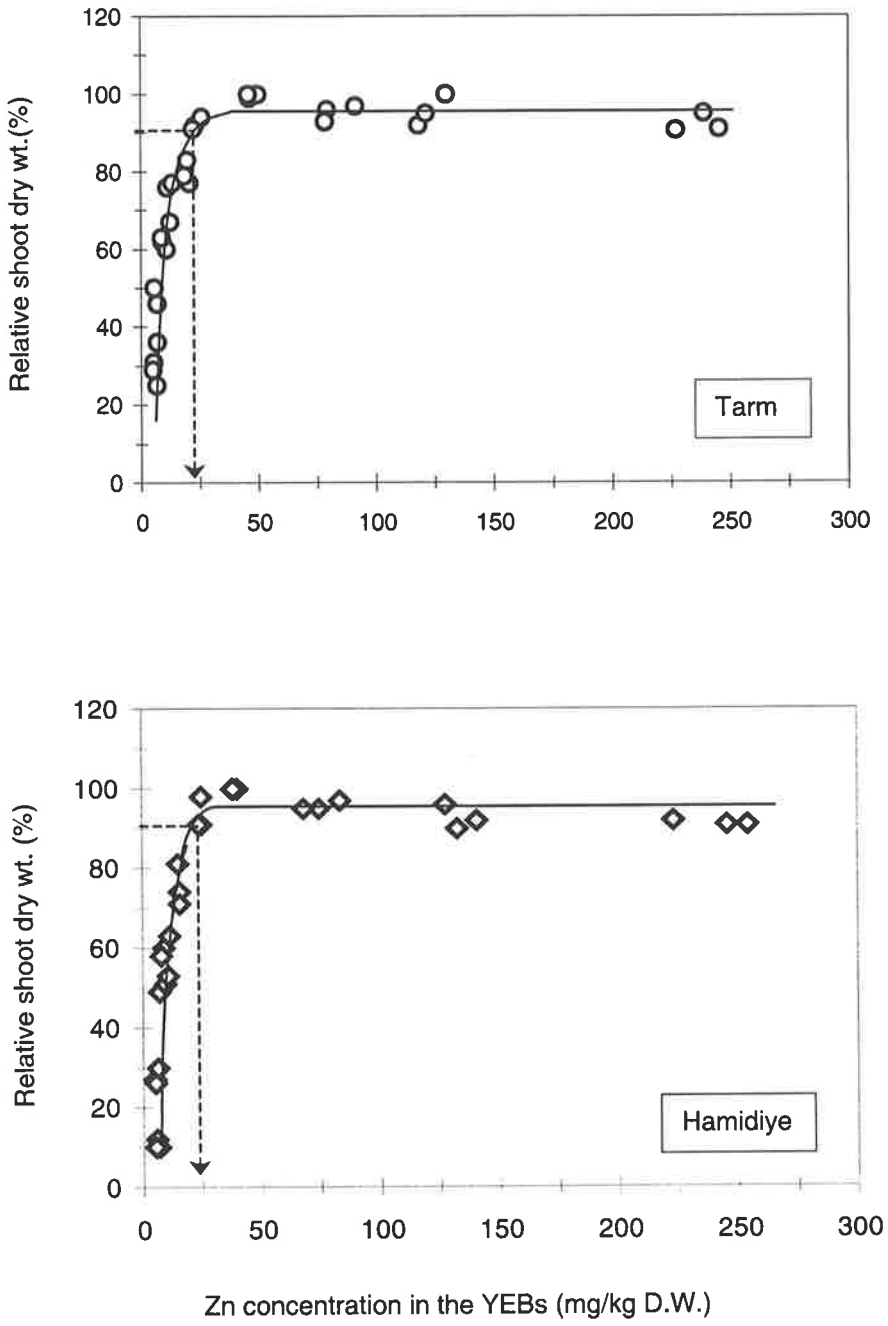


Figure 4.3. The relationship between relative shoot dry weight and Zn concentration in the YEBs of barley genotypes as a function of Zn fertilization. Critical deficiency concentrations of Zn for barley genotypes Tarm (upper graph) and Hamidiye (lower graph) are shown by dotted vertical lines.

4.3.2 Experiment 2

4.3.2.1 Visual symptoms

Unlike Experiment 1, no symptoms of Zn deficiency were observed among of the 20 genotypes including Hamidiye, which showed severe deficiency symptoms at nil Zn fertilization in Experiment 1 (see Plate 4.1b for symptom expression in Hamidiye and Tarm). In contrast to Experiment 1, boron toxicity symptoms were observed. The genotypes WI-2875, Yagan, WI-2597, Clipper, Tantangara, Forrest, and Chebec exhibited the most severe symptoms of boron toxicity which appeared mainly on old leaves. This occurred in plants grown at all Zn levels and the severity was not related to the Zn treatment.

4.3.2.2 Shoot and root dry matter

Zn fertilization significantly increased shoot and root dry matter production but the response was much less than that observed in Experiment 1 (Table 4.9). The genotype x Zn fertilization interaction was significant: at nil Zn fertilization, almost all genotypes had similar shoot dry matter production with the exception of greater dry matter of Yagan than WI-2875, Sahara and Haruna Nijo. When Zn fertilization was increased to 0.04 mg/kg soil, responses showed a different trend: CI3576 had greater dry matter accumulation than most of the genotypes (Table 4.9). At 0.8 mg Zn/kg soil, genotypic differences were still evident, which indicated differences in yield potential among genotypes.

The effect of Zn fertilization on root growth was less than that on shoot growth in that significant increases occurred only when plants were fertilized with 0.8 mg Zn/kg soil (Table 4.9). In contrast to shoot growth, no significant genotype x Zn fertilization interaction occurred (Table 4.9).

Table 4.9. Effects of Zn fertilization (mg/kg soil) on shoot and root dry matter (g/plant) of barley genotypes at 26 DAS in Experiment 2.

	Shoot dry wt.				Root dry wt.			
Genotype	0	0.04	0.8	Mean	0	0.04	0.8	Mean
Tarm	0.51	0.63	0.78	0.64	0.29	0.39	0.45	0.37
Hamidiye	0.59	0.78	0.81	0.72	0.24	0.34	0.33	0.30
Yesevi	0.59	0.68	0.78	0.68	0.34	0.38	0.43	0.38
Erginel	0.58	0.69	0.79	0.69	0.32	0.38	0.47	0.39
Galleon	0.50	0.67	0.82	0.66	0.29	0.31	0.38	0.33
WI-2875	0.40	0.46	0.57	0.48	0.20	0.22	0.25	0.22
Amagi Nijo	0.55	0.64	0.71	0.63	0.21	0.27	0.30	0.26
Yagan	0.70	0.76	0.89	0.78	0.37	0.33	0.45	0.38
WI-2597	0.61	0.68	0.80	0.70	0.30	0.29	0.33	0.31
Sahara	0.46	0.67	0.65	0.60	0.23	0.31	0.32	0.29
WI-2868	0.60	0.68	0.86	0.71	0.29	0.34	0.50	0.38
Harrington	0.57	0.60	0.69	0.62	0.34	0.32	0.36	0.34
Skiff	0.61	0.61	0.70	0.64	0.33	0.30	0.34	0.32
Clipper	0.51	0.59	0.68	0.60	0.32	0.34	0.34	0.33
Schooner	0.52	0.52	0.63	0.56	0.25	0.26	0.26	0.26
Tantangara	0.54	0.64	0.72	0.63	0.23	0.25	0.33	0.27
Forrest	0.61	0.64	0.82	0.69	0.36	0.39	0.45	0.40
CI3576	0.64	0.85	0.86	0.78	0.40	0.41	0.38	0.40
Haruna Nijo	0.49	0.67	0.70	0.62	0.29	0.31	0.37	0.33
Chebec	0.51	0.53	0.80	0.61	0.34	0.32	0.45	0.37
Mean	0.55	0.65	0.75		0.30	0.32	0.37	
Tukey's $HSD_{0.05}$								
Genotype (G)		0.10				0.08		
Zn fertilization (Zn)		0.03				0.02		
G x Zn		0.20				ns		

ns; non-significant.

Zn efficiency, calculated at 0 or 0.04 mg Zn/kg, varied with the level of Zn fertilization and genotype, but interaction genotype x Zn fertilization was not significant. Based on the shoot dry matter at nil fertilization, Zn efficiency ranged from 63% to 88%, with most of the genotypes in the range of 70-80%. At a second level of Zn fertilization, 0.04 mg Zn/kg, almost all the genotypes had similar efficiency in their response (80-90% efficiency) with the exception of Hamidiye, Sahara, CI3576 and Haruna Nijo which showed greater response (Figure 4.4).

4.3.2.3 Root:shoot dry weight ratio

Root:shoot dry weight ratios were greater in plants fertilized with no Zn than in those fertilized with Zn (0.04 and 0.8 mg/kg) (Appendix 4.1). There were also significant differences in the root:shoot dry weight ratio among genotypes (Appendix 4.1). However, the genotype x Zn fertilization interaction was not significant.

4.3.2.4 Concentration and content of Zn in shoots and roots of selected genotypes (Tarm and Hamidiye)

Both Zn concentration and content in shoots were increased significantly by Zn fertilization, but significant increases in Zn concentration occurred only at 0.8 mg Zn/kg while a continuous significant increase was observed for Zn content at all Zn levels (Tables 4.10 and 4.11). Neither genotype nor genotype x Zn fertilization for both Zn concentration and content was significant. Roots followed a different pattern: Hamidiye had greater Zn concentration when fertilized at above 0.04 mg/kg (Table 4.10), but had similar content to Tarm at all levels (Table 4.11).

4.3.2.5 Zn concentration in YEBs of selected genotypes (Tarm and Hamidiye)

Plants fertilized with no Zn had lower Zn concentrations than those fertilized with 0.04 and 0.8 mg/kg (Table 4.10). Interestingly, at 0.04 mg Zn/kg both genotypes had Zn concentrations (22.9 and 21.9 mg Zn/kg dry wt. for Tarm and Hamidiye, respectively) above the critical deficiency level as determined in Experiment 1. Significant genotypic differences were observed only at 0.8 mg/kg: Tarm had a greater Zn concentration than Hamidiye.

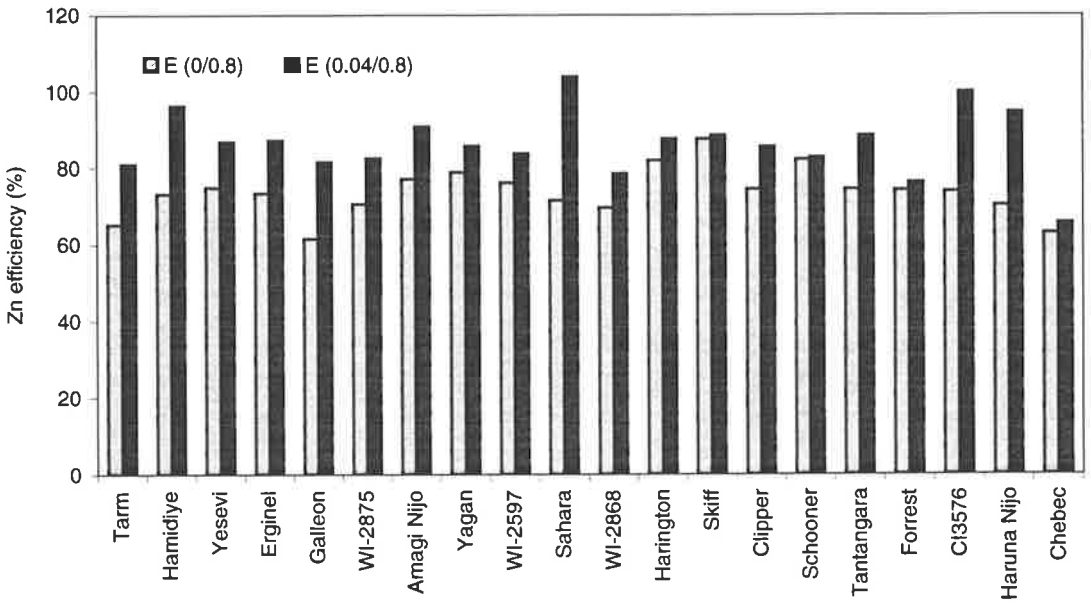


Figure 4.4. Zn efficiency (E) (ratio of shoot dry matter at 0 and 0.04 to 0.8 mg Zn/kg soil) of barley genotypes in Experiment 2. Plants were harvested at 26 DAS. Genotype x Zn fertilization was not significant for either ratio.

Table 4.10. Effects of Zn fertilization on Zn concentration (mg/kg D.W.) in the YEB, shoot and root of two barley genotypes at 26 DAS in Experiment 2.

	Zn concentration						
Zn fert.	YEB		Shoot			Root	
	Tarm	Hamidiye	Tarm	Hamidiye	Mean	Tarm	Hamidiye
0	14.4	16.4	16.2	17.1	16.6	16.0	16.7
0.04	22.9	21.9	25.1	24.1	24.6	25.8	31.0
0.8	48.4	38.1	46.0	42.7	44.4	54.1	83.1
Tukey's $HSD_{0.05}$							
Zn					8.4		
G x Zn	8.7			ns		10.6	

ns; non-significant.

Table 4.11. Effects of Zn fertilization (mg/kg soil) on Zn content (μg/plant) in the shoot and root of barley at 26 DAS in Experiment 2.

	Zn content	
Zn fertilization	Shoot	Root
0	10.3	4.3
0.04	19.1	10.2
0.8	38.5	27.0
Tukey's $HSD_{0.05}$		
Zn	7.6	5.8

4.4 Discussion

The level of Zn deficiency stress in the two experiments differed quite markedly, and as a consequence the response of genotypes to Zn fertilization varied between experiments. Zn deficiency in Experiment 1 was severe enough to investigate aspects of Zn nutrition such as plant response, critical deficiency concentration, and selection criteria for tolerance to Zn deficiency, but not severe enough in Experiment 2 to define clearly genotypic differences in tolerance to Zn deficiency among the barley genotypes studied.

The responses of the two barley genotypes to Zn deficiency in Experiment 1 in which Zn deficiency was severe were similar to those observed in the field (Yilmaz *et al.*, 1996), indicating that screening barley for Zn efficiency at the early growth stage in soil culture under controlled conditions could be a predictor of field response. The Zn-efficient genotype, Tarm, had better shoot and root growth than the Zn-inefficient genotype, Hamidiye, when plants were supplied with inadequate Zn. When Zn supply was adequate, both genotypes achieved similar dry matter yields. The differences in growth, therefore, may be attributed to the differences in Zn efficiency of genotypes, given that seed Zn

content, soil and growth conditions were uniform. The greater root growth of Tarm compared to that of Hamidiye under Zn deficiency may be an important contributing factor to Zn efficiency of this genotype. Enhanced root growth has also been suggested by Dong *et al.* (1995) to be an important mechanism of Zn efficiency in wheat presumably because it can greatly improve Zn uptake efficiency of plants. It was noteworthy that Tarm had a higher Zn uptake efficiency than Hamidiye (Table 4.5). This enhanced Zn uptake efficiency of Tarm over Hamidiye can be considered as one of possible mechanisms for its higher Zn efficiency in this experiment. Increased Zn uptake capacity of plants under Zn deficient conditions was shown as an important factor determining Zn efficiency of genotypes of rapeseed in the glasshouse (Grewal *et al.*, 1997) and wheat in field experiments (Cakmak *et al.*, 1997a). Also in nutrient solution experiments, differences in Zn efficiency between wheat genotypes were closely related to the capacity of genotypes to take up Zn and translocate it into shoots (Rengel and Graham, 1996; Cakmak *et al.*, 1996c).

Based on the observations such as visual symptoms and growth in genotypes common to both experiments namely Tarm and Hamidiye, a differential Zn deficiency stress, and accordingly response to Zn fertilization occurred between the two experiments (Plate 4.1a,b). In contrast to Experiment 1, at Zn_0 or $Zn_{0.04}$, the two genotypes did not develop any symptoms of Zn deficiency, and achieved greater shoot and root growth in Experiment 2. In an attempt to quantify this differential response to Zn fertilization between the two experiments, growth was expressed in a relative term, the ratio of shoot growth at low to that at adequate Zn supply, which is described as Zn efficiency, and marked differences were found between the two experiments. For example, the relative growth at Zn_0 was recorded to be 30% for Tarm and 10% for Hamidiye in Experiment 1

(Figure 4.2), while these values were 65% for Tarm and 70% for Hamidiye in Experiment 2 (Figure 4.3). Such high values of relative growth were also evident in other genotypes (70-80%; Figure 4.3) in Experiment 2. Relative growth up to 65-70% with no visible symptoms of Zn deficiency at Zn_0 in both genotypes in Experiment 2, indicated a lack of selection pressure, and accordingly an inability to clearly separate the Zn-efficient genotypes from the Zn-inefficient genotypes in Experiment 2. In an attempt to explain the unexpectedly poorer response to Zn in Experiment 2, soil from the batch was analyzed for available Zn; however, this was done after the experiment was terminated. DTPA-extractable Zn was 0.30 and 0.07 mg/kg soil for Experiment 2 and Experiment 1, respectively (Table 4.1).

Good correlations between DTPA-extractable Zn and response of crops to Zn fertilization in different soils have been reported previously (Stewart and Tahir, 1971; Brown *et al.*, 1971; Takkar and Mann, 1975; Takkar *et al.*, 1975 and Lindsay and Norvell, 1978). More recently, Brennan (1992) examined the relationship between critical DTPA extractable Zn and properties of 42 southwestern Australian soils which were responsive to applied Zn, and found that the critical level of DTPA extractable Zn from the soil for maximum wheat growth ranged from 0.12 to 0.27 mg/kg. The DTPA extractable Zn value of 0.30 mg/kg in Experiment 2 is closer to the values reported by Brennan (1992) but much lower than previously reported values of 0.6 (Bansal *et al.*, 1980) and 0.8 mg/kg (Lindsay and Norwell, 1978). According to Brennan (1992), the higher critical values by Bansal *et al.* (1980) and Lindsay and Norwell (1978) would be excessive for sandy soils. From the poor response to Zn fertilization in present study, Laffer sand may have a higher critical DTPA extractable value than the sandy soils studied by Brennan (1992), or it may reflect the greater Zn efficiency of barley compared to bread wheat.

That there were no genotypic differences in Zn concentration of YEBs or shoots while there were differences in severity of Zn deficiency symptoms in Experiment 1 suggests differential utilization or compartmentation of Zn in leaf cells. This was suggested as one of the mechanisms of Zn efficiency in cereals (Graham and Rengel, 1993). More recently, Rengel (1995) in his study examining the activity of a Zn-containing enzyme, carbonic anhydrase, observed differences in enzyme activity between a Zn-efficient and a Zn-inefficient wheat despite the same tissue Zn concentration: the carbonic anhydrase activity was higher in a Zn-efficient bread wheat than a Zn-inefficient durum wheat. From the results reported here and elsewhere Zn concentration in YEBs or shoots does not seem to be a reliable parameter for distinguishing between the Zn-efficient and Zn-inefficient genotypes, especially under Zn deficient conditions (Rengel, 1995; Cakmak *et al*, 1996a). However, it can be used to diagnose Zn status, accordingly Zn deficiency, within a genotype.

Similar critical Zn concentrations for the two genotypes (20.0 and 19.7 mg/kg dry wt. for Tarm and Hamidiye, respectively) provide further evidence for differences in their uptake of Zn rather than in their requirement as the basis of their difference in efficiency. Although these levels are within the universally accepted range (e.g. 15-20 mg/kg dry wt.) for a number of crop plants, care needs to be taken with the use of critical deficiency concentrations to distinguish between Zn-efficient and Zn-inefficient genotypes. Further research is needed to define the extent of variation in critical levels of Zn in a wider range of genotypes differing in tolerance to Zn deficiency and its usefulness in assessing genotypic variation in response to Zn deficiency. However, there seems to be a good correlation between Zn concentration in youngest leaves up to about 25 mk/kg and plant growth (Figure 4.3; Table 4.7). Therefore, it can be assumed that the Zn concentration in

youngest leaves can be used as an indicator of the Zn status of plants as demonstrated for a wide range of crops such as sugarbeet (Rosell and Ulrich, 1964), cotton (Ohki, 1984), soybeans (Ohki, 1977), and wheat (Brennan, 1992; Riley *et al.*, 1992).

4.5 Conclusion

Genetic differences in response to Zn deficiency reported previously in the field were reproduced under controlled conditions in pots using a Zn-deficient soil, Laffer sand. The differences were expressed in visual symptoms, shoot and root growth and uptake efficiency (Experiment 1). From the results reported here, it appears that soil culture under controlled conditions may offer potential improvements over field trials in screening for tolerance to Zn deficiency, provided the results are shown to be consistent in a wide range of genotypes. However, the success of the method will depend on the level of Zn deficiency stress and high soil Zn content will reduce the ability to distinguish between genotypes. Therefore, it is important that the relationship between a soil test (e.g. DTPA or EDTA) and plant growth, plant Zn concentration or Zn uptake be established for a specific soil to be used for screening purposes. The results also indicate that there is a good correlation between Zn concentration in youngest leaves and plant growth, therefore, Zn concentration in the youngest leaves can be used to diagnose the Zn status of barley genotypes. The results also highlight the possibility of variation in Zn status of different batches of Laffer sand, therefore the need to use soil of the same batch of known Zn response in future screening work.

CHAPTER 5

Screening for tolerance to zinc deficiency in Lancelin sand under glasshouse conditions

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5.1 Introduction

In the course of screening for tolerance to Zn deficiency using Laffer sand (Chapter 4), variation in the expression of Zn deficiency stress was encountered when two different batches of Laffer sand were used. Soil analyses indicated that the differences in severity of Zn deficiency were associated with differences in DTPA-extractable Zn between the batches (Chapter 4). It is well known that in the field, soils differ considerably in their expression of Zn deficiency stress and responses to Zn fertilization (Yilmaz *et al.*, 1996; Brennan, 1992). However, the possibility of such differences occurring within Laffer sand was not anticipated because this medium has been used successfully in Zn studies with other crops (Khan *et al*, 1998a,b; Grewal *et al.*, 1997; Rengel and Graham, 1995a,b).

The low response to Zn in Experiment 2 (Chapter 4) presented a dilemma: It may have been a very atypical result, in which case future screening work may be little affected if new batches of sand were used. However, on the other hand, if such variation was more common than previously thought, interpreting results of the screening studies would be hindered by the variation in the Zn content of the soil. One alternative would be to test each batch of soil for DTPA-extractable Zn because this would indicate the likely response of plants to Zn grown in the sand. This would first require the critical concentration of DTPA-extractable Zn to be determined for the soil used and the DTPA-extractable Zn measured for each soil batch used in the future experiments. Given the

time constraints in the present program this was not considered a feasible option and, it was decided to try a widely-used alternative to Laffer sand, Lancelin sand.

In a detailed study of the Zn status of 42 soils in Western Australia, Brennan (1992) found that Lancelin sand was responsive to Zn fertilization and had a critical DTPA-extractable Zn of 0.13 mg/kg. This sand has been used successfully as a growth medium for studies on Zn nutrition in a number of species including subterranean clover (Reuter *et al.*, 1982; Burker and Robson, 1994) and canola (Huang *et al.*, 1995), although there are no reports in the literature of it being used as a growth medium for studies on Zn responses in barley. Therefore, in the study presented here, Lancelin sand was assessed for its potential for screening for tolerance to Zn deficiency under controlled conditions as a possible alternative to Laffer sand.

5.2 Materials and methods

The potential for Lancelin sand as a growth medium to screen for Zn efficiency was explored in three experiments conducted during a short study visit to Department of Soil Science and Plant Nutrition of University of Western Australia in 1997. These experiments were conducted in a glasshouse at 20/15 °C day/night temperature and approximately 12 h photoperiod. In the first experiment, the response to Zn of barley grown in Lancelin sand was studied. The results of this experiment were used to examine growth of a range of barley genotypes grown at three levels of Zn. All three experiments were designed as randomized complete block designs with three replicates.

5.2.1 Experiment 1. Establishing zinc response in Lancelin sand for screening for tolerance to Zn deficiency

A Zn-deficient, sandy surface soil, collected from uncleared land near Lancelin, Western

Australia, (DTPA-extractable Zn=0.12 mg/kg soil), was sieved through a 2 mm stainless steel sieve and one kg of soil was placed into cardboard cartons (7x7x17.5 cm) of 600 ml volume following the addition of calcium carbonate powder (0.5 % w/w). Basal nutrients (in mg/kg dry soil) of NH_4NO_3, 95; K_2HPO_4, 90; K_2SO_4, 140; $MgSO_4.7H_2O$, 20; $CuSO_4.5H_2O$, 2; $MnSO_4.4H_2O$, 15; H_3BO_3, 0.7; $CoSO_4.7H_2O$, 0.4; $Na_2MoO_4.2H_2O$, 0.2, together with six zinc treatments (0.0, 0.04, 0.2, 0.8, 3.2 and 12.8 mg/Zn kg soil applied as $ZnSO_4.7H_2O$ and designated hereafter as Zn_0, $Zn_{0.04}$, $Zn_{0.2}$ $Zn_{0.8}$ $Zn_{3.2}$ and $Zn_{12.8}$) were applied to the surface of the soil, allowed to dry and mixed again throughout the soil. Additional N was applied as 95 mg/kg NH_4NO_3 at 14 and 26 DAS.

In this experiment, the barley genotypes Tarm and Hamidiye could not be used as previously done in the experiments using Laffer sand (Chapter 4) due to lack of seed. Instead, the barley genotypes Tantangara and Amagi Nijo were used. These genotypes were selected based on results from a preliminary experiment conducted in Laffer sand in which 22 genotypes of barley were tested: when no Zn was applied Amagi Nijo developed very severe chlorosis of whole plant while Tantangara exhibited mild symptoms and stunted shoot growth. The differences in symptoms occurred despite Amagi Nijo having a greater seed Zn content than Tantangara. Seed germination and sowing, seedling thinning, randomization and watering of pots were performed as described in Chapter 4.

Seeds of Tantangara and Amagi Nijo, with a seed Zn concentration of 10.5 mg/kg dry wt. were hand sorted to uniform size (43±2 mg/seed D.W.), surface-sterilized, and imbibed on moist paper in perti dishes for 24 h at room temperature, as described in Chapter 4. Plants were harvested four weeks after sowing (tillering, FS 5.0, Large, 1954). The

adhering soil was washed off under running tap water then dipped into deionized water. Following a quick rinse in double deionized (DD) water (18 Mohms/cm resistivity), plants were separated into roots and shoots. Plant samples were oven dried at 80 °C for 48 h for dry matter yields. Zn efficiencies were calculated as the ratio of shoot dry matter at each individual Zn level to that at the Zn level where maximum shoot dry matter was achieved ($Zn_{12.8}$). Plant samples were digested in 70% HNO_3 and analyzed by ICP, as described earlier (Chapter 3).

Data were analyzed using the GENSTAT statistical package (GENSTAT 5, 1988). Tukey's Honestly Significant Differences (HSD) at α=0.05 was used in pairwise comparisons (Steel and Torrie, 1960). To overcome non-homogeneity of variances, the data for tissue Zn concentration and content were transformed prior to analysis of variance. Log (x) was used for Zn concentration and log (x+1) for Zn content data.

5.2.2 Experiment 2. Genotypic variation in response of barley to Zn deficiency (16 genotypes)

The extent of genotypic variation in response to Zn deficiency was examined in 16 genotypes (Table 5.1). The Lancelin sand used in the experiment came from the same batch as Experiment 1. Most of the seed used in this experiment was obtained from field plots from Lameroo, South Australia, in which genotypes were grown without Zn fertilization.

Preparation of soil and basal solutions were the same as in Experiment 1. Three levels of Zn, selected from the results of Experiment 1 were used. The plants were grown at 0, 0.02 and 0.8 mg Zn/kg soil, which produced very severe, severe and no Zn stress. Based on the results of relative shoot growth in Experiment 1 (Figure 5.2), it was felt that a Zn level

between 0 and 0.04 mg Zn/kg soil would induce greater Zn deficiency stress, and therefore, better discrimination among genotypes than 0.04 mg Zn/kg soil; therefore, 0.02 mg Zn/kg soil was used in this experiment. A Zn fertilization of 0.8 mg/kg soil was considered adequate for normal growth since there was no significant increase in dry matter production above this level in Experiment 1 (Figures 5.1and 5.2). Plants were grown, harvested, and shoot and root samples were analyzed as described in Experiment 1. Zn efficiency was calculated as the ratio of shoot dry matter at 0 to 0.8 mg Zn/kg soil or 0.02 to 0.8 mg/kg soil.

Table 5.1. Seed weight (mg/seed), concentration (mg/kg D.W.) and content (µg/seed) of Zn in the seeds of barley genotypes used in Experiment 2. Standard errors are based on three replicates containing 10 seeds each.

Genotype	Weight	Zn concentration	Zn content
Amagi Nijo	45	14.7 ± 0.4	0.65 ± 0.04
Chebec	46	12.8 ± 0.1	0.59 ± 0.01
CI3576	41	10.7 ± 0.4	0.44 ± 0.02
Clipper	47	12.2 ± 0.6	0.57 ± 0.03
Forrest	50	13.0 ± 0.3	0.65 ± 0.02
Galleon	47	13.5 ± 0.5	0.63 ± 0.02
Harrington	45	13.6 ± 0.3	0.62 ± 0.01
Haruna Nijo	48	16.6 ± 1.3	0.80 ± 0.06
Sahara	29	41.6 ± 2.5	1.21 ± 0.07
Schooner	45	12.9 ± 0.5	0.58 ± 0.02
Skiff	45	15.0 ± 0.3	0.68 ± 0.01
Tantangara	47	13.9 ± 0.8	0.66 ± 0.04
WI-2597	47	11.6 ± 0.6	0.55 ± 0.03
WI-2868	48	10.9 ± 1.2	0.53 ± 0.06
WI-2875	46	15.2 ± 0.8	0.70 ± 0.04
Yagan	50	11.0 ± 0.2	0.55 ± 0.01

Plants were visually scored on a scale of 1 to 6, according to the following criteria (see also Appendix 5.1):

1= dark green, healthy leaves, (no symptoms),

2=pale green leaves (slight symptoms),

3=linear chlorotic areas appearing on young leaves (mild symptoms),

4=chlorotic areas extending to margins and leaves collapsing in the middle (mild to severe symptoms),

5=both young and old leaves turning pale yellow (severe symptoms) and,

6=dead growing points (very severe symptoms).

Data analysis, transformation and pairwise comparisons were performed as described in Experiment 1.

5.2 Experiment 3. Genotypic variation in response of barley to Zn deficiency (39 genotypes)

Thirty nine genotypes (Table 5.2) were tested using the same soil and growing conditions as Experiments 1 and 2. Zn treatments were the same as in Experiment 2, namely 0, 0.02 and 0.8 mg/kg soil.

The seed used in this experiment (Table 5.2) was obtained from the barley breeding programme of University of Western Australia, but could not be analyzed for elemental composition prior to experiment due to time constraints. The seed and tissue analysis were performed by the Waite Analytical Services (SA) after the completion of the experiment. Seed Zn concentration values were based on one replication containing 10 seeds per genotype due to limited availability of seed.

Plant harvest, drying and preparation for nutrient analysis were the same as in Experiment 1. Zn efficiency was calculated as the ratio of shoot dry matter at 0 and 0.8 mg Zn/kg soil or 0.02 and 0.8 mg/kg soil. Plants were scored visually on a scale of 1 to 6 as described in

Experiment 2. Data analysis, transformation and pairwise comparisons were the same as in Experiment 1.

Table 5.2. Seed weight (mg/seed), concentration (mg/kg D.W.) and content (μg/seed) of Zn in the seeds in genotypes used in Experiment 3.

Genotype	Weight	Zn concentration	Zn content
83SM522	57	21.8	1.24
Bearpaw	52	20.1	1.05
Blenheim	51	13.0	0.66
Capulet	53	59.4	3.16
Chariot	59	32.7	1.94
Chebec	56	18.5	1.04
Cheri	53	11.4	0.61
Clipper	61	86.3	5.23
Dicktoo	35	60.1	2.09
Ellice	57	63.0	3.61
Europa	49	17.1	0.83
Fitzgerald	54	21.5	1.16
Franklin	61	16.3	0.99
Gairdner	53	30.2	1.60
Galleon	57	22.2	1.27
Harrington	52	45.6	2.36
Haruna Nijo	56	21.7	1.21
Igri	56	25.3	1.43
Kinukei 15	42	17.3	0.72
Kinukei 19	52	16.4	0.85
Kinukei 21	58	122.0	7.07
Manley	45	18.1	0.81
Molloy	56	13.0	0.73
Mona	56	58.4	3.26
Morex	53	39.0	2.07
Mundah	64	17.6	1.12
Natasha	49	15.9	0.79
Nudinka	50	38.8	1.92
O'Connor	63	55.0	3.44
Onslow	43	26.1	1.12
Prisma	41	54.3	2.24
Proctor	48	40.3	1.95
Skiff	60	28.7	1.73
Stein	53	80.9	4.30
Steptoe	58	30.8	1.79
Stirling	62	20.0	1.24
TR-306	58	68.3	3.98
Waverney	48	20.0	0.97
Yagan	74	22.9	1.68

Due to limited availability of seed, seed Zn concentration values were based on one replication containing 10 seeds per genotype

5.3. Results

5.3.1. Experiment 1

5.3.1.1 Visual symptoms

Typical Zn deficiency symptoms, such as reduced shoot growth and development of pale yellow, chlorotic areas along the mid-rib of young leaves, as observed earlier (Experiment 1, Chapter 4), were visible in plants fertilized with no Zn, 12-14 days after sowing. At harvest, both genotypes exhibited severe Zn deficiency symptoms when grown with no Zn fertilization. A Zn fertilization rate of 0.04 mg/kg decreased the severity of deficiency symptoms (reduced growth, no visible chlorotic areas). There was no visible effect of Zn deficiency when Zn fertilization was increased beyond 0.2 mg/kg.

5.3.1.2 Shoot and root dry matter

Shoot and root dry matter were markedly decreased by Zn deficiency (Figure 5.1), and the effect was similar for both genotypes. Overall, Amagi Nijo had slightly greater shoot and root dry matter (shoot, 0.33 g/plant; root; 0.17 g/plant) than Tantangara (shoot, 0.29 g/plant; root, 0.17 g/plant). Based on the growth response in this experiment, $Zn_{0.8}$ can be considered adequate for optimum growth since there was no further significant increase in dry matter above this level (Figure 5.1). Zn efficiency was decreased significantly by Zn deficiency, $<Zn_{0.2}$, but the effect was similar for both genotypes (Figure 5.2). The shoot growth was recorded to be 24% and 58% of the maximum at Zn_0 and $Zn_{0.04}$, respectively.

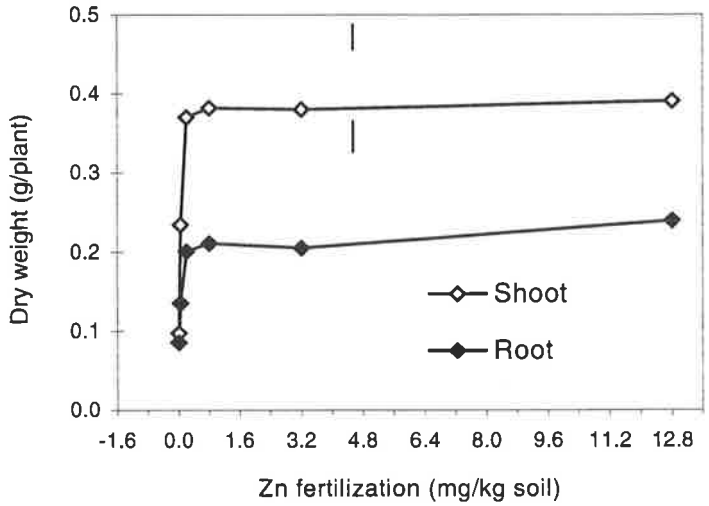


Figure 5.1. Effects of Zn fertilization on shoot and root dry weight of barley at 28 DAS in Experiment 1. The data were averaged over two genotypes because no interaction involving the genotype effect was significant. The vertical bars represent the Tukey's $HSD_{0.05}$ values for the Zn effect (upper and lower bars for shoot and root respectively).

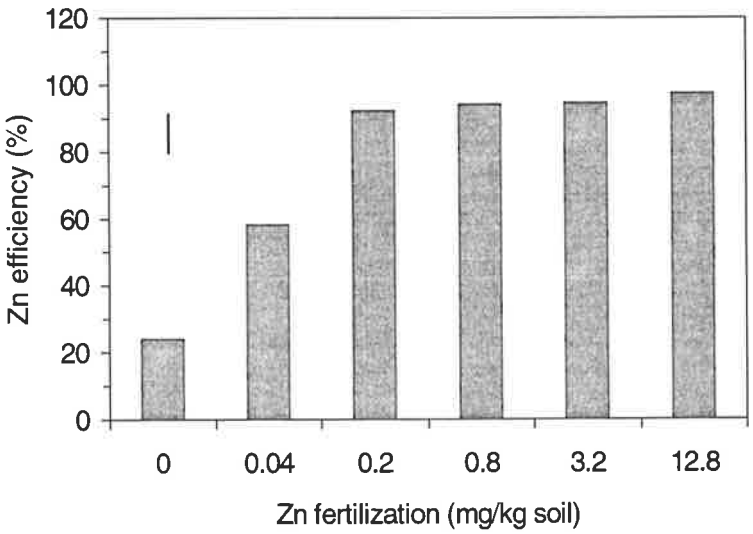


Figure 5.2. Zn efficiency of barley over a range of Zn levels in Experiment 1. Plants were harvested at 28 DAS. The vertical bar represents Tukey's $HSD_{0.05}$ value for the Zn effect (Genotype x Zn fertilization interaction is non-significant).

5.3.1.3 Root:shoot dry weight ratio

The root:shoot dry weight ratio was affected by the Zn status of plants: the ratio was higher in Zn-deficient compared to Zn-sufficient plants, and the response was similar for both genotypes. However, only plants grown with no soil applied Zn (root:shoot ratio= 0.88) had a significantly greater ratio than those grown with applied Zn (the ratio ranging from 0.58 to 0.61 with soil applied Zn ranging from $Zn_{0.04}$ to $Zn_{12.8}$).

5.3.2 Experiment 2

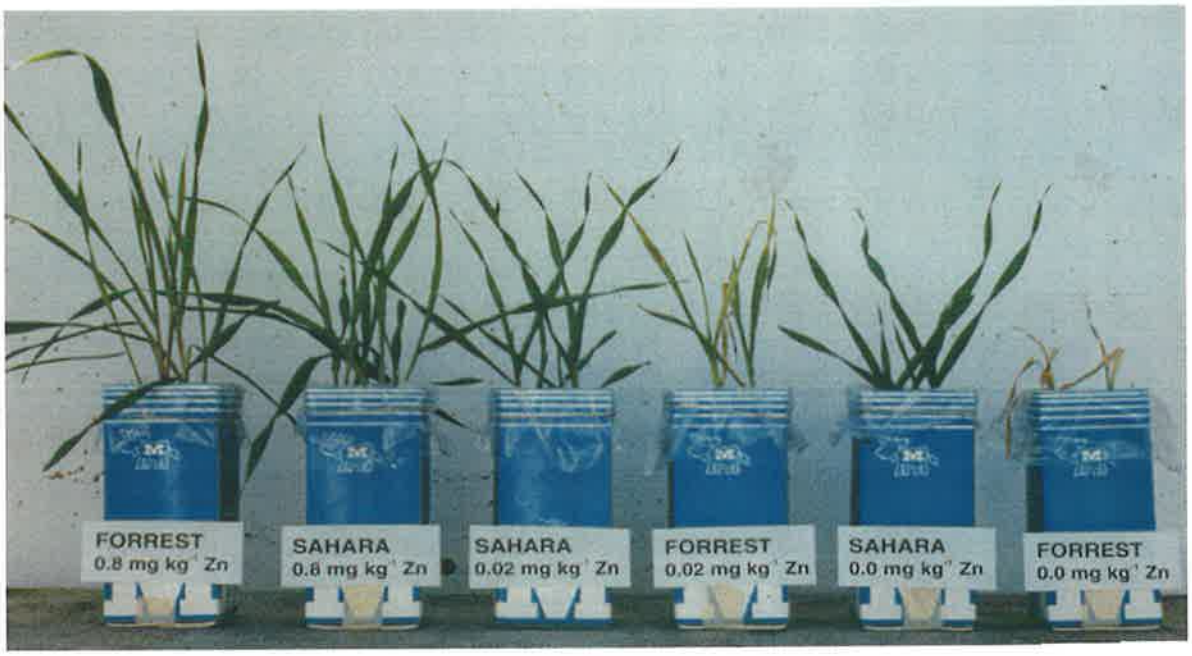
5.3.2.1 Visual symptoms

At harvest, most of genotypes developed severe to very severe Zn deficiency symptoms when no Zn was applied, except for Schooner, Skiff (mild symptoms) and Sahara (no symptoms) (Figure 5.3). Zinc fertilization of 0.02 mg/kg soil resulted in less severe symptoms. Symptoms were absent in Sahara and were mild in all other genotypes. No symptoms of Zn deficiency occurred at $Zn_{0.8}$ (see Plate 5.1a for variation in expression of deficiency symptoms observed in the extreme genotypes, Sahara and Forrest, at three Zn levels).

5.3.2.2 Shoot and root dry matter

Shoot dry matter was lower in Zn-deficient than in Zn-sufficient plants in all genotypes, but the differences in genotypes differed with the degree of Zn deficiency stress (Table 5.3). At Zn_0, the deficiency stress was so severe that very little dry matter accumulation was observed with no significant differences occurring among the genotypes. At $Zn_{0.02}$, there was a significant increase in shoot dry matter accumulation in almost all genotypes, and significant genotypic differences were observed: Haruna Nijo, Sahara, Skiff had greater shoot dry matter than Forrest and CI3576. At $Zn_{0.8}$, there were differences in early

a)



b)

Plate 5.1. Genotypic variation in expression of Zn deficiency symptoms in barley genotypes grown in Lancelin sand fertilized with different Zn levels at D28 in Experiment 2 (a) and Experiment 3 (b).

seedling vigour among the genotypes with Haruna Nijo, Yagan and Amagi Nijo showing the greatest, while Clipper and WI-2875 the lowest vigour.

Similar to shoot dry matter, Zn deficiency decreased root dry matter accumulation significantly in all genotypes, but the effect varied with the level of Zn deficiency stress. Under very severe Zn deficiency, Zn_0, all the genotypes produced very little dry matter, and genetic differences were not significant. However, at $Zn_{0.02}$, dry matter accumulation increased in all genotypes, and significant genetic differences were observed: Skiff and Haruna Nijo had greater root dry matter than Forrest and Clipper (Table 5.3).

Zinc efficiency varied with the level of Zn deficiency stress. As observed in earlier experiments (Chapters 3 and 4), efficiency increased with increasing soil Zn supply: under severe Zn deficiency, Zn_0, efficiency was recorded in the range of 14 to 32%, while under less severe deficiency stress, $Zn_{0.02}$, it ranged from 28% to 55% (Figure 5.4).

5.3.2.3 Root:shoot dry weight ratio

The root:shoot dry weight ratio was generally higher in Zn-deficient than in Zn-sufficient plants, but there were few significant differences between Zn rates. Sahara, Skiff, and Tantangara were the only genotypes that had significantly greater root:shoot dry weight ratio when grown under severe Zn deficiency, Zn_0, compared with no Zn deficiency, $Zn_{0.8}$ (Table 5.3).

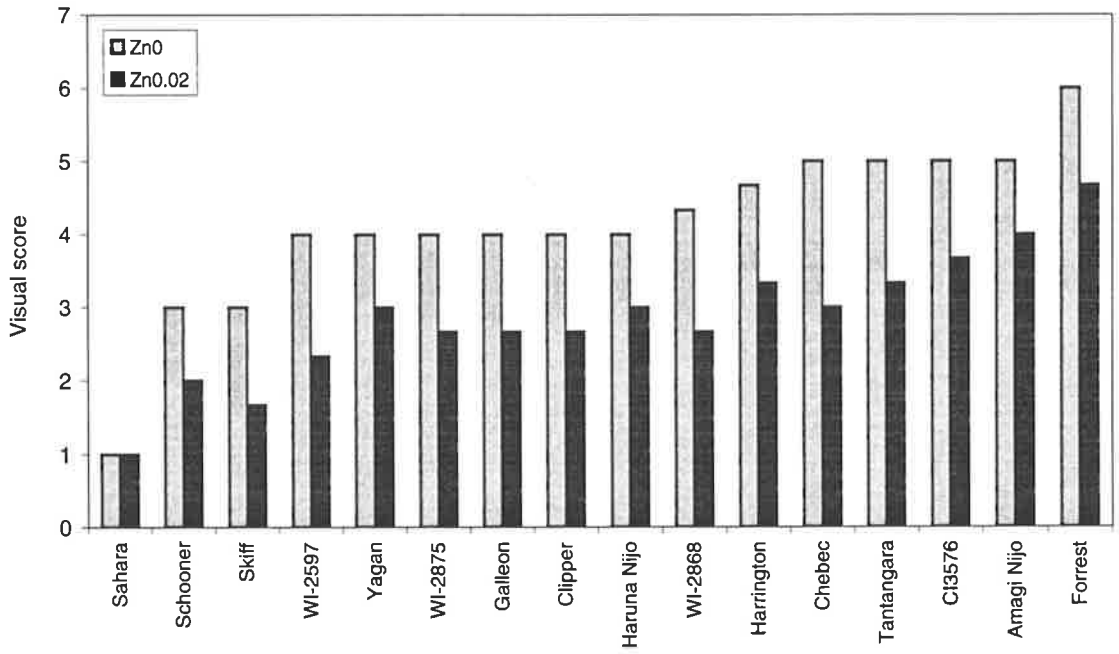


Figure 5.3. Ranking of barley genotypes based on Zn deficiency symptoms (visual scores) when fertilized with Zn_0 and $Zn_{0.02}$ at harvest (28 DAS) in Experiment 2. Plants were scored on a scale of 1 to 6 (see section 5.2.2 for details). The vertical bars represent Tukey's $HSD_{0.05}$ value for genotype effect.

Table 5.3. Shoot and root dry weight (g/plant) of barley genotypes grown at 3 levels of Zn (mg/kg soil) at 28 DAS in Experiment 2.

Genotype	Shoot dry wt. Zn fertilization			Root dry wt. Zn fertilization			Root:shoot ratio Zn fertilization		
	0	0.02	0.8	0	0.02	0.8	0	0.02	0.8
Amagi Nijo	0.07	0.14	0.41	0.06	0.10	0.32	0.81	0.74	0.78
Chebec	0.07	0.12	0.37	0.05	0.08	0.23	0.73	0.68	0.63
CI3576	0.05	0.11	0.36	0.05	0.07	0.23	0.96	0.58	0.65
Clipper	0.06	0.14	0.32	0.05	0.10	0.27	0.88	0.77	0.83
Forrest	0.06	0.11	0.39	0.04	0.07	0.29	0.75	0.64	0.76
Galleon	0.06	0.14	0.34	0.06	0.11	0.25	0.89	0.77	0.73
Harrington	0.08	0.13	0.38	0.08	0.12	0.27	1.00	0.89	0.71
Haruna Nijo	0.08	0.22	0.45	0.08	0.14	0.26	0.91	0.66	0.58
Sahara	0.11	0.18	0.34	0.10	0.13	0.2	0.99	0.75	0.58
Schooner	0.07	0.12	0.35	0.07	0.09	0.26	0.99	0.75	0.72
Skiff	0.08	0.18	0.33	0.09	0.14	0.24	1.17	0.75	0.71
Tantangara	0.07	0.16	0.38	0.07	0.12	0.24	1.00	0.78	0.63
WI-2597	0.06	0.14	0.34	0.06	0.11	0.25	1.04	0.76	0.72
WI-2868	0.08	0.16	0.37	0.08	0.12	0.27	0.92	0.76	0.72
WI-2875	0.07	0.15	0.30	0.06	0.11	0.26	0.87	0.73	0.85
Yagan	0.09	0.17	0.40	0.06	0.10	0.19	0.63	0.59	0.47
Tukey's $HSD_{0.05}$ G x Zn		0.07			0.07			0.36	

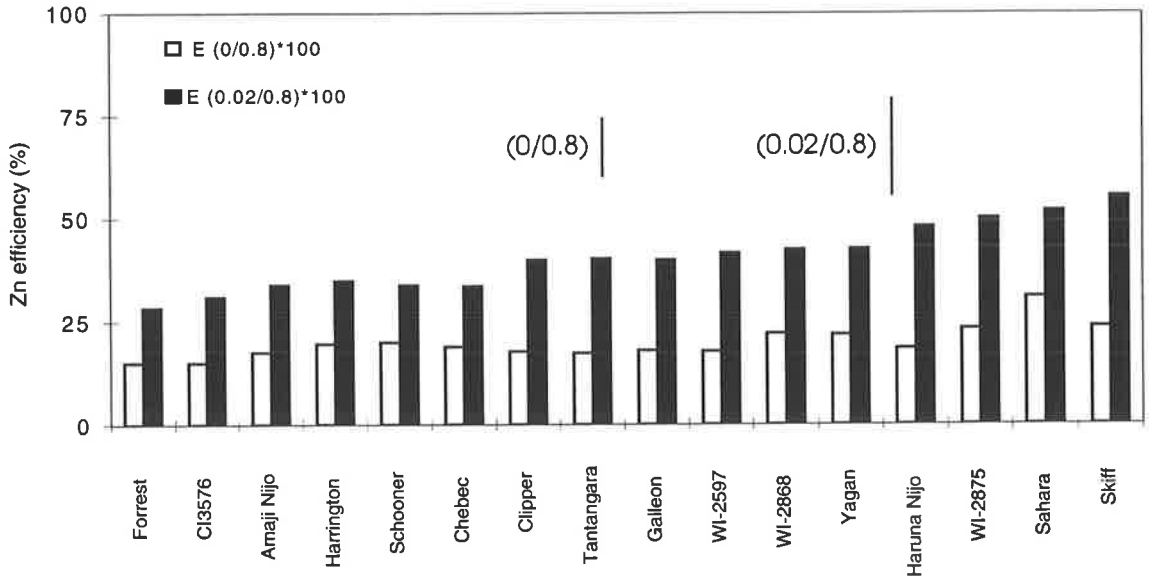


Figure 5.4. Zn efficiency (E) (ratio of shoot dry matter at 0 and 0.02 to 0.8 mg Zn/kg soil) of barley genotypes at 28 DAS in Experiment 2. Vertical bars represent Tukey's $HSD_{0.05}$ values for genotype effect for the two ratios.

5.3.2.4 Concentration and content of Zn in shoots

In all genotypes, concentrations of Zn were much higher in plants supplied with adequate Zn ($Zn_{0.8}$) than plants with inadequate Zn (Zn≤0.02 mg/kg soil) (Table 5.4). Zinc concentrations were similar among genotypes at each level of Zn except for Sahara at $Zn_{0.8}$ and Forrest at Zn_0, both of which had high Zn concentration at these levels. Zinc contents in shoots were also greater in plants supplied with adequate than inadequate Zn (Table 5.4). Under Zn deficiency, genotypes did not differ significantly in Zn content. With Zn fertilization of 0.02 mg/kg soil, some variation occurred; Haruna Nijo had a higher Zn content than most of the genotypes; WI-2875 and Sahara had higher Zn content than some other genotypes. When supplied with adequate Zn ($Zn_{0.8}$), Sahara had the greatest and Skiff the lowest Zn content in shoot.

Table 5.4. Zinc concentration (mg/kg D.W.) and content (µg/plant) in the shoots of barley genotypes grown at 3 levels of Zn (mg/kg soil) at 28 DAS in Experiment 2.

Genotype	Zn concentration			Zn content		
	Zn fertilization			Zn fertilization		
	0	0.02	0.8	0	0.02	0.8
Amaji Nijo	3.7 (1.30)[a]	3.9 (1.35)	36.9 (3.59)	0.27 (0.24)	0.52 (0.42)	15.07 (2.76)
Chebec	5.1 (1.60)	5.3 (1.64)	46.5 (3.82)	0.33 (0.29)	0.66 (0.50)	16.30 (2.85)
CI3576	4.6 (1.52)	5.5 (1.71)	43.6 (3.77)	0.25 (0.22)	0.62 (0.48)	15.59 (2.81)
Clipper	4.2 (1.44)	4.9 (1.60)	49.9 (3.90)	0.24 (0.22)	0.62 (0.48)	15.96 (2.83)
Yagan	4.4 (1.45)	5.3 (1.67)	36.1 (3.59)	0.37 (0.32)	0.91 (0.65)	14.45 (2.74)
Forrest	6.7 (1.91)	5.9 (1.77)	40.4 (3.68)	0.39 (0.33)	0.64 (0.50)	15.74 (2.79)
Galleon	3.3 (1.19)	5.1 (1.62)	41.2 (3.71)	0.21 (0.19)	0.69 (0.52)	14.10 (2.71)
Harrington	3.3 (1.17)	4.8 (1.57)	33.3 (3.50)	0.24 (0.22)	0.63 (0.49)	12.84 (2.59)
Haruna Nijo	4.9 (1.58)	6.3 (1.82)	35.8 (3.57)	0.41 (0.34)	1.35 (0.85)	16.22 (2.84)
Sahara	4.0 (1.38)	5.4 (1.70)	67.3 (4.21)	0.42 (0.35)	0.98 (0.68)	23.14 (3.18)
Schooner	3.8 (1.31)	4.7 (1.55)	46.3 (3.82)	0.27 (0.24)	0.55 (0.44)	16.31 (2.83)
Skiff	3.2 (1.18)	4.2 (1.44)	31.5 (3.43)	0.25 (0.23)	0.76 (0.57)	10.42 (2.42)
Tantangara	4.1 (1.41)	4.9 (1.58)	42.5 (3.75)	0.28 (0.24)	0.75 (0.56)	16.37 (2.85)
WI-2597	4.0 (1.39)	4.7 (1.54)	43.8 (3.77)	0.24 (0.22)	0.68 (0.51)	14.99 (2.77)
WI-2868	3.2 (1.17)	4.5 (1.50)	41.1 (3.70)	0.27 (0.24)	0.72 (0.54)	15.28 (2.79)
WI-2875	3.4 (1.20)	6.1 (1.81)	49.7 (3.90)	0.23 (0.21)	0.93 (0.66)	15.01 (2.77)
Tukey's $HSD_{0.05}$[b]						
G x Zn		(0.47)			(0.39)	

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

[b] The $HSD_{0.05}$ values are applicable to log-transformed data (in parentheses).

5.3.3. Experiment 3

5.3.3.1 Visual symptoms

At harvest, there were large differences in the severity of Zn deficiency symptoms among genotypes when they were grown without Zn fertilization (Figure 5.5; Plate 5.1b). Most of the genotypes developed mild symptoms, while some exhibited severe symptoms (e.g. Kinukei 15, Blenheim, Franklin, and Haruna Nijo) and some others no symptoms (e.g. Stein, Ellice, Mona and Kinukei 21). An application of 0.02 mg Zn/kg soil, decreased severity of deficiency symptoms (Figure 5.5). At Zn0.8, all the genotypes had a healthy appearance.

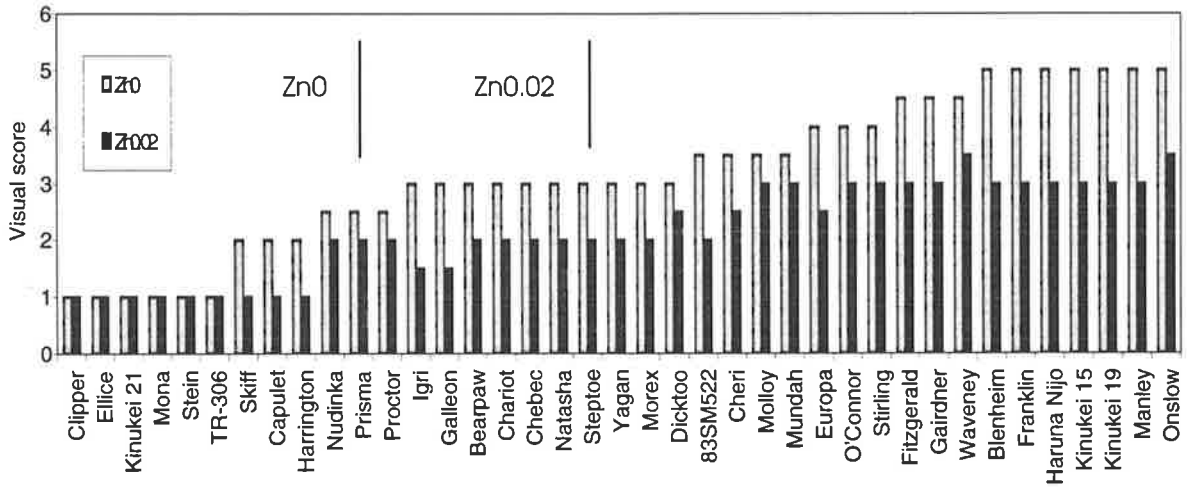


Figure 5.5. Ranking of barley genotypes based on Zn deficiency symptoms (visual scores) when fertilized with Zn_0 and $Zn_{0.02}$ at harvest (28 DAS) in Experiment 3. Plants were scored on a scale of 1to 6 (see section 5.2.2 for details). The vertical bars represent Tukey's $HSD_{0.05}$ value for genotype effect.

5.3.3.2. Shoot and root dry matter

Shoot dry matter production was reduced by Zn deficiency in all genotypes but the relative reduction was more marked in Kinukei 19, Waverney, Blenheim, Manley, Kinukei 15, Onslow, O'Connor and Franklin. In contrast, Kinukei 21, Stein, Mona and Ellice performed well under Zn deficiency (Table 5.5). Based on the $Zn_0/Zn_{0.8}$ ratio, Zn efficiency ranged from 16 % for Manley to 75 % for Stein (Figure 5.6). An application of 0.02 mg Zn/kg increased shoot dry matter and consequently, Zn efficiency: values of Zn efficiency based on the $Zn_{0.02}/Zn_{0.8}$ ratio ranged from 26% (Manley) to 91% (Kinukei 21), however, Zn efficiency ranking remained the same for almost all genotypes.

Root dry matter production was also significantly decreased by Zn deficiency in almost all genotypes, but significant reductions occurred only in Franklin, Kinukei 19, Manley and Onslow at Zn_0 as compared with $Zn_{0.8}$. Ellice, Kinukei 21, Mona, Stein and Steptoe had higher root dry matter production at Zn_0 than many other genotypes (Table 5.5), but not

significantly. Generally, under Zn deficiency, genotypes which produced higher shoot dry matter also produced greater root dry matter.

5.3.3.3. Root:shoot dry weight ratio

In general, the root:shoot ratio was higher under Zn-deficient than Zn-sufficient conditions. This ratio was greatest when plants were not fertilized with Zn, and it gradually decreased with increasing Zn fertilization: this ratio was significantly greater in Blenheim, Chebec, Cheri, Europa, Fitzgerald, Kinukei 15, Natasha, Proctor, Skiff and Waveney at Zn_0 as compared to $Zn_{0.8}$.

5.3.3.4. Concentration and content of Zn in shoots

The concentration of Zn in the shoot was significantly increased only when plants were supplied with sufficient Zn ($Zn_{0.8}$). Under Zn deficiency ($\leq Zn_{0.02}$), shoot concentrations of Zn were similar in almost all genotypes with the exception of Harrington, which had a value at Zn_0 much greater than the other genotypes. Based on its Zn concentration and content at Zn0.02, it was felt that this value of Harrington was erroneously high and therefore, it was excluded in the subsequent analysis. The reason for this high value remains unknown.

The total amount of Zn in shoots was also significantly enhanced by Zn fertilization (Table 5.6). Similar to Zn concentration, genotypes differed in their capacity to accumulate Zn only when grown in Zn-sufficient conditions: Kinukei 21 had the highest and Nudinka the lowest Zn accumulation in shoots (Table 5.6).

Table 5.5. Shoot and root dry weight, and root:shoot dry weight ratio of barley genotypes grown at three levels of Zn fertilization at 28 DAS in Experiment 3.

Genotype	Shoot dry wt. Zn fertilization			Root dry wt. Zn fertilization			Root:shoot ratio Zn fertilization		
	0	0.02	0.8	0	0.02	0.8	0	0.02	0.8
83SM522	0.13	0.18	0.37	0.10	0.11	0.22	0.80	0.61	0.60
Bearpaw	0.10	0.20	0.44	0.10	0.16	0.27	1.01	0.79	0.62
Blenheim	0.07	0.12	0.43	0.08	0.09	0.20	1.14	0.77	0.47
Capulet	0.22	0.24	0.38	0.18	0.17	0.25	0.82	0.70	0.66
Chariot	0.18	0.25	0.39	0.14	0.15	0.27	0.77	0.62	0.71
Chebec	0.09	0.13	0.41	0.11	0.10	0.22	1.28	0.78	0.54
Cheri	0.11	0.21	0.42	0.11	0.14	0.25	1.03	0.68	0.60
Clipper	0.30	0.32	0.40	0.19	0.19	0.27	0.62	0.59	0.68
Dicktoo	0.18	0.24	0.38	0.16	0.15	0.23	0.90	0.63	0.61
Ellice	0.29	0.35	0.41	0.21	0.20	0.27	0.75	0.59	0.65
Europa	0.08	0.18	0.45	0.08	0.10	0.22	1.01	0.55	0.50
Fitzgerald	0.14	0.22	0.42	0.15	0.15	0.26	1.09	0.69	0.61
Franklin	0.08	0.18	0.43	0.08	0.11	0.25	0.98	0.63	0.58
Gairdner	0.15	0.24	0.42	0.16	0.16	0.29	1.06	0.67	0.67
Galleon	0.13	0.23	0.41	0.14	0.17	0.28	1.04	0.77	0.69
Harrington	0.19	0.25	0.42	0.18	0.19	0.26	0.90	0.76	0.62
Haruna Nijo	0.14	0.22	0.49	0.11	0.13	0.24	0.79	0.60	0.49
Igri	0.16	0.23	0.38	0.12	0.11	0.15	0.77	0.46	0.39
Kinukei 15	0.08	0.17	0.43	0.07	0.10	0.23	0.98	0.60	0.53
Kinukei 19	0.08	0.24	0.54	0.07	0.12	0.26	0.84	0.50	0.49
Kinukei 21	0.41	0.50	0.55	0.22	0.26	0.30	0.58	0.53	0.55
Manley	0.08	0.11	0.44	0.07	0.10	0.27	0.82	0.87	0.62
Molloy	0.09	0.17	0.37	0.08	0.12	0.20	0.82	0.75	0.55
Mona	0.32	0.39	0.50	0.20	0.18	0.21	0.62	0.48	0.43
Morex	0.15	0.24	0.44	0.12	0.19	0.25	0.81	0.79	0.57
Mundah	0.12	0.19	0.42	0.09	0.10	0.20	0.72	0.50	0.48
Natasha	0.12	0.21	0.50	0.12	0.11	0.25	0.95	0.53	0.50
Nudinka	0.17	0.23	0.33	0.15	0.15	0.17	0.87	0.65	0.51
O'Connor	0.14	0.23	0.42	0.12	0.15	0.26	0.90	0.66	0.62
Onslow	0.09	0.18	0.43	0.08	0.12	0.27	0.97	0.71	0.62
Prisma	0.19	0.26	0.46	0.16	0.17	0.20	0.85	0.66	0.44
Proctor	0.16	0.27	0.37	0.16	0.18	0.17	1.02	0.66	0.48
Skiff	0.16	0.26	0.48	0.16	0.17	0.27	1.00	0.65	0.56
Stein	0.32	0.38	0.48	0.24	0.28	0.31	0.75	0.72	0.64
Steptoe	0.20	0.28	0.40	0.21	0.22	0.26	1.06	0.79	0.66
Stirling	0.11	0.21	0.39	0.10	0.13	0.25	0.89	0.63	0.65
TR-306	0.22	0.31	0.45	0.13	0.19	0.23	0.60	0.61	0.51
Waverney	0.07	0.20	0.42	0.08	0.10	0.20	1.07	0.54	0.48
Yagan	0.17	0.30	0.50	0.16	0.19	0.30	0.93	0.63	0.60
Tukey's $HSD_{0.05}$									
G x Zn		0.19			0.17			0.41	

Table 5.6. Zn concentration (mg/kg D.W.) and content (µg/plant) in the shoots of barley genotypes grown at three levels of Zn (mg/kg soil) at 28 DAS in Experiment 3.

Genotype	Zn concentration Zn fertilization			Zn content Zn fertilization		
	0.0	0.02	0.8	0	0.02	0.8
83SM522	4.8 (1.57)[a]	6.6 (1.89)	52.8 (3.97)	0.6 (0.48)	1.2 (0.79)	19.6 (3.02)
Bearpaw	4.0 (1.37)	5.5 (1.71)	31.7 (3.45)	0.4 (0.33)	1.1 (0.74)	14.0 (2.71)
Blenheim	5.9 (1.77)	3.6 (1.21)	36.3 (3.59)	0.4 (0.35)	0.4 (0.35)	15.7 (2.82)
Capulet	5.8 (1.75)	7.0 (1.94)	36.5 (3.59)	1.2 (0.80)	1.7 (0.98)	13.7 (2.69)
Chariot	6.9 (1.93)	6.5 (1.88)	37.3 (3.62)	1.2 (0.80)	1.6 (0.96)	14.7 (2.75)
Chebec	4.5 (1.50)	4.9 (1.57)	40.9 (3.71)	0.4 (0.33)	0.7 (0.51)	16.6 (2.87)
Cheri	3.9 (1.37)	4.8 (1.57)	32.2 (3.47)	0.4 (0.34)	1.0 (0.69)	13.7 (2.68)
Clipper	5.8 (1.76)	7.5 (2.00)	46.3 (3.83)	1.8 (1.01)	2.4 (1.23)	18.4 (2.96)
Dicktoo	6.4 (1.85)	5.6 (1.71)	35.5 (3.57)	1.2 (0.77)	1.3 (0.84)	13.4 (2.67)
Ellice	5.5 (1.71)	6.7 (1.91)	31.1 (3.44)	1.6 (0.94)	2.3 (1.20)	12.8 (2.62)
Europa	3.8 (1.23)	5.9 (1.77)	38.9 (3.66)	0.3 (0.26)	1.1 (0.72)	17.6 (2.92)
Fitzgerald	4.4 (1.48)	6.4 (1.86)	36.5 (3.60)	0.6 (0.46)	1.4 (0.89)	15.4 (2.80)
Franklin	3.7 (1.32)	5.8 (1.76)	53.4 (3.98)	0.3 (0.27)	1.0 (0.71)	22.8 (3.17)
Gairdner	4.6 (1.52)	5.9 (1.78)	42.5 (3.78)	0.7 (0.52)	1.4 (0.89)	18.1 (2.94)
Galleon	4.2 (1.41)	6.8 (1.91)	46.6 (3.84)	0.6 (0.44)	1.5 (0.93)	18.9 (2.99)
Harrington	11.4 (2.43)	5.8 (1.76)	31.7 (3.46)	2.2 (1.16)	1.5 (0.94)	13.2 (2.65)
Haruna Nijo	7.0 (1.94)	6.0 (1.79)	43.6 (3.77)	1.0 (0.67)	1.3 (0.85)	21.2 (3.10)
Igri	3.9 (1.35)	6.0 (1.78)	30.9 (3.43)	0.6 (0.47)	1.4 (0.86)	11.6 (2.53)
Kinukei 15	5.3 (1.67)	6.9 (1.93)	42.2 (3.74)	0.4 (0.34)	1.2 (0.77)	18.3 (2.96)
Kinukei 19	6.5 (1.87)	5.8 (1.75)	39.4 (3.68)	0.6 (0.44)	1.4 (0.88)	21.1 (3.10)
Kinukei 21	6.6 (1.87)	6.4 (1.86)	44.3 (3.79)	2.7 (1.29)	3.2 (1.43)	24.4 (3.23)
Manley	4.4 (1.47)	5.6 (1.70)	39.7 (3.68)	0.4 (0.30)	0.6 (0.48)	17.3 (2.90)
Molloy	4.2 (1.44)	6.9 (1.92)	37.2 (3.62)	0.4 (0.33)	1.1 (0.76)	13.7 (2.68)
Mona	4.1 (1.40)	6.0 (1.80)	38.5 (3.64)	1.3 (0.83)	2.3 (1.20)	19.2 (3.00)
Morex	6.1 (1.81)	6.0 (1.77)	31.3 (3.44)	0.9 (0.65)	1.4 (0.88)	14.0 (2.69)
Mundah	3.2 (1.16)	5.6 (1.72)	43.0 (3.76)	0.4 (0.31)	1.1 (0.73)	18.1 (2.95)
Natasha	4.1 (1.40)	5.2 (1.64)	39.8 (3.68)	0.5 (0.41)	1.1 (0.74)	19.9 (3.03)
Nudinka	5.9 (1.77)	6.0 (1.78)	31.2 (3.44)	1.0 (0.70)	1.4 (0.87)	10.1 (2.41)
O'Connor	4.4 (1.47)	6.8 (1.91)	41.5 (3.72)	0.6 (0.47)	1.5 (0.93)	17.5 (2.92)
Onslow	4.5 (1.50)	5.7 (1.74)	32.4 (3.47)	0.4 (0.33)	1.0 (0.70)	13.9 (2.69)
Prisma	5.1 (1.62)	5.9 (1.78)	31.4 (3.45)	0.9 (0.66)	1.5 (0.93)	14.4 (2.73)
Proctor	7.4 (1.95)	6.3 (1.84)	34.2 (3.53)	1.2 (0.75)	1.7 (0.99)	12.5 (2.60)
Skiff	6.1 (1.78)	5.7 (1.74)	39.8 (3.68)	1.0 (0.67)	1.5 (0.91)	18.7 (2.99)
Stein	5.3 (1.66)	6.6 (1.87)	36.7 (3.60)	1.7 (0.79)	2.6 (1.05)	17.7 (3.10)
Steptoe	5.4 (1.69)	5.9 (1.77)	43.3 (3.77)	1.1 (0.72)	1.6 (0.97)	17.4 (2.91)
Stirling	4.0 (1.38)	5.8 (1.76)	32.6 (3.48)	0.4 (0.36)	1.2 (0.78)	12.6 (2.61)
TR-306	5.5 (1.70)	5.9 (1.78)	47.9 (3.87)	1.2 (1.24)	1.9 (1.55)	21.5 (3.77)
Waveney	3.9 (1.36)	6.1 (1.80)	31.0 (3.43)	0.3 (0.25)	1.2 (0.78)	12.9 (2.63)
Yagan	5.3 (1.63)	4.9 (1.58)	33.2 (3.50)	0.9 (0.65)	1.5 (0.90)	16.7 (2.87)
Tukey's $HSD_{0.05}$ [b]						
Genotype x Zn fertilization	(0.65)			(0.43)		

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

[b] The $HSD_{0.05}$ values are applicable to log-transformed data (in parentheses).

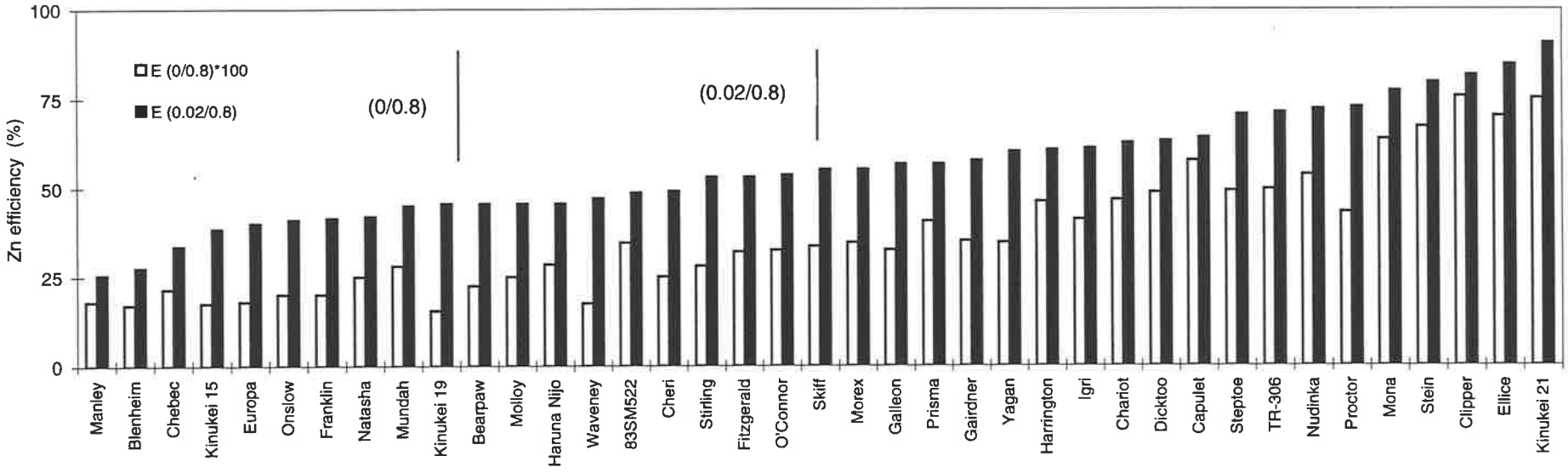


Figure 5.6. Zn efficiency (E) (ratio of shoot dry matter at 0 and 0.02 to 0.8 mg/Zn kg soil) of barley genotypes in Experiment 3. Vertical bars represent Tukey's $HSD_{0.05}$ values for genotype effect for the two ratios

5.4 Discussion

The rapid development of severe Zn deficiency symptoms and large responses to Zn fertilization in all three experiments demonstrated that Lancelin sand generated severe Zn deficiency stress, and therefore, it could be used to screen for tolerance to Zn deficiency in barley. At Zn_0, Tantangara and Amagi Nijo, tested in Experiments 1 and 2, developed severe symptoms of Zn deficiency and had a low relative shoot growth (24% for both genotypes). Similarly, the existence of genotypes developing mild to severe Zn deficiency symptoms and having a low relative shoot growth in Experiments 2 and 3 (e.g. 14-32% in Experiment 2) when grown at Zn_0 indicated severe Zn deficiency stress in Lancelin sand. The majority of genotypes in Experiment 2 were also tested in Laffer sand (Experiment 2, Chapter 4), but did not develop any visible symptoms of Zn deficiency and achieved a greater relative shoot growth (63-88%). This indicates Lancelin sand generated a greater Zn deficiency stress than the Laffer sand used in Experiment 2 in Chapter 4. However, the experiments with Laffer sand and Lancelin sand were conducted under different experimental conditions, and a further study under identical experimental conditions is needed before direct comparisons of Zn responses between the two soils can be made.

The results demonstrated that genotypes differed considerably in appearance and severity of Zn deficiency symptoms. In Experiment 2, based on severity of deficiency symptoms, Forrest was the most sensitive, Schooner, Skiff and WI-2875 were moderately tolerant and Sahara the most tolerant to Zn deficiency. The tolerance of Sahara to low soil Zn in this experiment may be associated with its higher Zn content in seed (Table 5.1). This result is in accordance with the results obtained in Chapter 3 where Zn deficiency symptoms were absent or less evident in barley plants grown from seed with high Zn content in Zn-deficient conditions. However, there was little variation in seed Zn content among other genotypes

(Table 5.1), and therefore, the differences observed among these genotypes are likely to be inherent and not confounded markedly by the variation in seed Zn content.

In contrast, in Experiment 3, a greater proportion of the differences in visual symptoms were attributable to the larger variation in seed Zn content. For instance, Kinukei 21 had a 10-fold higher seed Zn content than Kinukei 15 and, it is possible that a seed Zn content of this magnitude may have contributed to the absence of symptoms in this genotype over Kinukei 15. When genotypes having similar Zn contents in Experiment 2 and different Zn contents in Experiment 3 were compared (Harrington, Clipper, Galleon and Yagan; Table 5.7), it was found that the higher seed Zn content in Experiment 3 decreased the severity of deficiency symptoms markedly and consequently altered the ranking based on deficiency symptoms.

Table 5.7. The effect of seed Zn content (µg/seed D.W.) and Zn fertilization (mg/kg soil) on expression of deficiency symptoms (visual scores) and Zn efficiency (0/0.08 and 0.02/0.8) in selected genotypes in Experiments 2 and 3.

	Seed Zn	Zn efficiency		Visual score	
		(0/0.8)	(0.02/0.8)	0	0.02
Experiment 2					
Chebec	0.59	19	34	5.0	3.0
Harington	0.62	20	35	4.7	3.3
Clipper	0.57	18	40	4.0	2.7
Galleon	0.63	18	40	4.0	2.7
Yagan	0.55	22	43	4.0	3.0
Experiment 3					
Chebec	1.04	22	34	3.0	2.0
Harington	2.36	46	61	2.0	1.0
Clipper	5.23	76	82	1.0	1.0
Galleon	1.27	33	57	3.0	1.5
Yagan	1.68	35	60	3.0	2.0

These and earlier results in Chapter 3 indicated that genotypes in Experiment 3 could not be properly assessed and classified for their sensitivity to Zn deficiency due to considerable variation in seed Zn content among these genotypes. Nevertheless, comparisons can be made within groups of genotypes with similar Zn content. For example, O'Connor, Mona and Capulet had similar seed Zn contents (~3.3 μg Zn/seed D.W.; Table 5.2), but O'Connor developed more severe symptoms than Mona and Capulet; therefore, O'Connor can be classified as more sensitive to Zn deficiency than Mona and Capulet. Similarly, Haruna Nijo, 83SM522 (~1.2 μg Zn/seed D.W.), Franklin, Chebec (~1.0 μg Zn/seed D.W.) and Manley, Natasha (~0.8 μg Zn/seed D.W.) had similar seed Zn contents, but the severity of deficiency symptoms was in the order Haruna Nijo > 83SM522, Franklin > Chebec and Manley > Natasha) (Figure 5.5).

Zn efficiency was also influenced by seed Zn content (Table 5.7). At both levels of Zn deficiency stress ($Zn_0/Zn_{0.8}$, $Zn_{0.02}/Zn_{0.8}$), the increase in Zn efficiency was greater in Clipper than Chebec, Harrington, Galleon, and Yagan in Experiment 3 than in Experiment 2, which could be explained by the greater Zn content in seed of Clipper than the other genotypes in Experiment 3 (Table 5.7). As with deficiency symptoms, it is difficult to determine the extent of genotypic variation in response to Zn deficiency in Experiment 3 independent of seed Zn content. However, genotypes with similar Zn content can be compared to examine genotypic variation independent of seed Zn content. For instance, at a similar Zn content, O'Connor was 50% less efficient than Mona and Ellice. O'Connor also had greater Zn in seed but was less Zn efficient than Steptoe, Nudinka and Proctor. Therefore, the much of the observed differences in Zn efficiency among these genotypes may result from inherent genotypic variation, independent of seed Zn content.

The differences in seed Zn content among genotypes used in these experiments can be attributed to the growing conditions of the parent plants: plants grown in a normal potting mix under glasshouse conditions accumulated considerably more Zn in the seed than those grown in the field. In the present study, Clipper had a much higher grain Zn concentration when grown in a normal potting mix (~86 mg Zn/kg seed D.W. in Experiment 3) than when grown in the field (~12 mg Zn/kg seed D.W. in Experiment 2). Similarly, Kinukei 21 and Kinukei 19 had a 6-fold higher Zn concentration in the seed when grown in a normal potting mix than when grown in the field. A survey of nutrient levels in barley grain grown in South Australia and north-western Victoria in the 1983/1993 period, reported a range of 13-22 mg Zn/kg seed D.W. (Reuter, 1995). A similar concentration, 15 mg Zn/kg seed D.W., was reported in another study of nutrient levels in barley grain in the 1983/84 season in South Australia and the 1989/90 season in South Australia and north-western Victoria (Spouncer *et al.*, 1990). The lower levels of Zn content in field grown barley grain is further supported by a recent survey with 33 genotypes grown in 1997/98 season in South Australia. This study found that Zn concentration in barley grain ranged from 12 to 21 mg Zn/kg seed D.W. when fertilized with Zn, and from 10 to 18 mg Zn/kg seed when fertilized with no Zn (R. D. Graham and J. Lewis, pers. commun.). Based on these field surveys, a range of 10-20 mg Zn/kg seed D.W. can be considered as the average range, which would mean a range of 0.45-0.90 µg/seed in seed Zn content, if average grain weight for barley is considered to be 0.45 mg/seed D.W. From the earlier results in Chapter 3 (Figure 5.6.), the effect of such a range of seed Zn content on Zn efficiency may be relatively small (e.g. 10% increase in Zn efficiency as seed Zn content increases from 0.45 to 0.90 µg) and therefore grain within this range could be used without much concern that seed Zn content would affect Zn efficiency ranking considerably.

The fact that Zn concentration, and consequently Zn content, of barley can differ remarkably depending on growing conditions suggests that barley genotypes obtained from different sources may not be properly compared for Zn efficiency if they differ substantially in seed weight or Zn concentration in the seed. This has implications for breeding programmes aimed at selecting genotypes with higher Zn efficiency. In order to minimize variation in the seed Zn content, parent plants should be grown under identical conditions such as field or in glasshouse but at a low level of Zn. Seed of comparable Zn content can be obtained by growing parent plants in a Zn-deficient soil fertilized with a number of Zn levels, and then selecting similar range of seed Zn concentration. However, when a large number of genotypes are considered, the task becomes very difficult (if not practically impossible) to accomplish. Therefore, research is needed to develop a screening technique independent of seed Zn.

Like this study, a number of workers examined response of barley to Zn deficiency under field and glasshouse conditions, however, the number of genotypes tested has been small. In a field study with six barley genotypes, Takkar *et al.* (1983) reported that all the genotypes were tolerant to Zn deficiency. In contrast, Pathak *et al.* (1978) in glasshouse (ten genotypes) and Yilmaz *et al.* (1996) in field studies (ten genotypes), demonstrated that barley genotypes differed considerably in response to Zn deficiency. The differential responses reported in the literature may be due to inherent differences among barley genotypes or to differences in experimental conditions. Unlike, the present study, none of these studies reported the seed Zn content, but it has been reported that higher seed Zn content improves both vegetative growth and grain yield of wheat (Rengel and Graham, 1995a,b). A similar result has been found for vegetative growth in barley (Chapter 3) and it

is likely that the seed Zn effect persists to grain yield in barley. Therefore, it is possible that the results from previous studies could have been confounded by seed Zn levels.

The large genetic differences in visual deficiency symptoms at similar Zn concentrations in the shoot, supported the earlier results in Chapter 4 that Zn concentration is not a good parameter for assessing genotypic variation in Zn efficiency. In this study, under Zn deficiency, (Zn_0) in Experiment 3, there was a poor relationship between visual symptoms and Zn concentration in the shoot (r=0.20, n.s.). When grown with no Zn fertilization, Blenheim and Kinukei 15 developed severe symptoms of deficiency, whereas Stein and Ellice did not show any visual symptoms, despite all four genotypes having similar shoot Zn concentration. This lack of correlation between visual symptoms and Zn concentration in the shoot was even more apparent in plants fertilized with $Zn_{0.02}$: compared with Zn_0, severity of deficiency symptoms reduced considerably, while Zn concentrations in the shoot did not change much (Table 5.6; Figure 5.6). In contrast to Zn concentration, Zn content correlated better with severity of Zn deficiency symptoms (r=0.73, $P<0.01$): genotypes developing severe symptoms had much lower Zn content (Blenheim, Kinukei 15) than those showing no symptoms (Stein and Ellice).

Root:shoot dry weight ratio was examined as a selection parameter, but it does not appear to be useful because it did not separate the Zn-efficient from the Zn-inefficient genotypes. In this study, the ratio did not differ with sensitivity of genotypes (Table 5.5; sensitive: Blenheim, Franklin, Haruna Nijo: tolerant: Stein Ellice and Mona) and in some cases it was greater in sensitive (Blenheim) than tolerant genotypes (Kinukei 21) (Table 5.5).

5.5 Conclusion

The results from all three experiments consistently showed that Lancelin sand generated severe Zn deficiency stress, suggesting that it can be used as a growth medium for screening for tolerance to Zn deficiency. The results also demonstrated that there was considerable variation in response to Zn deficiency in barley. However, the present results were confounded markedly by seed Zn content. Therefore, a further study using seed with similar Zn contents is needed to assess properly genotypic variation in tolerance to Zn deficiency. The results suggested that visual symptoms and Zn content are better parameters for assessing genotypic variation than Zn concentration and root:shoot dry weight ratio. The results also highlighted the difficulty of achieving similar seed Zn content for a large number of genotypes; therefore a selection method independent of seed Zn content (e.g. development of biochemical/molecular marker) is needed. However, given the difficulties of selection for Zn efficiency in the field, the current method seems to be useful to further our knowledge of mechanisms of Zn efficiency provided that precautions related to seed Zn content and soil type are taken. A reliable screening technique may then be used to develop biochemical/molecular markers that may facilitate screening process and speed up breeding for Zn efficiency in the near future.

CHAPTER 6

A comparison of differential expression of zinc deficiency stress and plant growth of barley genotypes in two soils, Lancelin sand and Laffer sand, used in screening studies for tolerance to zinc deficiency

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6.1 Introduction

Field trials have been used extensively in Australia and elsewhere to examine the growth and yield responses of cereals to application of zinc fertilizer. However, an inherent problem with field experiments is the large seasonal variation in yield and variability in zinc responses. Soil-based systems, either in a glasshouse or a growth room have been preferred because of the greater degree of control they give and increased reliability in the results. In trace element work in Australia, two soils, Laffer sand and Lancelin sand, have been used a great deal in studies of zinc responses (Chapters 4 and 5).

Laffer sand has been used by the Waite Plant Nutrition Group for many years and has been used to screen for Zn efficiency in a range of crops, such as bread and durum wheat (Graham *et al.*, 1992), rapeseed (Grewal *et al.*, 1997), chickpea (Khan *et al.*, 1998a,b), and annual medics (Streeter, 1998). Laffer sand was used also in the first experiments of the current study (Chapters 3 and 4) and although zinc responses were obtained, it was also found that there was considerable variation in the responsiveness between different batches of soil. This variation was quite unexpected and it was attributed to differences in extractable zinc (DTPA) between different batches of soil (Chapter 4).

Lancelin sand has also been used by researchers in Western Australia in studies on zinc deficiency for many crop species such as wheat (Robson and Snowball, 1989; Burker

and Robson, 1994), subterranean clover (Reuter *et al.*, 1982), and canola (Huang *et al.*, 1995), and it has also been shown to give good responses to zinc in barley (Chapter 5). It also appeared from the experiments in Chapter 5, that severity of zinc deficiency in Lancelin sand may be greater than that in Laffer sand; however, there has not been a direct comparison of the two soil types. Therefore experiments were conducted to examine the zinc responses in these soils, which historically have been the basis of much of the zinc work in South Australia and Western Australia.

6.2. Experiment 1

Previous experiments provided circumstantial evidence that severity of Zn deficiency in Lancelin sand is greater than that in Laffer sand (Chapters 4 and 5). This experiment was conducted to verify this observation. Different combinations of the two soils were used to determine an ideal growth medium for screening for tolerance to Zn deficiency (e.g. a reasonable level of Zn deficiency stress which allows a good separation of genotypes).

6.2.1 Materials and methods

Two Zn-deficient soils, Lancelin and Laffer sands (for soil properties see Table 6.1; for detailed soil collection and preparation see Chapters 4 and 5), and their various mixtures were used in this experiment. The soil treatments used were, 100% Lancelin sand; 75% Lancelin, 25% Laffer; 50% Lancelin, 50% Laffer; 25 % Lancelin, 75% Laffer, 100% Laffer which were designated **a**, **b**, **c**, **d**, and **e** respectively. The Laffer sand used in this experiment came from a different batch than that used in experiments in Chapter 4, and had a DTPA-extractable Zn of 0.14 mg Zn/kg soil (Table 6.1), while Lancelin sand came from the same batch as that used in experiments in Chapter 5.

Table 6.1. Properties of Laffer sand and Lancelin sand used in Experiment 1 and Experiment 2.

Properties	Laffer sand	Lancelin sand
pH (water)	6.7	6.3
Organic carbon (%)	0.07	0.63
Nitrate-nitrogen (mg/kg)	1	2
Phosphorus (mg/kg)	4	3
Potassium (mg/kg)	25	11
DTPA-extractable (mg/kg)		
Zn	0.14	0.12
Mn	0.2	0.67
Fe	7	54
Cu	0.08	0.05

One kg of each soil or their mixtures was placed into cardboard cartons (7 x 7 x 17.7 cm) of 600 ml volume following the addition of calcium carbonate powder (0.5 % w/w). Basal nutrients (in mg/kg dry soil) of NH_4NO_3, 350; K_2SO_4, 120; K_2HPO_4, 150; $MgSO_4.7H_2O$, 90; $CuSO_4.5H_2O$, 10; $MnSO_4.4H_2O$, 7; H_3BO_3, 1; $CoSO_4.7H_2O$, 1; $FeSO_4.7H_2O$, 0.15, together with zinc treatments (0, 0.02 and 0.8 mg/kg soil) were applied in solution to the surface of the soil, allowed to dry and mixed again throughout the soil.

Four pre-germinated seeds of barley genotypes, Forrest, Galleon and Skiff, with similar Zn content (0.65 ± 0.02, 0.63 ± 0.02, and 0.68 ± 0.01 µg/seed, respectively) were sown in each pot and thinned to two plants after emergence. These genotypes were chosen because they represented the range of Zn efficiencies observed in Experiment 2 in Chapter 5 (Forrest, low; Galleon, intermediate; Skiff, high).

Pots were randomized and watered daily to keep the soil water content at 12% (w/w). Plants were grown in a growth room at 20/15 °C day/night temperature, with a 14/10 h

light/dark period and 300 µmol m^2/s light intensity at plant height. Plants were visually scored on a scale of 1 to 6 (see 5.2.2).

Plants were harvested 26 DAS, at tillering (FS 5.0, Large, 1954). At harvest, soil was washed from the roots under running tap water. Both roots and shoots were then rinsed in deionized water followed by dipping into DD water, separated, dried at 65 °C for 48 h, and the dry weights were recorded. Dried shoot samples were digested in nitric acid and analyzed for elemental composition by ICP as described previously (Chapter 3).

The experiment was set up as a factorial design (3 genotypes x 3 Zn levels x 5 soil treatments) with two replicates. Results were analyzed by using the GENSTAT statistical package (GENSTAT, 1988). Tukey's Honestly Significant Differences (HSD) at α=0.05 was employed to examine pairwise comparisons (Steel and Torrie, 1960).

6.2.2 Results

6.2.2.1 Visual symptoms

At 12 DAS, typical Zn deficiency symptoms (linear chlorotic areas in the upper half of the leaf) developed on the middle leaves of Forrest and Galleon plants grown with no applied Zn in soils **a** and **b**. At this stage, symptoms were not visible in Skiff plants. At 18 DAS, when no Zn was applied, both Forrest and Galleon developed severe symptoms when grown in soils **a** and **b** but mild symptoms when grown in soils **c**, **d**, and **e**. At 0.02 mg Zn/kg soil, these genotypes developed less severe symptoms in soils **a** and **b** and no symptoms in soils **c**, **d**, and **e** as compared to no applied Zn. Symptoms in Skiff became evident only at 24 DAS when grown in **a** with no applied Zn.

At harvest, 26 DAS, plants of Forrest and Galleon exhibited very severe Zn deficiency symptoms when grown with no applied Zn in soil **a** while symptoms were mild when they were grown in soil **e** (Figure 6.1; Plate 6.1, only Forrest is shown). Skiff exhibited mild symptoms only when grown in soils **a**, **b** and **c** at Zn≤0.02 mg Zn/kg soil. Overall, the severity of Zn deficiency symptoms decreased as the proportion of Laffer sand increased (Figure 6.1; Plate 6.2).

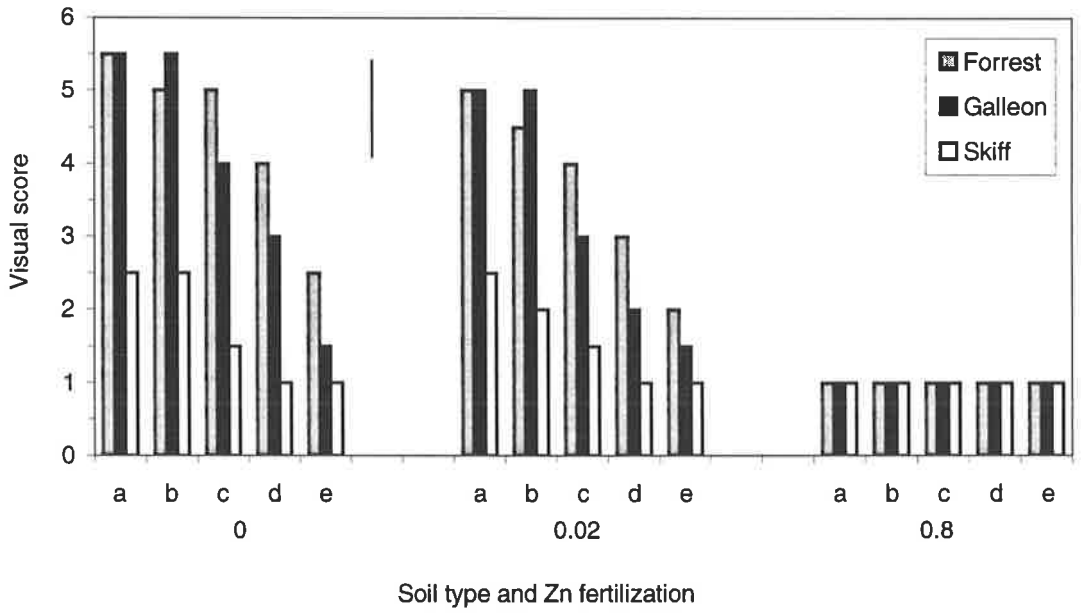


Figure 6.1. Effects of soil type (a,b,c,d,and e) and Zn fertilization (mg/kg soil) on expression of Zn deficiency symptoms of barley genotypes at D26 (26 days after sowing). Symptoms were scored on a scale of 1 to 6 (1=no symptoms; 6= very severe symptoms) (a=100% Lancelin; b=75% Lancelin, 25% Laffer; c=50% Lancelin, 50% Laffer; d=25% Lancelin, 75% Laffer; e=100% Laffer). The vertical bar represents Tukey's $HSD_{0.05}$ value for interaction Genotype x Zn fertilization x Soil type.

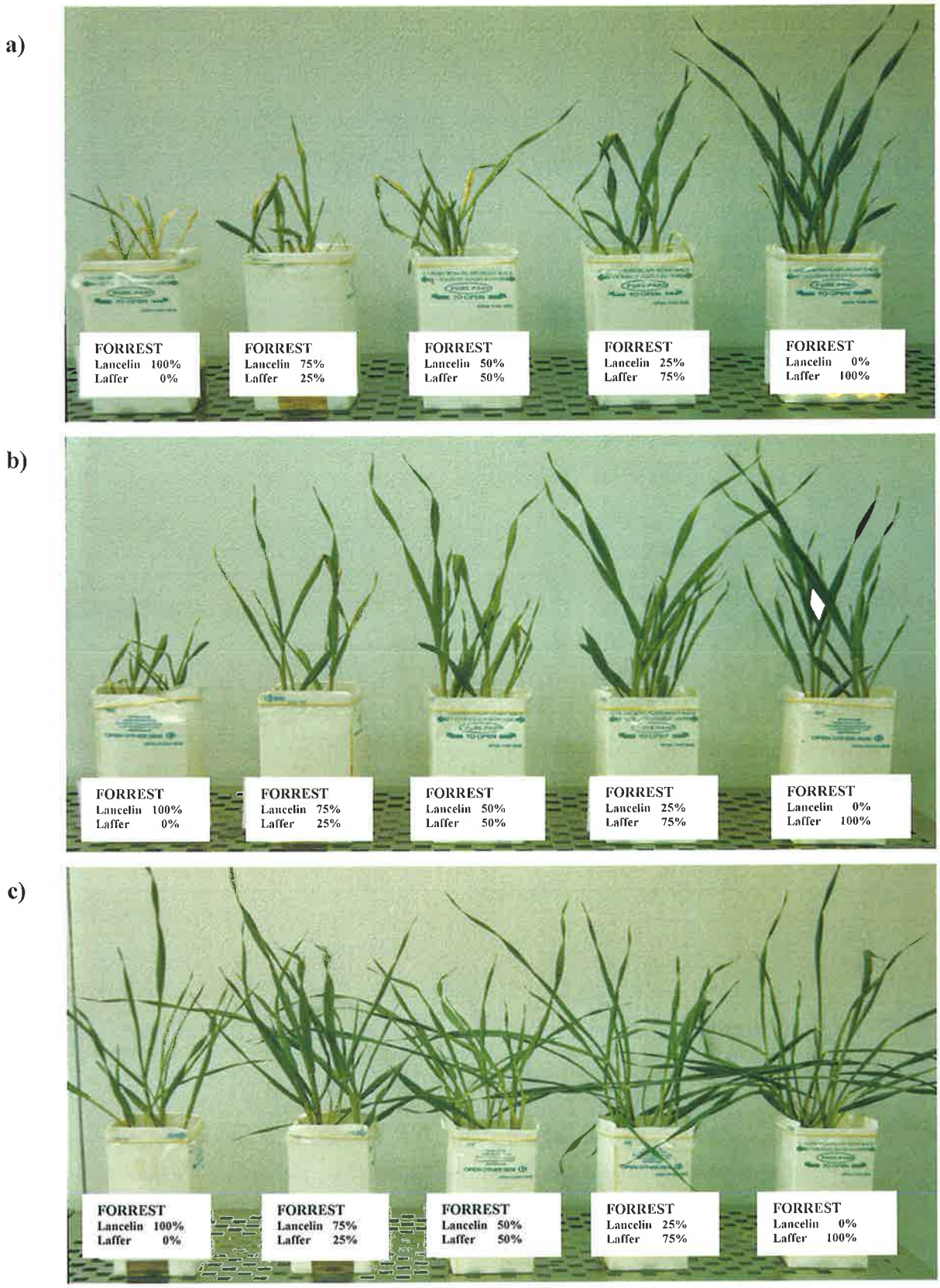


Plate 6.1. Effects of soil type and Zn fertilization (a, b, c; 0, 0.02, 0.8 mg Zn/kg soil, respectively) on expression of Zn deficiency symptoms in barley genotype Forrest at D26.

Plate 6.2. Effects of soil type and Zn fertilization (a, b, c; 0,0.02, 0.8 mg Zn/kg soil, respectively) on expression of Zn deficiency symptoms in barley genotype Skiff at D26.

6.2.2.1 Shoot and root dry matter

The trend for root and shoot dry matter yield was similar to that for visual symptoms: shoot and root dry matter yields increased as the proportion of Laffer sand increased at all Zn levels (Figure 6.2), but the response differed with genotype. Galleon showed a larger response in shoot and root dry matter than Skiff and Forrest when the proportion of Laffer sand with no applied Zn was increased. The greater dry matter yields of plants grown in Laffer sand compared to those of Lancelin sand grown plants even when Zn supply was adequate (0.8 mg/kg) implied that plant growth in Lancelin sand was limited by a factor other than Zn.

6.2.2.3 Concentration and content of Zn in shoots

Zn fertilization increased significantly both the concentration and content of Zn in shoots but only at 0.8 mg Zn/kg soil. At this level, plants grown in the two soils and their various mixtures achieved Zn concentrations of between 28 and 53 mg/kg D.W. (Table 6.2), above the critical level (20.0 mg/kg D.W., Chapter 4) which indicated that a Zn fertilization of 0.8 mg/kg soil was adequate for normal growth under the conditions of the current experiment. Of the genotypes, Galleon had a higher Zn concentration than Forrest when grown in soil **c** with adequate Zn fertilization (0.8 mg/kg). Zn content showed a similar trend to that of Zn concentration: shoot Zn content increased significantly with increasing proportion of Laffer sand at 0.8 mg Zn/kg (Table 6.3).

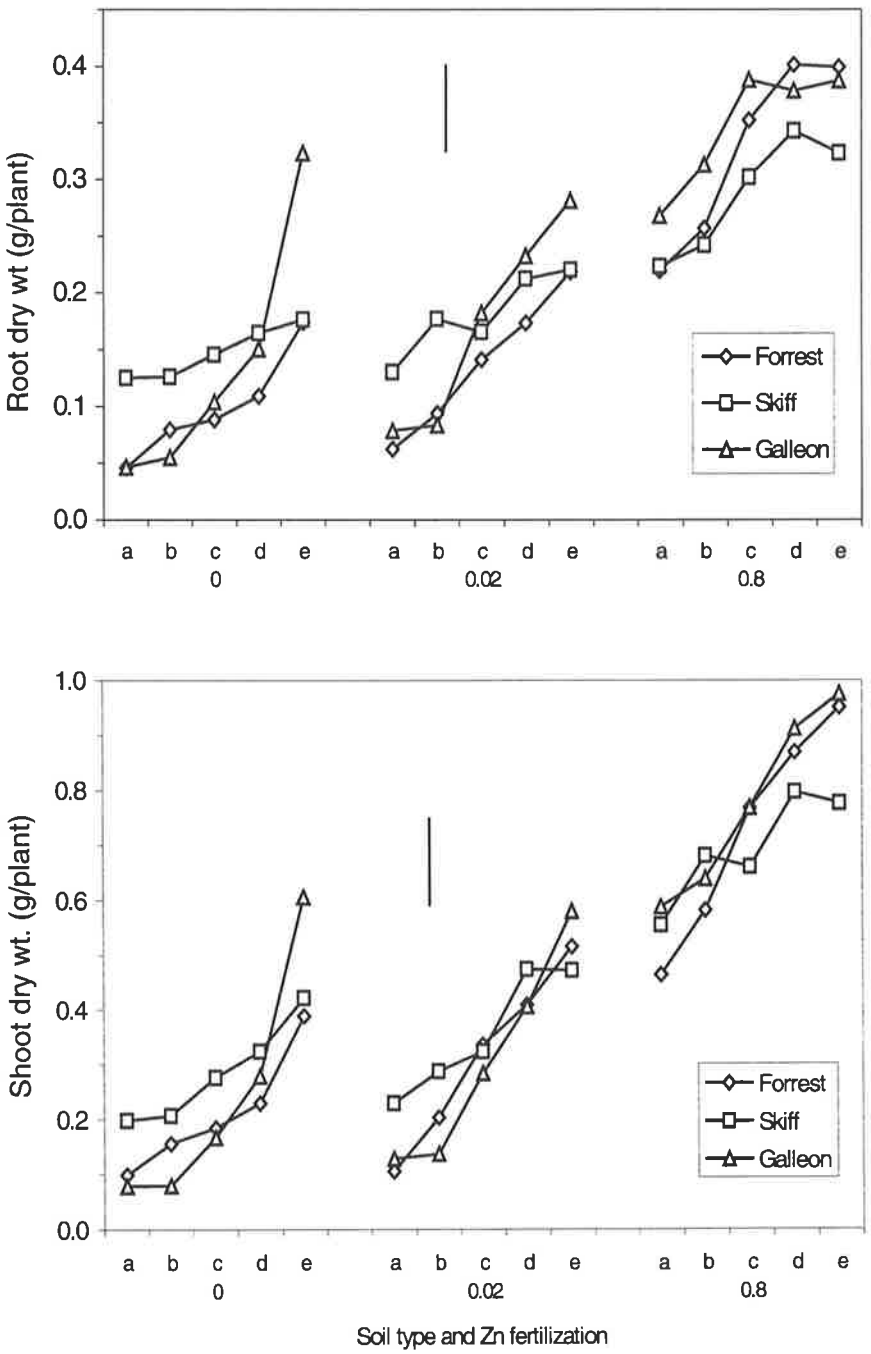


Figure 6.2. Effects of soil type (a, b, c, d, and e) and Zn fertilization (mg/kg soil) on shoot and root dry weight of barley genotypes (a=100% Lancelin, b=75% Lancelin, 25% Laffer; c=50% Lancelin, 50% Laffer; d=25% Lancelin,75% Laffer; e=100% Laffer). Vertical bars represent Tukey's $HSD_{0.05}$ values for interaction Genotype x Zn fertilization x Soil type.

Table 6.2. Effects of soil type and Zn fertilization (mg/kg D.W.) on shoot Zn concentration (mg/kg D.W.) in barley genotypes at 26 DAS in Experiment 1.

		Soil type[A]				
Zn fert.	Genotype	a	b	c	d	e
0	Forrest	5.3 (1.67)[B]	4.8 (1.55)	5.4 (1.69)	6.0 (1.78)	6.4 (1.86)
	Skiff	4.1 (1.41)	4.3 (1.46)	5.0 (1.61)	6.1 (1.81)	9.1 (2.21)
	Galleon	4.5 (1.51)	5.8 (1.77)	7.0 (1.95)	7.9 (2.06)	12.1 (2.49)
0.02	Forrest	6.5 (1.87)	5.5 (1.71)	6.1 (1.81)	6.7 (1.89)	12.2 (2.50)
	Skiff	4.6 (1.51)	5.2 (1.64)	7.6 (2.02)	7.4 (2.00)	11.1 (2.41)
	Galleon	4.5 (1.51)	3.8 (1.33)	5.1 (1.62)	10.0 (2.30)	13.3 (2.59)
0.8	Forrest	39.9 (3.68)	31.5 (3.45)	31.2 (3.44)	32.0 (3.46)	27.5 (3.31)
	Skiff	39.8 (3.65)	39.7 (3.68)	43.5 (3.77)	38.6 (3.65)	43.2 (3.76)
	Galleon	53.4 (3.98)	47.6 (3.86)	50.7 (3.93)	47.8 (3.87)	42.3 (3.74)
Tukey's $HSD_{0.05}$[C]						
Genotype x Zn fertilization x Soil type				(0.46)		

[A] a=100% Lancelin; b=75% Lancelin, 25% Laffer;c=50% Lancelin, 50% Laffer; d=25% Lancelin, 75% Laffer;e=100% Laffer).
[B] Values in parentheses are log-transformed.
[C] $HSD_{0.05}$ values are applicable to log-transformed data.

Table 6.3. Effects of soil type and Zn fertilization (mg/kg soil) on shoot Zn content (µg/plant) in barley genotypes at 26 DAS in Experiment 1.

		Soil type[A]				
Zn fert.	Genotype	a	b	c	d	e
0	Forrest	0.68 (0.42)[B]	0.84 (0.56)	1.00 (0.69)	1.36 (0.86)	2.50 (1.25)
	Skiff	0.81 (0.59)	0.89 (0.64)	1.37 (0.87)	2.00 (1.09)	3.84 (1.58)
	Galleon	0.36 (0.30)	0.59 (0.38)	1.17 (0.77)	2.20 (1.16)	7.31 (2.12)
0.02	Forrest	0.69 (0.52)	1.12 (0.75)	2.07 (1.12)	2.74 (1.31)	6.25 (1.98)
	Skiff	1.04 (0.71)	1.50 (0.91)	2.45 (1.23)	3.52 (1.51)	5.24 (1.83)
	Galleon	0.58 (0.45)	0.52 (0.42)	1.47 (0.89)	4.05 (1.62)	7.72 (2.17)
0.8	Forrest	18.34 (2.96)	18.33 (2.96)	24.01 (3.21)	27.58 (3.35)	26.39 (3.30)
	Skiff	21.57 (3.11)	27.03 (3.33)	31.14 (3.46)	30.76 (3.46)	33.55 (3.54)
	Galleon	31.41 (3.48)	30.34 (3.44)	38.94 (3.69)	43.55 (3.79)	41.26 (3.74)
Tukey's $HSD_{0.05}$[C]						
Genotype x Zn fertilization x Soil type				0.47		

[A] a=100% Lancelin; b=75% Lancelin, 25% Laffer; c=50% Lancelin, 50% Laffer; d=25% Lancelin, 75% Laffer; e=100% Laffer).
[B] Values in parentheses are log-transformed (log+1).
[C] $HSD_{0.05}$ values are applicable to log-transformed data.

6.2.2.4 Concentration of Fe, P and Mn in shoots

Under Zn deficiency (≤0.02 mg Zn/kg soil), the concentrations of Fe in shoots were extremely high in plants grown in Lancelin sand, but decreased gradually with increasing percentage of Laffer sand in the growth medium (Table 6.4). At adequate Zn supply (0.8 mg/kg), Fe concentrations were within the adequate range and did not change, regardless of soil type and the composition of the mixture (Table 6.4).

The P concentrations in shoot were affected by soil type and Zn fertilization. Overall, plants grown in soils **d** and **e** tended to have higher P values than those grown in soils **a**, **b** and **c** but significant difference occurred only at adequate Zn supply (Figure 6.3). The striking effect was that at adequate Zn supply, plants grown in growth medium in which Lancelin was present (**a, b, c, d**), had P concentrations below the critical range while those grown in Laffer sand achieved P concentrations within the critical range (0.35-0.6%) (Reuter and Robinson, 1997). This suggested that plants grown in the growth medium in which Lancelin sand was present were P-deficient.

The concentration of Mn in shoot also differed significantly between plants grown in soils **a** and **e** but only at adequate Zn fertilization: Mn values in plants grown in soil **a** were much lower when compared to those grown in soil **e** (Figure 6.3). This indicates a possibility of Mn deficiency in Lancelin sand when plants are grown for longer than that in the present study.

Table 6.4. Effects of soil type and Zn fertilization (mg/kg soil) on shoot Fe concentration (mg/kg D.W.) in barley genotypes at 26 DAS in Experiment 1.

		Soil type[A]				
Zn fert.	Genotype	a	b	c	d	e
0	Forrest	1034 (6.93)[B]	508 (6.23)	398 (5.99)	275 (5.62)	130 (4.87)
	Skiff	929 (6.88)	506 (6.20)	422 (6.05)	326 (5.79)	161 (5.08)
	Galleon	1002 (6.91)	829 (6.71)	414 (6.02)	338 (5.82)	180 (5.19)
0.02	Forrest	874 (6.77)	488 (6.19)	313 (5.74)	206 (5.33)	115 (4.75)
	Skiff	900 (6.80)	622 (6.43)	398 (5.98)	239 (5.48)	142 (4.94)
	Galleon	781 (6.66)	347 (5.84)	286 (5.65)	303 (5.72)	114 (4.74)
0.8	Forrest	93 (4.53)	62 (4.13)	65 (4.18)	56 (4.03)	48 (3.87)
	Skiff	73 (4.28)	71 (4.25)	69 (4.22)	61 (4.09)	60 (4.08)
	Galleon	103 (4.64)	70 (4.25)	64 (4.15)	64 (4.15)	57 (4.04)
Tukey's $HSD_{0.05}$[C]						
Genotype x Zn fertilization x Soil type				(0.48)		

[A]a=Lancelin; b=75% Lancelin, 25% Laffer;c=50% Lancelin, 50% Laffer; d=25% Lancelin, 75% Laffer; e=100% Laffer.
[B] Values in parentheses are log-transformed.
[C]$HSD_{0.05}$ values are applicable to log-transformed data.

6.2.3 Discussion and conclusion

The present results established that the two soils showed marked differences in plant growth and severity of Zn deficiency symptoms. The differences in growth as well as deficiency symptoms between the two soils may be explained by the characteristics of these soils. Of the soil characteristics, Fe and organic carbon varied considerably between the two soils (Table 6.1). It is well known that Zn has an affinity for soil oxides as well as for organic matter. It is, therefore, possible that greater Zn deficiency stress at low Zn supply, and less growth at low and adequate Zn supply in Lancelin sand as compared with that in Laffer sand may be due to high Fe and organic carbon in this soil.

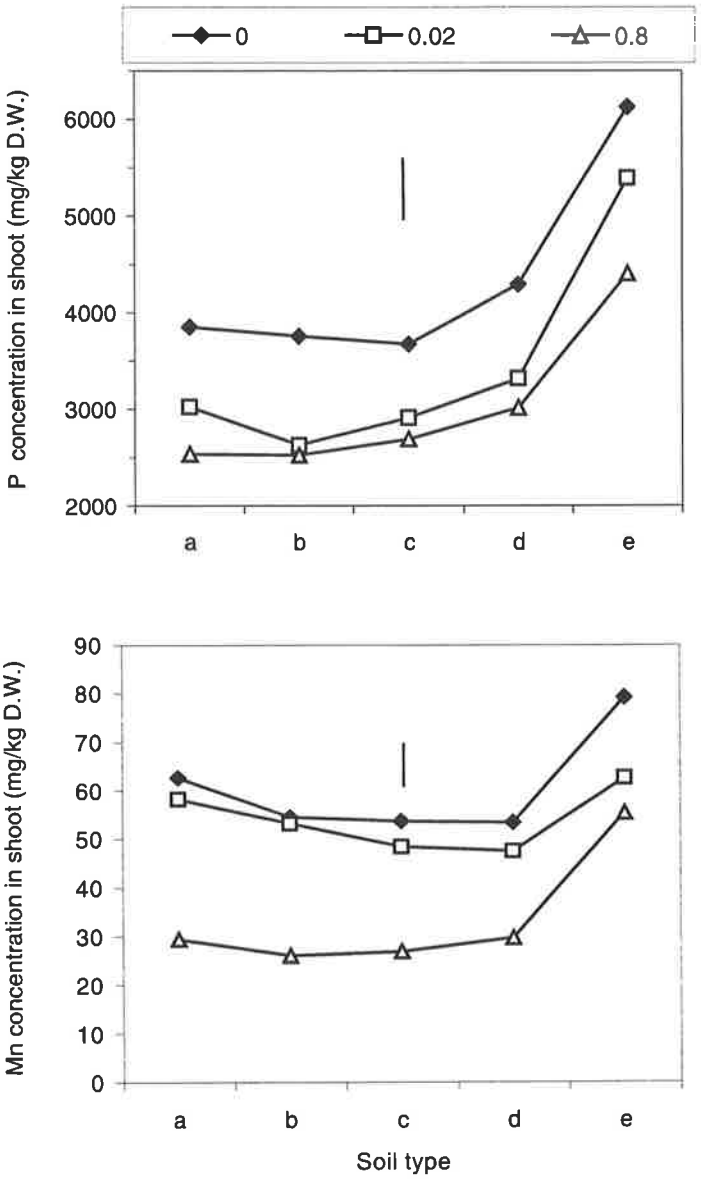


Figure 6.3. Effects of soil type and Zn fertilization on concentrations of P and Mn in shoot (a=100% Lancelin; b=75% Lancelin, 25% Laffer; c=50% Lancelin, 50% Laffer; d=25% Lancelin, 75% Laffer; e=100% Laffer). The vertical bars represent Tukey's $HSD_{0.05}$ values for the interaction Soil type x Zn fertilization.

In severely Zn-deficient plants grown in Lancelin sand, Fe accumulated to high levels, and the accumulation of Fe decreased with increasing Laffer sand in the growth medium. Although high Fe accumulation in Zn-deficient plants has been reported previously in sugar beat (Rosell and Ulrich, 1964), corn (Jackson *et al.*, 1967) and barley (Schwartz *et al.*, 1987), the actual cause for this high Fe accumulation is not known (e.g. is it a nutritional imbalance caused by Zn deficiency or a high Fe response).

The greater dry matter yield in Laffer sand than in Lancelin sand, even at adequate Zn supply led to the conclusion that growth in Lancelin sand was limited by factor(s) other than Zn. Plant tissue analysis provided evidence that P may have limited the growth, and thus plants would have responded to P, as indicated by the P concentrations below the critical range (Figure 6.3). Further research is required to verify this observation.

In conclusion, for an ideal soil screening, it is important that all the nutrients other than the nutrient in question (e.g. Zn) be adequate for normal growth, and growth is not affected by deficiencies of toxicities of other nutrients in the soil system. In the current soil system based on Lancelin sand, therefore, the Fe and P effects need further verification.

6.3 Experiment 2

In the previous experiment, it was found that when no Zn was added, Fe concentrations were unusually high in plants grown in 100% Lancelin sand (**a**) compared to those grown in 100% Laffer sand (**e**). It was also found that when Zn was not limiting growth, dry matter production in 100% Lancelin sand was significantly less than that in 100% Laffer sand. Tissue analysis suggest low P to be a factor causing this.

In order to use Lancelin sand, (i) the Fe levels need to be reduced, and (ii) the P levels need to be increased. An experiment was therefore conducted in Lancelin and Laffer sand to verify the Fe and P effects, and develop a soil system for screening.

6.3.1 Materials and methods

In this experiment, 400 g of each of the two soils, Lancelin sand and Laffer sand, was placed into cylindrical containers of 300 ml volume after both soils were thoroughly mixed with calcium carbonate powder (w/w). These pots were much smaller than those used previously, but were used due to limited quantities of Laffer sand. A preliminary experiment with two genotypes, Skiff, Forrest, and 6 Zn fertilization rates 0, 0.02, 0.04, 0.08, 0.2, 0.8 mg/kg soil established that seedlings could be grown for 21 days in containers of this type under growth cabinet conditions without showing other nutrient deficiencies and Zn fertilization of 0.8 mg/kg is adequate for normal growth (Appendices 6.1 and 6.2). Due to the limited amounts of Laffer sand available for this experiment, only Skiff and Forrest were used. In this experiment, both Laffer sand and Lancelin sand came from the same source as Experiment 1.

Basal nutrients (see 6.2.1), Zn (0 and 0.8 mg/kg as $ZnSO_4$), Fe and P treatments (Table 6.5) were applied in solution to the surface of the two soils. Double P and 1% $CaCO_3$ were aimed to reduce high Fe levels found in Zn-deficient plants grown in Lancelin sand in previous experiments. It was also felt that the application of double P would increase low P levels found in Zn-sufficient plants grown in Lancelin sand in previous experiment to adequate levels. Fe increments in Laffer sand were used to examine whether high Fe could reduce growth to the level of Lancelin sand. The treatments have been designated A-E (Lancelin sand) and F-J (Laffer sand) (Table 6.5).

Table 6.5. Treatment structure* in Experiment 2.

Lancelin sand
A-0.5% $CaCO_3$ + 0.7 mg/kg $FeSO_4$***
B-0.5% $CaCO_3$ only
C-0.5% $CaCO_3$ + 0.7 mg/kg $FeSO_4$+ double P**
D-1% $CaCO_3$ + 0.7 mg/kg $FeSO_4$
E-1% $CaCO_3$ + 0.7 mg/kg $FeSO_4$ + double P
Laffer sand
F-0.5% $CaCO_3$ + 0.7 mg/kg $FeSO_4$***
G-0.5% $CaCO_3$ only
H-0.5% $CaCO_3$ + 7 mg/kg $FeSO_4$
I-0.5% $CaCO_3$ + 70 mg/kg $FeSO_4$
J-0.5% $CaCO_3$ + 700 mg/kg $FeSO_4$

* Zn treatments (0 and 0.8 mg/kg soil) were superimposed onto the treatment structure shown in this table.
** as KH_2PO_4, *** control treatment,

After all the salt solutions had dried, the contents of each pot were thoroughly mixed in plastic containers in order to limit contamination, the containers were acid-washed between treatments.

Seeds of two barley genotypes, Skiff and Forrest, with similar Zn content (0.68 ± 0.01 and 0.65 ± 0.02 mg/kg per seed respectively) were pre-germinated (see 3.2.1). Two pre-germinated seeds of each genotype were sown in each pot. Seedlings were thinned to one per pot after emergence. Pots were randomized and watered daily by maintaining water content at 12% (w/w).

Plants were grown under the same conditions as in Experiment 1 and harvested 21 days after sowing (FS 5.0, Large, 1954). Roots and shoots were sampled and processed for nutrient analyses as described in Experiment 1.

The experiment was set up as factorial randomized complete block design with three replicates (2 genotypes x 2 Zn levels x 10 soil treatments). Analyses of data and comparisons were performed as described in Experiment 1.

6.3.2 Results

6.3.2.1 Visual symptoms

Symptoms of Zn deficiency developed more rapidly in Lancelin sand. At day 10, tiny brown spots were visible on the edges of the oldest leaves of Forrest growing in treatments **C**, **D**, and **E** of Lancelin sand and treatment **J** of Laffer sand when no Zn was applied. At 12 DAS, Zn deficiency symptoms in the form of chlorotic areas became apparent in Forrest grown in treatments **A** and **C** of Lancelin sand when no Zn was applied. At this stage, Forrest plants growing in treatments **H** and **I** of Laffer sand also developed brown spots on the edges of old leaves. At day 14, browning was evident in Forrest across all the treatments of Lancelin sand, (**A**, **B**, **C**, **D**, and **E**), and treatments **H**, **I** and **J** of Laffer sand at nil Zn fertilization. At day 16, Skiff growing in treatment **C** of Lancelin sand developed Zn deficiency symptoms while plants of Forrest in treatment of **F**, Laffer sand, with no applied Zn, were already showing Zn deficiency symptoms which became visible in treatments **H** and **I** at 18 DAS.

By harvest (21 DAS), plants of Forrest growing in Lancelin sand with no applied Zn developed severe symptoms of Zn deficiency (**A**, **B**, and **C**) or had died (**D** and **E**) whereas those growing in Laffer sand exhibited mild symptoms (**F**, **G**, **H**, **I** and **J**) (Figure 6.4). Skiff plants growing in Lancelin sand with no applied Zn showed mild (**A**, **B** and **D**) to severe (**C** and **E**) symptoms of Zn deficiency. In Laffer sand, browning was confined to the edges of old leaves of plants growing in treatments **F** and **G** (Figure 6.4).

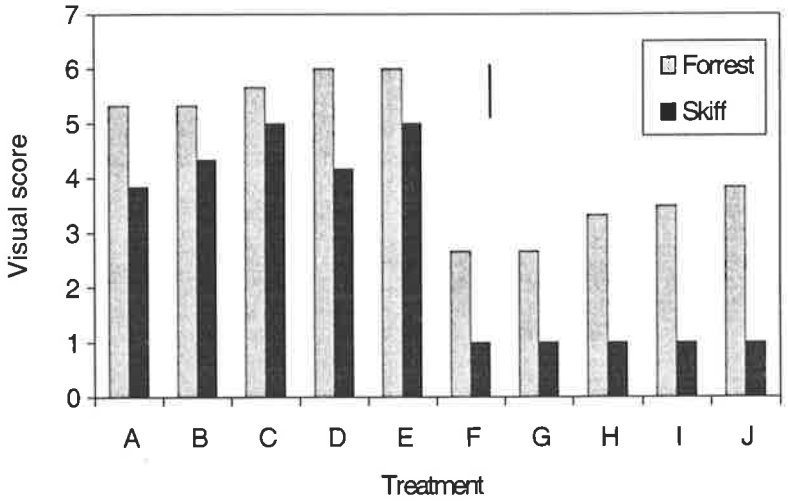


Figure 6.4. Effects of various treatments of Fe, P and calcium carbonate on expression of Zn deficiency symptoms (visual scores) of barley genotypes grown with no added Zn in Lancelin sand (A, B, C, D, and E) and Laffer sand (F, G, H, I and J) 21 days after sowing.Symptoms were scored on a scale of 1 to 6 (1=no symptoms, 6=very severe symptoms). The vertical bar represents Tukey's $HSD_{0.05}$ value for treatment effect.

6.3.2.2 Shoot and root dry matter

Dry matter yields of roots and shoots of both genotypes were increased significantly by Zn fertilization. Generally, the response to Zn fertilization was greater in treatments of Lancelin sand (**A**, **B**, **C**, **D** and **E**), than those of Laffer sand, (**F**, **G**, **H**, **I**, and **J**) (Figure 6.5). Under Zn deficiency in Lancelin sand, an increase in P fertilization alone, (**C**), or in combination with increased $CaCO_3$, (**E**), did not have an effect on shoot and root dry matter accumulation. In contrast, when Zn supply was adequate, increased P fertilization, (**C**), increased dry matter of Skiff only, but the combination of increased P and $CaCO_3$, **E**, increased dry matter of both Skiff and Forrest, which indicated differential variation in response to P fertilization, and P fertilization accompanied by elevated carbonate level.

In Laffer sand, high Fe fertilization, (**J**), reduced dry matter yield when compared to the control (**F**), but significant reduction occurred only in shoot dry matter yield at adequate Zn supply (Figure 6.5). However, reduction must be an indirect effect of high Fe in the soil, since the concentration of Fe in the shoots did not change significantly, despite significant reduction in shoot dry matter (Figures 6.5 and 6.7). A close association between shoot dry matter yield and P concentration in shoots (Figures 6.5 and 6.7) suggested that the reduction was associated with the decrease in P concentration in the shoots as a result of high Fe fertilization.

6.3.2.3 Concentration and content of Zn in shoots

Zn concentrations were increased significantly by Zn fertilization but the increase varied with treatment: Skiff had a greater concentration of Zn in **F**, **G**, **H** and **I** than **C**, and Forrest had a greater concentration of Zn in **F**, **G**, and **H** than **C** at 0.8 mg/kg. The application of P alone, (**C**), or in combination with increased $CaCO_3$, (**E**), did not influence Zn concentration in shoots significantly. Increasing Fe supply to Laffer sand decreased Zn concentrations of both Skiff and Forrest when applied at the high levels, **I**, **J** compared to control (**F**) (Figure 6.6). Shoot Zn content followed a pattern similar to that of shoot Zn concentration (Figure 6.6).

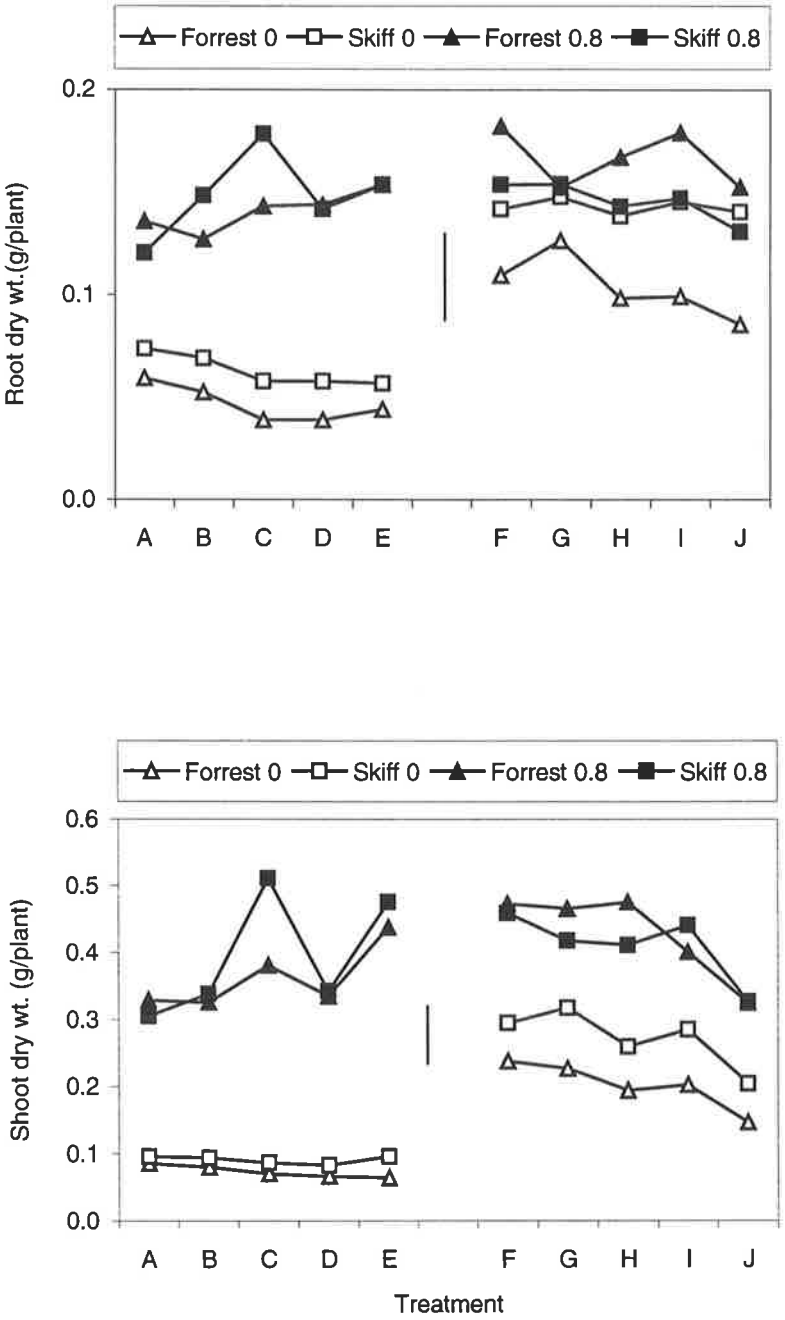


Figure 6.5. Effects of Zn fertilization and various treatments of Fe, P and calcium carbonate on dry matter yield of barley genotypes grown in Lancelin (A, B, C, D, and E) and Laffer sand (F, G, H, I and J) at 21 DAS in Experiment 2. Vertical bars represent Tukey's $HSD_{0.05}$ value for Genotype x Zn fertilization x Treatment interaction.

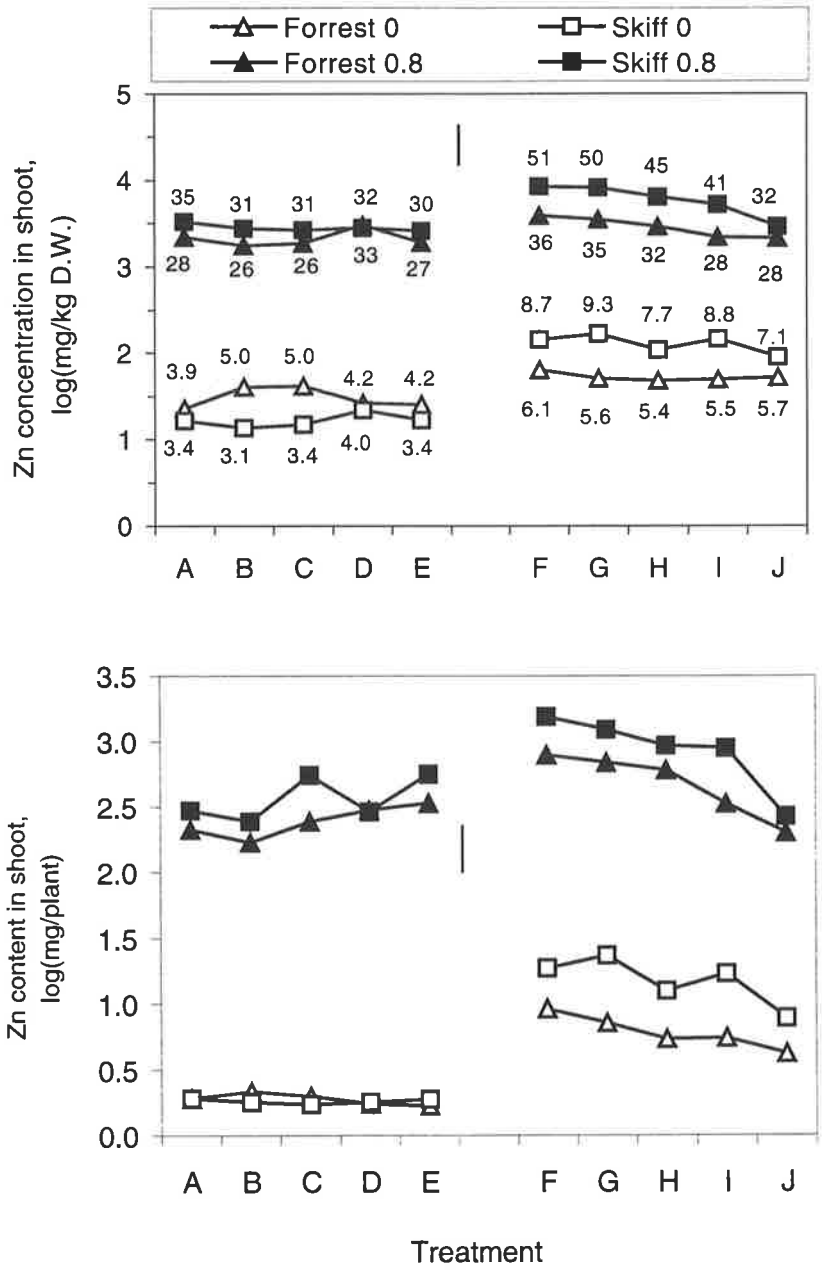


Figure 6.6. Effects of Zn fertilization and various treatments of Fe, P and calcium carbonate on concentration and content of Zn in shoots of plants grown in Lancelin (A, B, C, D and E) and Laffer sand (F, G, H, I and J) at D21 (21 days after sowing) in Experiment 2. Vertical bars represent Tukey's $HSD_{0.05}$ values for interaction Genotype x Zn fertilization x Treatment. Actual values for Zn concentration are displayed on the chart but $HSD_{0.05}$ values are applicable to log-transformed data.

6.3.2.4 Concentration of Fe, P and Mn in shoots

When no Zn was applied, Fe accumulated to very high concentrations in Lancelin sand (Figure 6.7). Skiff had a greater concentration of Fe (1010-1130 mg/kg D.W.) than Forrest (500-830 mg/kg D.W.). In contrast, when grown in Laffer sand with no applied Zn, both genotypes achieved similar concentrations of Fe in shoots. When Zn was applied, regardless of treatment, Fe concentrations in both Lancelin and Laffer sand grown plants fell dramatically to adequate levels (60-100 mg/kg D.W.; Brown, 1982). P concentrations differed significantly between the two soils, regardless of Zn fertilization. Plants grown in control treatment of Laffer sand, (**F**), had significantly greater concentrations of P than those grown in control treatment of Lancelin sand, (**A**), (Figure 6.7). The application of P alone, (**C**), or in combination with increased $CaCO_3$, (**E**), enhanced P concentrations of both genotypes significantly, especially at the lower level of Zn fertilization and increased them to levels comparable to that found in Laffer sand.

Increasing the supply of Fe to Laffer sand depressed P concentrations in shoots significantly. The effect of Fe supply in depressing P concentrations in Laffer sand was more marked than the effect of P in depressing Fe concentrations in Lancelin sand. P concentrations of plants grown in Lancelin sand without additional P (**A**, **B**, **D**), with adequate Zn were below the concentrations required for normal growth (0.35-0.60 % D.W., FS 5). Plants responded to an increase in P added to the soil, (**C**, **E**), which indicated that Lancelin sand might have been marginally P-deficient at this growth stage (FS 5.0).

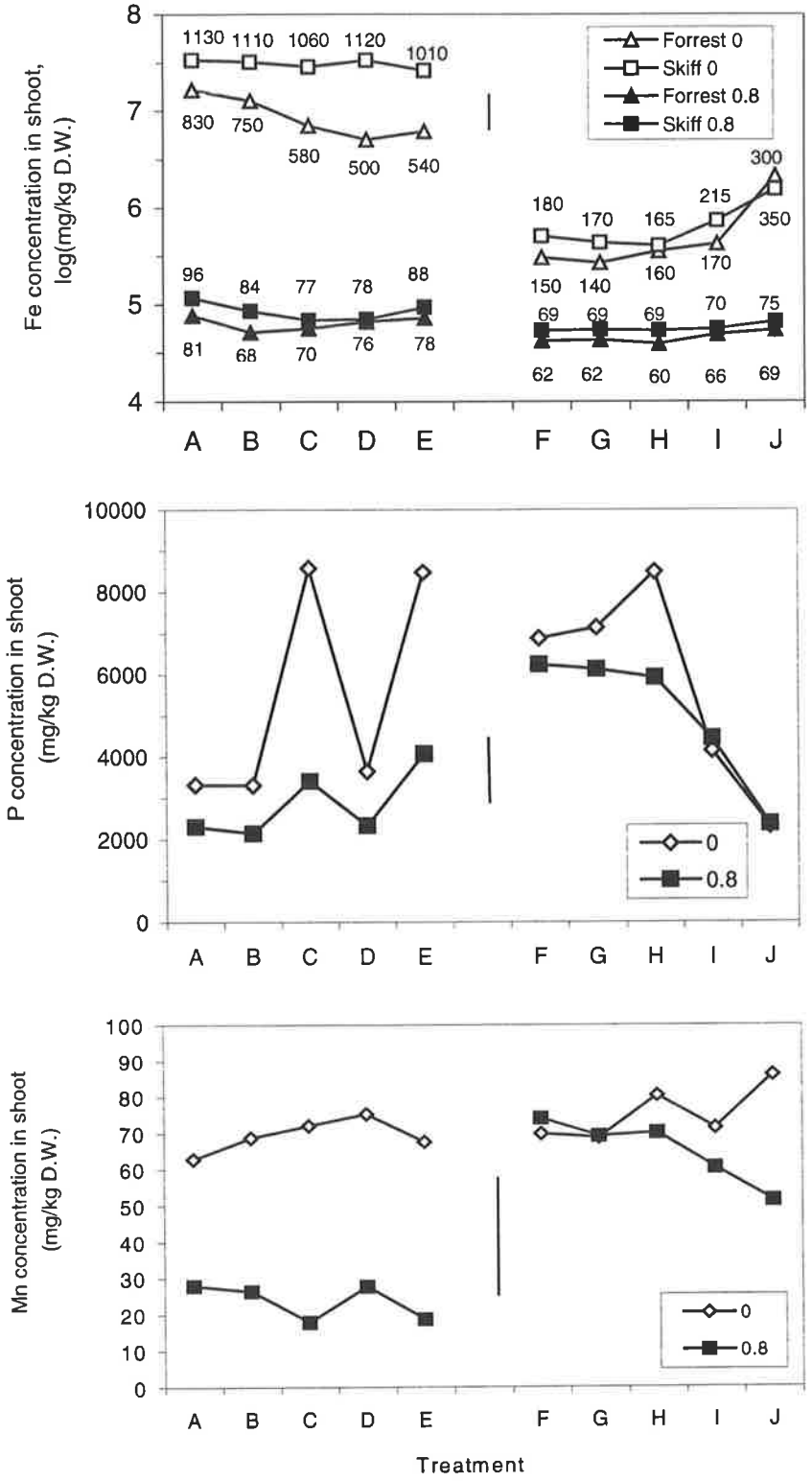


Figure 6.7. Effects of Zn fertilization and various treatments of Fe, P and calcium carbonate on concentrations of Fe, P and Mn in shoots of plants grown in Lancelin (A, B, C, D, and E) and Laffer sand (F, G, H, I and J) at D21 (21 days after sowing) in Experiment 2. Vertical bars represent Tukey's $HSD_{0.05}$ value for interaction genotype x Zn fertilization x Treatment for Fe and Zn fertilization x treatment for P and Mn. Actual Fe values are displayed on the chart but $HSD_{0.05}$ values are applicable to log-transformed data.

Mn concentrations in shoots varied significantly between the two soils only when fertilized with adequate Zn: plants grown in Laffer sand had greater concentrations of Mn than plants in Lancelin sand. However, the concentrations were above the critical deficiency level (12-12.4 mg/kg dry wt.) (Hannam *et al.*, 1987), which indicated that plants were not Mn-deficient at this growth stage. Of the treatments, Fe had the largest effect on shoot Mn concentrations of plants when Zn was added to the growth medium. In general, Fe fertilization depressed Mn concentrations but depression was not significant (Figure 6.7). The other treatments, P, $CaCO_3$, did not influence shoot Mn concentrations.

6.3.3 Discussion and conclusion

The results showed that increased applications of $CaCO_3$ and P depressed shoot Fe concentrations of Zn-deficient plants grown in Lancelin sand but were not effective in reducing the toxic Fe concentrations to normal levels. Despite having a greater Fe concentration than Forrest, Skiff did not develop any brown spots, which suggested differential variation in accumulation and internal tolerance to high Fe levels in shoots under Zn deficiency. A close association between browning and Fe concentration in shoots of Forrest suggested that browning was caused by high Fe accumulation. However, browning was in the form of small brown spots confined to the edges of the old leaves and did not spread over leaves, indicating that it was not as severe as Fe toxicity reported in rice (Sahrawat, 1996), where in severe cases, leaves turn purplish brown.

In an effort to verify the Fe effect observed in Lancelin sand, it was found that high Fe fertilization in Laffer sand increased Fe concentrations in shoots of plants grown with

no Zn fertilization. Although the increase in Fe concentration was not to the extent of that found in Zn-deficient plants grown in Lancelin sand (Figure 6.7), this provided further evidence that the high Fe accumulation in Zn-deficient plants may be a result rather than a cause of Zn deficiency.

When grown with adequate Zn fertilization, 0.8 mg Zn /kg, high Fe supply to Laffer sand did not affect the Fe concentration in shoots, but reduced shoot dry matter yield to the level of Lancelin sand, suggesting that reduction in Lancelin sand was probably caused by a factor other than Fe. Shoot analysis found a close association between a reduction in shoot dry matter and a decrease in P concentration, providing evidence that the growth at adequate Zn fertilization in Lancelin sand was probably limited by P (Figure 6.7). This observation is supported by yield responses to P addition in Lancelin sand in the present study. The addition of Fe at adequate Zn fertilization also decreased concentrations of Mn and Zn but concentrations of these nutrients were still above the critical levels, which suggested that plant growth was not limited by either nutrient. The findings of the present study suggest that the adverse effect of high Fe supply on plant growth is through its ability to reduce concentrations of other nutrients such as P (Yoshida, 1981) and Zn.

6.4 General discussion and conclusion

The present results indicate that Zn deficiency stress differs significantly with the soil type. The differential Zn-deficiency stress (expressed in Zn deficiency symptoms and growth) between the two soils, Lancelin sand and Laffer sand, may be explained by the differences in the organic carbon and DTPA-extractable Fe between the two soils (Table 6.1). For instance, Lancelin sand has a greater organic carbon content than Laffer sand. It

is well known that Zn has an affinity for organic matter as well as for soil oxides. It is therefore possible that the greater Zn deficiency stress observed in Lancelin sand may be associated with the greater organic matter content and its complexation with Zn. In the case of Fe, its high availability is also known to depress plant levels of Zn. However, given the high initial pH, (>7.5 at 0.5% and 1% $CaCO_3$ levels in both sands), measured prior to the experiment, and therefore, presumably low solubility of Fe, and the small responses to soil applications of Fe in Experiment 2 (also seen by Gupta, 1991), high Fe induced Zn deficiency may be excluded as the primary cause of greater Zn deficiency stress in Lancelin sand.

From the results reported here, it is likely that the toxic Fe concentrations found in Zn-deficient plants is an induced nutritional imbalance which is a consequence of the Zn deficiency rather than a direct toxicity due to high soil Fe, since the sufficient supply of Zn to the soil brought Fe concentrations to adequate levels in the present study. If Fe toxicity was the main reason for the greatly reduced growth in Lancelin sand, then one would expect to see the effect of Fe toxicity even in Zn-sufficient plants. High Fe concentrations in Zn-deficient plants have been previously reported by numerous investigators. Jackson *et al.* (1967) reported an abnormally high level of Fe in Zn-deficient corn plants but a marked reduction of high Fe level when Zn was applied. In a nutrient solution study with sugar beet, Rosell and Ulrich (1964) found that Fe concentrations of the mature blades of Zn-deficient plants were 10-fold greater than those frequently found in normal blades. Schwartz *et al.* (1987) observed significantly higher Fe concentrations in Zn-deficient than in Zn-sufficient plants. Although the actual cause for increased Fe uptake by Zn-deficient plants is not known, it can be speculated that the passive uptake of Fe may be intensified by increased root cell membrane

permeability as has been suggested for increased uptake of P by Zn-deficient plants (Paribok and Alekseeva-Popova, 1965). The greater Fe accumulation in Zn-deficient plants grown in Lancelin sand than those grown in Laffer sand may be an artifact resulting from greater Fe content of Lancelin sand over Laffer sand (Table 5.1).

When grown in Lancelin sand with no added Zn, Forrest showed severe Zn deficiency symptoms in both experiments, whereas Skiff developed fewer Zn deficiency symptoms in Experiment 1 when compared to in Experiment 2. This can be attributed to the greater capacity of Skiff to utilise the extra native Zn in Experiment 1: the soil volume in Experiment 1 was 25 % higher than that in Experiment 2. Therefore, it can be assumed that an increase in total soil Zn content as a result of increased soil volume may have favoured Skiff.

The greater dry matter yield of plants grown in Laffer sand compared with those grown in Lancelin sand, even when Zn supply was sufficient, indicated that factors other than Zn may have limited growth in Lancelin sand. Elemental composition of the plants grown in both soils showed that plants grown in Lancelin sand had concentrations of P, below the critical range (0.35-0.6% dry wt.) (Reuter and Robinson, 1997), while plants grown in Laffer sand had P concentrations within the adequate range. This difference was confirmed by the yield responses to P addition to Lancelin sand in Experiment 2. Based on published values, the other nutrients appeared to be adequate for normal growth at this growth stage (FS 5.0, Large, 1954). It is clear from the results that P was responsible for differential growth observed between the two soils as evidenced by responses to P application.

In conclusion, the results suggest that soils of high organic matter content may be low in plant available Zn, possibly due to Zn-organic matter complexes. Although experimental evidence is lacking, it is possible that Zn in Zn-organic matter complexes may be extracted by the extractant, DTPA-method, but may not be available to plant uptake. This may explain why Zn deficiency stress in Lancelin sand was greater than that of Laffer sand, despite similar values for DTPA-extractable Zn.

The results also suggest that high Fe accumulation under Zn deficiency is likely to reflect a nutritional imbalance rather than a real toxicity. This result together with greater Zn deficiency stress of Lancelin sand over Laffer sand suggest that Lancelin sand can be used for assessing genotypes in their tolerance to Zn deficiency. At this stage, use of a mixture between the two soils for screening (e.g. **c** or **d**) may not be necessary, and it is anticipated that 100% Lancelin sand will be used in future screening work since Zn deficiency stress was greatest in 100% Lancelin sand.

Deficiencies of nutrients other than Zn (e.g. P) can also contribute to Fe accumulation to toxic levels, as has been observed in the present study. It is also concluded that there is a negative effect of high soil Fe on the availability of nutrients such as P, Mn, and Zn, which was evident in the present study in which a significant increase in dry matter yield was achieved by application of P. This finding bears practical implications for future screening using Lancelin sand as a growth medium. In instances where Lancelin sand is to be used for screening, a greater supply of P than that used in Laffer sand should be considered, in order to minimize excessive Fe accumulation for which P deficiency is partly responsible, and to supply plants with adequate levels of P for normal growth

under the conditions of the present study. Further research into mechanisms of excessive accumulation of Fe under Zn deficiency is warranted.

CHAPTER 7

Refining the soil-based method to screen for zinc efficiency in seedlings and verifying its ability to predict yield responses to zinc

7.1 Introduction

Field screening has been the main means of selecting Zn-efficient genotypes but there are a number of experimental limitations with this method. These problems were discussed in detail in Chapter 4, but mainly they are related to the inherent variability in the development of Zn deficiency under field conditions and the large seasonal variation in responsiveness. There is a need to develop alternative methods of selection for Zn-efficient genotypes to produce more reliable results. One method, screening using soil culture, has received a great deal of attention. Considerable improvements in selecting for greater nutrient efficiency using pot-based systems have been achieved for a number of nutrients in many crop species: Cu efficiency in wheat (Graham, 1978), Mn in barley (Longnecker *et al.*, 1990) and durum wheat (Saberi, 1999), Zn in bread wheat (Graham *et al.*, 1992), rapeseed (Grewal *et al.*, 1997) and chickpeas (Khan *et al.*, 1998a). Graham (1984) reported that in screening for micronutrient efficiency, potted soil generally ranked genotypes in the same order as field studies. However, factors such as seed nutrient content, the choice of appropriate criteria for diagnosing deficiency and the relationship between seedling responses and responses in grain yield need to be considered.

With respect to screening wheat in potted soil, Graham (1984) pointed out that seed nutrient content can significantly influence the seedling growth rate when the nutrient in question is limiting in the soil. Likewise, it was found in Chapter 3 that seed Zn content can markedly affect the severity of Zn deficiency symptoms and early growth of barley. Therefore, in the current studies, the seed of all genotypes has been hand-sorted to a

uniform weight to minimize the variation in seed Zn content among genotypes. Although seed selection based on seed weight has proven to be useful in screening studies, it is cumbersome when a large number of genotypes is being screened. An alternative to sorting by seed weight could be to use seed of uniform size, which can be achieved simply by mechanically grading seed into specific sizes such as small, medium, and large. Seed size, in contrast to seed weight, and its relationship to screening has received little attention and has not been examined for barley.

To enable routine screening of genotypes or segregating populations, the method needs to be able to handle large numbers of plants, be based on a quick and reliable selection criterion, which, ideally, should be non-destructive to allow recovery of seed. In Chapter 5, it was shown that a scoring system based on visual symptoms of Zn deficiency could be used to identify efficient and inefficient genotypes. However, interpretation of the true level of genotypic variation in Zn efficiency was affected by the large range in seed Zn contents and there was a need to repeat this work using seed with similar Zn contents. Later in Chapter 6, it was found that growing seedlings in small pots containing 400 g soil did not affect the expression of Zn deficiency symptoms or the relative differences among genotypes. This contrasts to the reported effects of pot size on Mn efficiency (Huang *et al.*, 1996). Therefore, a screening method for Zn efficiency based on 3-4 week old seedlings using small pots and based on expression of visual symptoms of deficiency shows considerable promise.

The seedling test will be most useful if it can predict differences in grain yield responses to Zn deficiency. In wheat, there is a positive relationship between grain yield and Zn concentrations in the youngest expanded leaf blades sampled at 6 weeks of growth (Rengel and Graham, 1995a). Also, in solution culture, it has been reported that at a growth stage as early as 22 days after sowing, classification of wheat genotypes into Zn-

efficient and Zn-inefficient groups correlated well with classification obtained in field experiments conducted on a Zn-deficient soil (Rengel and Graham, 1995b). However, field data with barley suggest that it is inherently more efficient than wheat and therefore the relationship observed with wheat may not be as strongly expressed in barley. There have been no reports of the relationship between seedling and yield responses to Zn for barley in the literature, either in pots or field grown plants.

In the studies described in this chapter, a diverse range of barley genotypes was screened in small pots and several selection criteria were compared. A subset of genotypes was then selected and grown to maturity under different levels of Zn stress to examine the relationship between the seedling test and yield responses. The ability of mechanically grading seed to produce seed of uniform Zn content suitable for large-scale screening was also investigated. The aim of this work was to develop final recommendations on a pot-based method that would be suitable for routine screening of genetic material as well as to characterize the Zn efficiency of a range of genotypes. If the methodology proved to be reliable, it would be used in a future study of the genetic control of Zn efficiency.

7.2 Materials and methods

7.2.1 Experiment 1. Determination of tolerance of barley genotypes to Zn deficiency at an early growth stage

A Zn deficient, sandy surface soil, collected from uncleared land near Lancelin, Western Australia, (DTPA-extractable Zn=0.12 mg/kg soil), as described in Chapters 5 and 6, was used in the experiments of this chapter. An equivalent of 400 g of soil was packed into plastic containers of 300 ml volume following the addition of calcium carbonate powder (1% w/w). Basal nutrients (in mg/kg dry soil) of NH_4NO_3, 350; K_2PO_4, 375; K_2SO_4, 120; $MgSO_4$, 90; $MnSO_4$, 10.5; $CuSO_4$, 5, together with Zn treatments 0.02 and 0.8 mg Zn/kg

soil as $ZnSO_4$ were applied to the surface of the soil and allowed to dry, and mixed throughout the soil.

Fifty-six genotypes of barley were grown with the two Zn treatments in a growth cabinet (20/15 °C day/night temperature and 14 h photoperiod). To better characterize the level of Zn efficiency among the barley genotypes, 4 wheat genotypes (Excalibur, Trident, Songlen and Yallaroi) of known Zn efficiency were also used in the experiment. Based on results from field and growth cabinet experiments in which Zn-deficient Laffer sand was used as the growth medium, Excalibur and Trident have been classified as Zn efficient, Songlen and Yallaroi as Zn-inefficient (Grewal and Graham, 1994; Grewal *et. al.*, 1996).

Seeds of all genotypes (Appendix 7.1) were hand-graded to a uniform weight (48±2 mg/seed), surface sterilized and pre-germinated on filter papers in petri dishes, as reported in previous chapters. Two pre-germinated seeds of each genotype were sown into each pot and thinned to one per pot following emergence. Plants were watered on a daily basis maintaining water content at 12% (w/w) during the experiment, which was harvested 21 days after sowing.

At harvest, plants were visually scored on a scale of 1 to 6 (see 5.2.2). Shoots were cut off at the soil surface and soil was washed from the roots with tap water. Both shoots and roots were rinsed in deionized water, oven-dried at 65 °C for 48 h and, weighed for dry matter. Tissues from genotypes that covered the range in responses to Zn were digested in 70% nitric acid (HNO_3) and analyzed for elemental composition (Appendix 7.2) by ICP spectrometry (Zarcinas *et al.*, 1987), as described earlier (Chapter 3).

The experiment was set up in a completely randomized block design with four replicates. Results were analyzed using the GENSTAT statistical package (GENSTAT 5, 1988).

Tukey's Honestly Significant Difference (HSD) at α=0.05 was employed in pairwise comparisons (Steel and Torrie, 1960).

7.2.2 Experiment 2. The effect of seed size on the early growth of barley under low and adequate Zn conditions

The soil preparation and nutrient treatments were the same as in Experiment 1. Six barley genotypes were chosen to represent the spectrum of tolerance to Zn deficiency obtained in previous experiments (Chapter 5). These genotypes were Forrest, Amagi Nijo (sensitive), Schooner, WI-2868 (intermediate), Tantangara and Skiff (tolerant). Seed was obtained from a field experiment conducted at Bute, South Australia in 1997. The seed for all genotypes was graded into three groups by sieving, namely large (> 2.8 mm in diameter), medium (2.5-2.8 mm) and small (2.25-2.5). Seed Zn content for each seed size is given in Table 7.1 (see Appendix 7.3, for seed weight, and complete elemental analysis of individual seed size classes). Surface sterilization, germination, sowing, thinning, growing and harvest were performed as described in Experiment 1. The experiment was set up in a completely randomized block design with four replicates. Statistical analyses and pairwise comparisons were performed as described in Experiment 1.

Table 7.1. Seed Zn content (µg/seed) of barley genotypes in Experiment 2. Standard errors are based on three replicates.

	Zn Content		
Genotype	Small	Medium	Large
Forrest	0.80 ± 0.04	1.03 ± 0.06	0.92 ± 0.01
Skiff	0.61 ± 0.02	0.70 ± 0.03	0.86 ± 0.02
Amagi Nijo	0.56 ± 0.05	0.66 ± 0.04	0.80 ± 0.04
Tantangara	0.58 ± 0.03	0.69 ± 0.05	1.01 ± 0.14
Schooner	0.58 ± 0.01	0.71 ± 0.01	0.73 ± 0.01
WI-2868	0.59 ± 0.01	0.75 ± 0.03	0.91 ± 0.04

7.2.3 Experiment 3. Correlation between vegetative and grain measures of tolerance to Zn deficiency in barley

In this experiment, an equivalent of 3 kg of Lancelin sand was packed into cylindrical PVC pots with approximate dimensions (diameter x length) of 11 x 28 cm following the addition of calcium carbonate powder (1% w/w) as described in Experiment 1. Basal nutrients were also applied at the same rates as in Experiment 1. Zn treatments were 0.1 mg/kg (low) and 2.4 mg/kg (adequate). These rates were based on the results of earlier experiment in large pots that examined responses of two barley genotypes (Amagi Nijo and Tantangara) to Zn fertilization (0.04, 0.2, 0.8, 3.2 and 12.8 mg Zn/kg soil) in Laffer sand under controlled conditions (Y. Genc, unpublished data). The results showed that an application of 0.04 mg Zn/kg soil was severely deficient for grain production, and grain yield at this level was in the range of 6% (Amagi Nijo) to 22% (Tantangara) of the maximum grain yield which was reached at 0.8 mg Zn/kg (Amagi Nijo) and 12.8 mg Zn/kg (Tantangara). In previous experiments (Chapter 5), it was found that Lancelin sand induced more severe Zn deficiency in barley than Laffer sand, therefore, it was decided that the application of Zn to Lancelin sand should be greater than that to Laffer sand, if a similar degree of Zn deficiency is to be achieved. As for the adequate treatment, 2.4 mg Zn/kg soil, it was selected based on the fact that there was no further significant increase in grain yield above 0.8 mg Zn/kg soil in Laffer sand, and to achieve similar responses in Lancelin sand, soil Zn supply should be greater than that to Laffer sand.

Fifteen barley genotypes together with two wheat genotypes, Excalibur, Yallaroi, were selected to represent the full spectrum of tolerance to Zn deficiency observed in Experiment 1. The seed used in this experiment came from the same source as the seed used in Experiment 1 (see Appendix 7.1). It was anticipated that using seed of similar Zn content would allow better genotypic comparisons.

Two harvests were performed, one at the awn just visible (40-73 days after sowing depending on genotype) and the other at maturity. At the first harvest, shoots were cut off at the soil-surface and rinsed with deionized water following a brief dipping into deionized water. The samples then were dried at 60 °C for 48 h in a forced draft oven, and weighed. At maturity, shoots were cut about 1 cm above the soil surface, fertile and infertile tillers were counted, heads were separated and threshed by hand. Straw and grain were dried at 80 °C for 48 h, and weighed. The harvest index was calculated as the ratio of grain weight to the total weight of above-ground parts (grain + straw).

The experiment was set up as a completely randomized block design with three replicates. Results were analyzed using GENSTAT statistical package (GENSTAT, 1988) and Tukey's Honestly Significant Difference (HSD) at α=0.05 was employed in pairwise comparisons, as in Experiment 1.

7.3 Results

7.3.1 Experiment 1

7.3.1.1 Visual symptoms

Thirteen days after sowing, typical symptoms of Zn deficiency such as stunted shoot growth and pale yellow linear chlorotic areas were observed on young leaves of the Zn-inefficient wheat genotypes Yallaroi, Songlen, and barley genotypes SA93013, Onslow, Europa, Natasha, Cheri and Kinukei 19 when grown at 0.02 mg Zn/kg. At day 14, deficiency symptoms were visible in barley genotypes, WA28784, Fitzgerald, Arapiles, Gairdner, Haruna Nijo, VIC9307, VIC86045B and at day 15, VIC9524, WI-3051, NSW WB 190R, Manley, Franklin, WA 28776, Harrington and WI-2597, and in the wheat genotype Excalibur. Symptoms continued to develop and progressed towards the tip and the base of the leaves. As the symptoms developed, the leaf blades often collapsed in the

mid-section and displayed a "scorched" appearance. In severe cases of Zn deficiency, the whole plant turned pale yellow.

By 21 days after sowing, there was considerable variation in the severity of Zn deficiency symptoms among the genotypes when fertilized with 0.02 mg Zn/kg soil (Plate 7.1). Most of the genotypes developed mild to severe Zn-deficiency symptoms (score 4-5) (Figure 7.1). Those which exhibited the least severe Zn-deficiency symptoms included Tarm, Tantangara, Galleon, WI-2976 and Skiff. Among the wheat genotypes, Excalibur, Songlen and Yallaroi showed severe symptoms, while Trident developed mild symptoms of Zn deficiency.

7.3.1.2 Shoot and root dry matter

Both shoot and root dry matter were reduced under low Zn supply compared with adequate Zn supply in all genotypes but the reduction differed with genotype (Figure 7.2). The reduction in shoot dry matter ranged from 48% for WI-2976 to 82% for SA93013. The reduction in root dry matter ranged from 4% for Blenheim to 68% for SA93013. Overall, root growth was less affected by Zn deficiency than shoot growth. Generally, the reduction in shoot and root growth was greater in bread wheat than in barley. Among the wheat genotypes, shoot and root dry matter were less affected by Zn deficiency in Trident than in other genotypes. There were differences in early seedling vigour when adequate Zn was applied, with SA93013, Haruna Nijo, Amagi Nijo, and Franklin showing the greatest vigour.

The ratio of shoot growth at low Zn to that at adequate Zn supply, which is an expression of Zn efficiency, ranged from 18% for SA93013 to 52% for WI-2976 (Figure 7.3). Based on efficiency ranking, three groups can be classified as Zn-inefficient (<30%), moderately Zn-efficient (30-40%) and Zn-efficient (>40%).

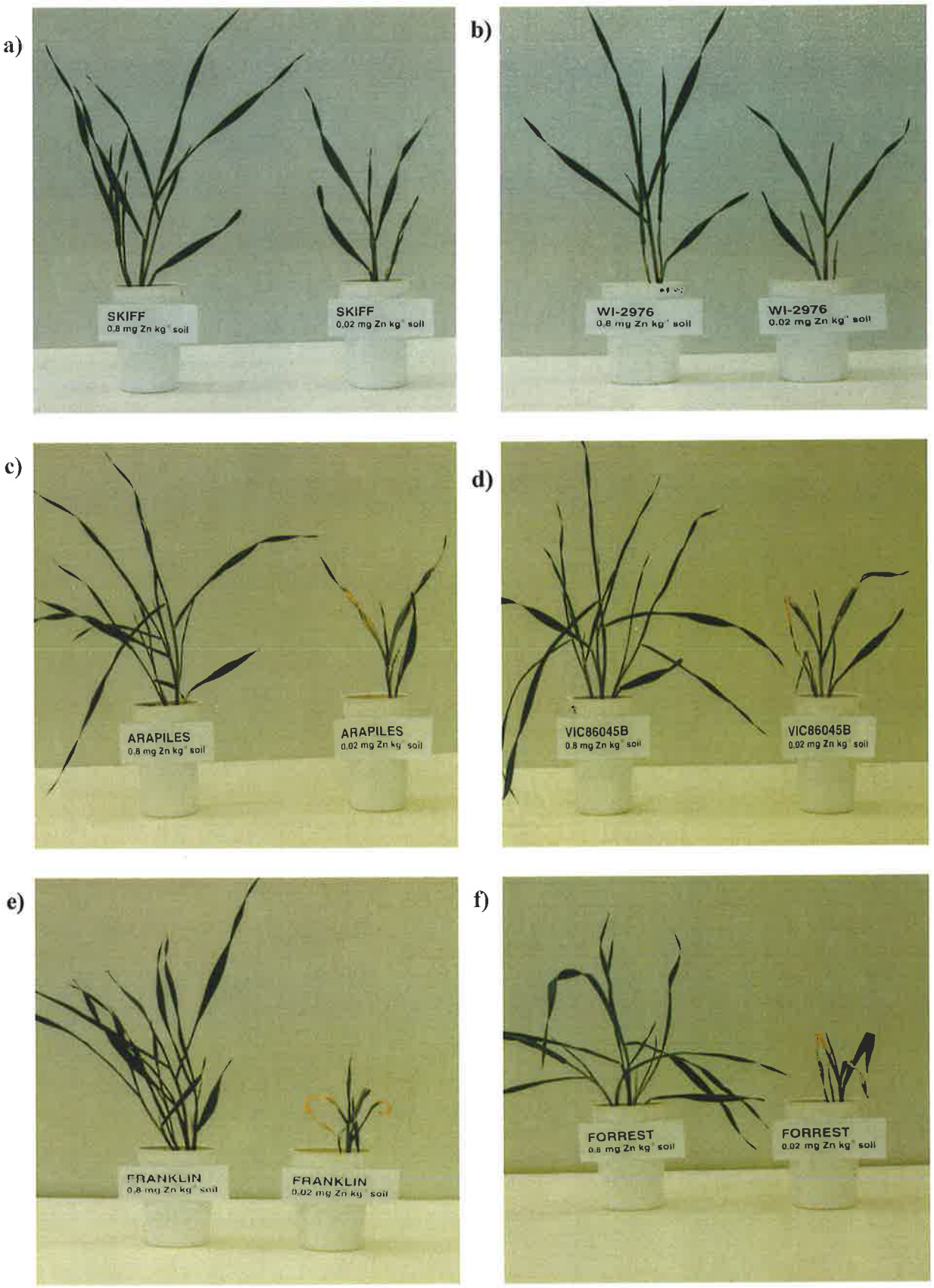


Plate 7.1. Effect of Zn fertilization on expression of Zn deficiency symptoms at 21 DAS in some of the barley genotypes tested in Experiment 1 (a, b, slight symptoms; c, d, mild to severe symptoms; d, e, severe symptoms).

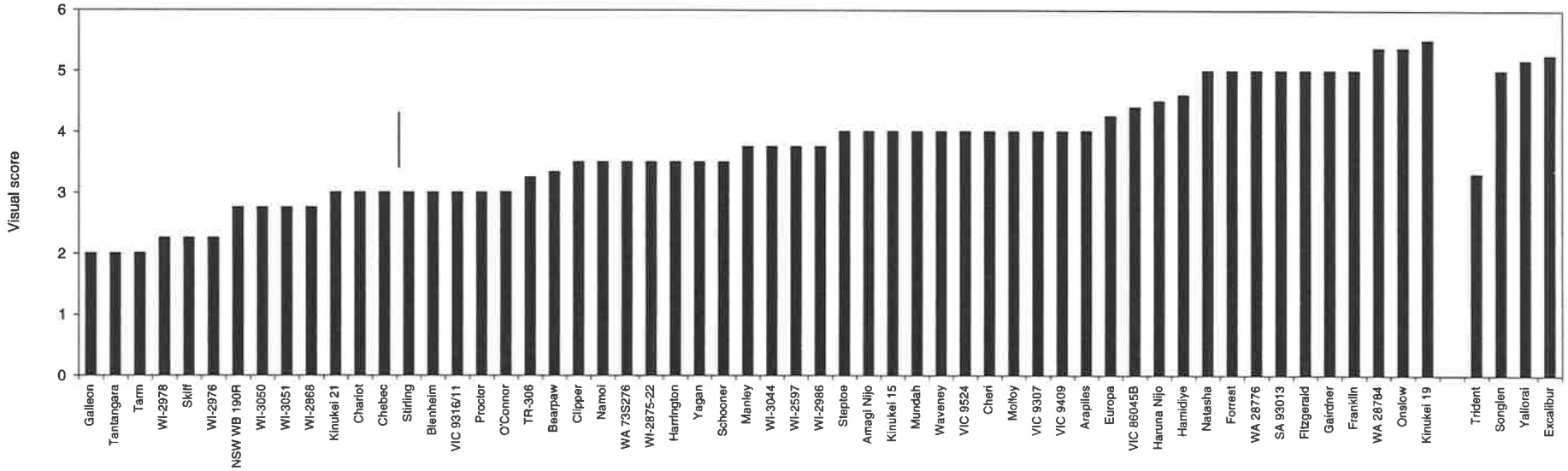


Figure 7.1. Ranking of barley genotypes based on Zn deficiency symptoms (visual scores) obtained at a Zn fertilization of 0.02 mg/kg soil at 21 DAS in Experiment 1 (1=dark green leaf, 2=pale green leaf, 3=linear chlorotic areas appearing on young leaves, 4= chlorotic areas extending to the margins and leaves collapsing in the middle, 5=both young and old leaves turning pale yellow, 6=dead growing points). The vertical bar represents Tukey's $HSD_{0.05}$ value for genotype effect.

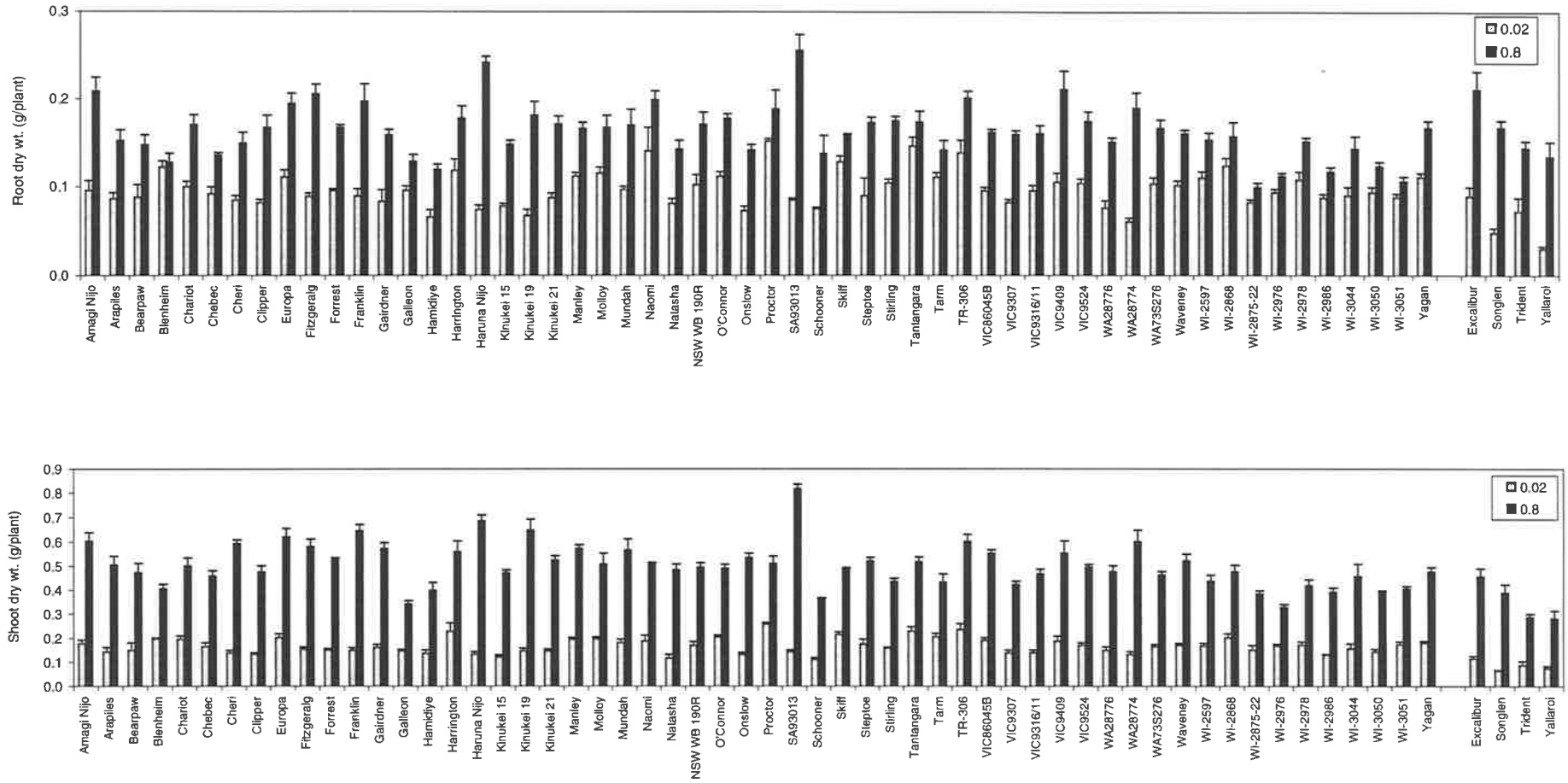


Figure 7.2. Effects of Zn fertilization (mg/kg soil) on shoot and root dry matter of barley and wheat genotypes at the early growth stage in Experiment 1. Barley and wheat genotypes were harvested at 21 DAS and 25 DAS, respectively.The vertical bars represent standard errors based on four replicates.

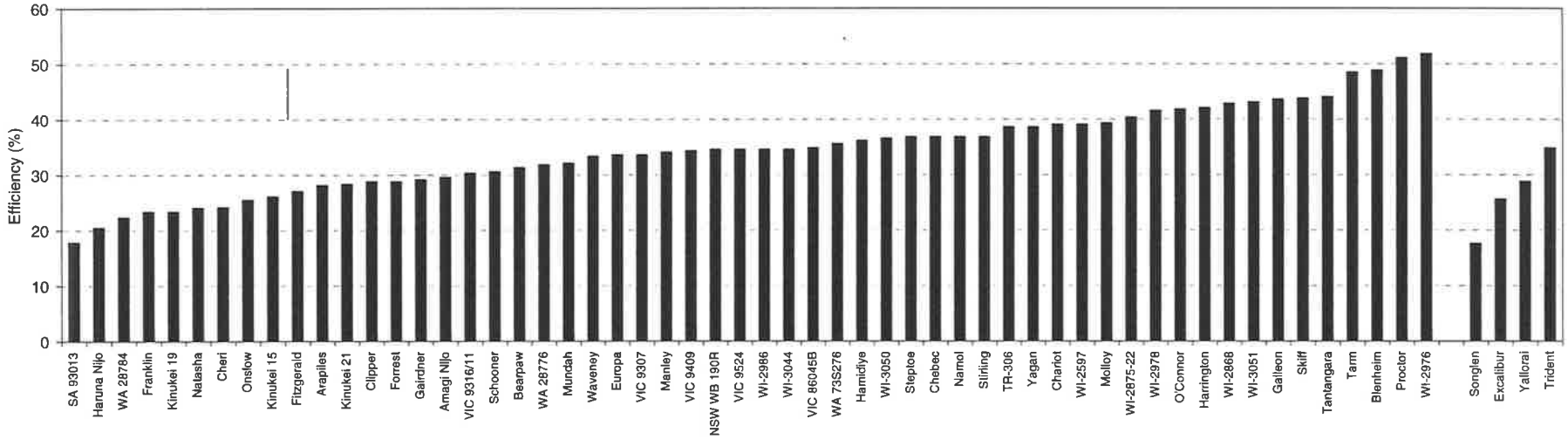


Figure 7.3. Ranking of barley and wheat genotypes for Zn efficiency, based on relative shoot dry matter [(0.02/0.8)*100] at the early growth stage. Barley and wheat genotypes were harvested at 21 DAS and 25 DAS, respectively. The vertical bar represents Tukey's $HSD_{0.05}$ value for the genotype effect.

7.3.1.3 Root:shoot dry weight ratio

Root: shoot D.W. ratio was influenced significantly by Zn fertilization in all genotypes with the exception of the durum wheat genotype Yallaroi, and the ratio was greater in Zn-deficient plants than in Zn-sufficient plants (Figure 7.4). However, genotypes differing in expression of deficiency symptoms were not always distinctly different in root: shoot D.W. ratio. For example, under Zn deficiency, there were slight symptoms in Tantangara, Skiff, Proctor and Blenheim, and severe symptoms in Forrest, Franklin, Kinukei 15 and SA93013, but they all had a similar root: shoot D.W. ratio. Even among those genotypes showing severe deficiency symptoms, some genotypes had higher root: shoot D.W. ratios (Forrest, Franklin, Kinukei 15 and SA93013) than the others (Gairdner, Kinukei 19, Onslow, Steptoe and WA28784).

7.3.1.4 Concentration and content of Zn in shoots

As expected, concentrations of Zn were much higher in plants supplied with adequate Zn than in the plants with inadequate Zn (Table 7.2). Barley genotypes differed in their Zn concentrations in shoots but only slightly when Zn supply was inadequate. For example, Proctor, Skiff, Tarm and WI-2976 had higher concentrations of Zn (5.1-5.8 mg/kg D.W.) than Amagi Nijo, Franklin, SA93013, Cheri and Yagan (3.4-3.6 mg/kg D.W.). In contrast to barley genotypes, regardless of Zn fertilization, wheat genotypes, Excalibur (Zn-efficient), and Songlen (Zn-inefficient) did not differ in their Zn concentrations in shoots. Overall, the two wheat genotypes contained higher Zn concentrations than barley genotypes.

Zn content in shoots was also much higher in plants supplied with adequate Zn than in plants supplied with inadequate Zn (Table 7.2) but the response differed with genotype. Under severe Zn deficiency, Proctor had a higher Zn content than Amagi Nijo, Cheri, Clipper, Franklin, Onslow, SA93013 and WA28784. When Zn supply was adequate,

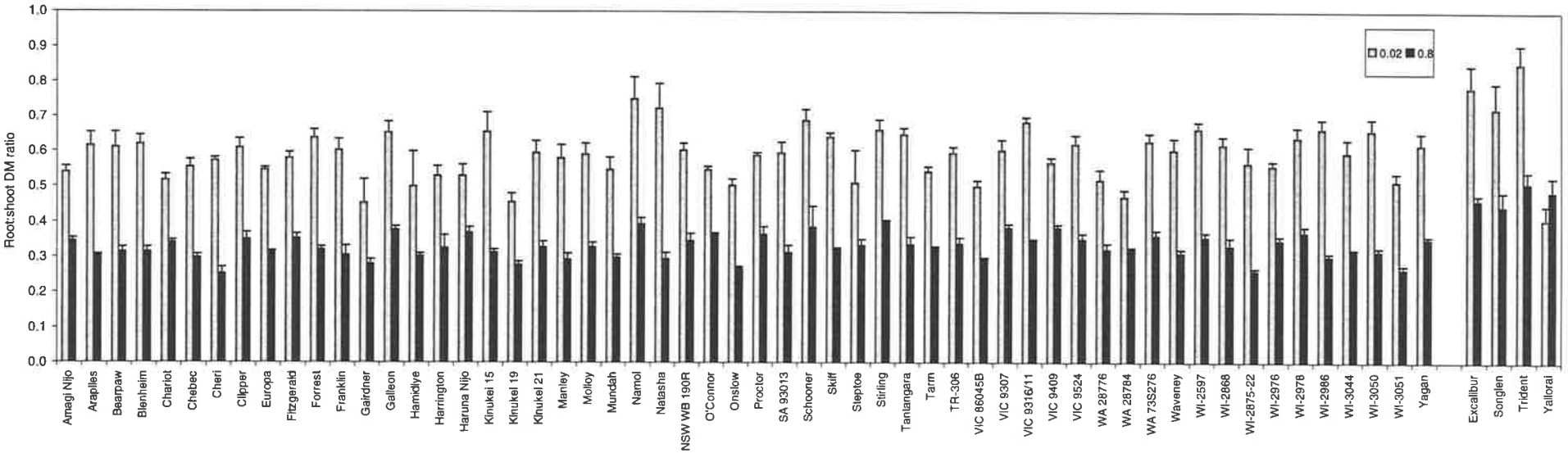


Figure 7.4. Effects of Zn fertilization (mg/kg soil) on the root:shoot dry matter ratio of barley and wheat genotypes at the early growth stage in Experiment 1. Barley and wheat genotypes were harvested at D21 and D25, respectively. The vertical bars represent standard error bars based on four replicates.

SA93013 had a higher Zn content than Skiff, Tarm, WI-2976 and Yagan due mainly to greater shoot dry matter production. Of the wheat genotypes, Excalibur (Zn-efficient) tended to have a greater Zn content than Songlen (Zn-inefficient) under severe Zn deficiency.

Table 7.2. Effects of Zn fertilization (mg/kg soil) on Zn concentration (mg/kg DW) and content (μg/plant) in the shoot of selected genotypes at 21 DAS in Experiment 1.

Genotype	Zn concentration		Zn content	
	Zn fertilization		Zn fertilization	
	0.02	0.8	0.02	0.8
Amagi Nijo	3.4 (1.22)[a]	23.2 (3.14)	0.6 (0.45)	14.5 (2.72)
Chariot	4.8 (1.57)	26.0 (3.25)	0.9 (0.66)	14.0 (2.69)
Cheri	3.5 (1.23)	22.0 (3.09)	0.5 (0.40)	13.0 (2.64)
Clipper	4.1 (1.40)	26.0 (3.26)	0.6 (0.45)	12.8 (2.62)
Forrest	4.3 (1.46)	21.5 (3.07)	0.7 (0.51)	13.0 (2.51)
Franklin	3.5 (1.24)	24.0 (3.17)	0.5 (0.43)	15.4 (2.79)
Harrington	4.2 (1.43)	22.2 (3.11)	1.0 (0.70)	13.3 (2.65)
Kinukei 21	4.4 (1.47)	25.0 (3.22)	0.6 (0.49)	13.5 (2.67)
Manley	4.4 (1.48)	23.2 (3.14)	0.9 (0.62)	13.6 (2.66)
Onslow	4.1 (1.42)	24.0 (3.18)	0.6 (0.45)	12.9 (2.63)
Proctor	5.3 (1.67)	25.0 (3.21)	1.4 (0.88)	13.4 (2.66)
SA93013	3.6 (1.28)	19.2 (2.95)	0.6 (0.45)	16.2 (2.84)
Skiff	5.6 (1.72)	22.0 (3.09)	1.2 (0.77)	9.6 (2.47)
Tantangara	4.5 (1.50)	25.2 (3.21)	1.1 (0.74)	13.2 (2.63)
Tarm	5.1 (1.64)	23.3 (3.15)	1.1 (0.74)	10.2 (2.41)
WA28784	4.2 (1.42)	20.1 (3.04)	0.6 (0.45)	13.2 (2.63)
WI-2976	5.8 (1.76)	21.3 (3.06)	1.0 (0.69)	7.1 (2.08)
Yagan	3.4 (1.21)	21.7 (3.08)	0.6 (0.49)	10.4 (2.43)
Excalibur	5.1 (1.63)	29.0 (3.37)	0.5 (0.42)	13.0 (2.63)
Songlen	5.1 (1.62)	32.7 (3.48)	0.3 (0.29)	13.1 (2.64)
Tukey's $HSD_{0.05}$[b]				
Genotype x Zn fertilization		(0.38)		(0.39)

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.
[b] The HSD values are applicable to log-transformed data (in parentheses).

7.3.1.5 Concentrations of Fe, P and Mn in shoots

Zn fertilization influenced concentration of other nutrients, but marked differences were noted in Fe, P and Mn concentrations (Table 7.3). Generally, concentrations of Fe, P and Mn were much greater in Zn-deficient plants than in Zn-sufficient plants. Significant

genetic differences occurred only when plants were Zn-deficient. In the barley genotypes grown under Zn deficient conditions, Franklin and Cheri had considerably higher Fe concentrations than Proctor and WI-2976. In contrast to barley, under Zn deficiency, wheat genotypes did not differ significantly in Fe concentration of shoots. However, wheat genotypes had lower concentrations of Fe than barley genotypes.

Phosphorus and Mn concentrations in shoots followed a different pattern to that of Fe concentration: among the barley genotypes, under Zn deficiency, WA28784 had the highest, while Kinukei 21 had the lowest P concentration. In the case of Mn, Franklin had a high concentration compared with Amagi Nijo, Onslow and Tarm. Wheat genotypes did not differ in P and Mn concentration; however, they tended to have greater Mn and P concentrations than barley genotypes under low Zn supply.

Table 7.3. Effects of Zn fertilization (mg/kg soil) on concentration of Fe, P and Mn (mg/kg DW) in the shoot of selected genotypes at 21 DAS in Experiment 1.

Genotype	Fe concentration		P concentration		Mn concentration	
	Zn fertilization		Zn fertilization		Zn fertilization	
	0.02	0.8	0.02	0.8	0.02	0.8
Amagi Nijo	277 (5.62)[a]	71 (4.27)	11730 (9.36)	5400 (8.59)	43.3 (3.67)	24.3 (3.19)
Chariot	330 (5.80)	84 (4.43)	12200 (9.41)	5800 (8.67)	52.0 (3.95)	18.8 (2.92)
Cheri	557 (6.32)	76 (4.32)	9700 (9.18)	5570 (8.62)	51.7 (3.95)	18.0 (2.86)
Clipper	303 (5.69)	79 (4.37)	11630 (9.36)	6500 (8.78)	49.0 (3.86)	18.7 (2.90)
Forrest	303 (5.71)	74 (4.29)	11970 (9.38)	4700 (8.45)	44.7 (3.78)	15.8 (2.76)
Franklin	717 (6.57)	75 (4.32)	9000 (9.09)	5870 (8.68)	86.0 (4.50)	18.5 (2.91)
Harrington	373 (5.92)	77 (4.34)	9370 (9.14)	5700 (8.65)	43.7 (3.76)	13.8 (2.62)
Kinukei 21	290 (5.64)	59 (4.08)	6700 (8.78)	5130 (8.54)	42.0 (3.71)	21.8 (3.05)
Manley	433 (6.06)	87 (4.47)	9100 (9.09)	6300 (8.74)	68.3 (4.12)	14.9 (2.70)
Onslow	287 (5.65)	77 (4.34)	13700 (9.48)	5800 (8.66)	40.0 (3.65)	16.3 (2.79)
Proctor	257 (5.54)	90 (4.50)	7430 (8.86)	6130 (8.72)	46.0 (3.81)	20.6 (3.02)
SA93015	290 (5.67)	67 (4.21)	10600 (9.16)	5500 (8.61)	52.3 (3.94)	17.3 (2.85)
Skiff	453 (6.05)	79 (4.37)	10400 (9.24)	5800 (8.66)	60.0 (3.98)	17.5 (2.85)
Tantangara	473 (6.16)	99 (4.56)	7630 (8.91)	6670 (8.80)	69.7 (4.24)	19.9 (2.98)
Tarm	357 (5.87)	83 (4.42)	8570 (9.05)	5930 (8.68)	32.3 (3.45)	17.8 (2.88)
WA28784	390 (5.96)	81 (4.39)	16900 (9.73)	5500 (8.61)	51.7 (3.93)	14.6 (2.67)
WI-2976	227 (5.42)	88 (4.48)	9270 (9.09)	5300 (8.58)	55.7 (4.02)	20.4 (3.01)
Yagan	366 5.67)	83 (4.41)	7800 (8.95)	6170 (8.55)	56.7 (4.02)	14.4 (2.67)
Excalibur	183 (5.21)	96 (4.57)	18570 (9.82)	6430 (8.77)	148.3 (4.99)	43.0 (3.74)
Songlen	211 (5.33)	109 (4.68)	14270 (9.56)	6300 (8.75)	122.7 (4.75)	36.0 (3.58)
Tukey's $HSD_{0.05}$[b]						
Genotype x Zn fertilization	(0.72)		(0.63)		(0.83)	

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

[b] The $HSD_{0.05}$ values are applicable to log-transformed data (in parentheses).

7.3.2 Experiment 2

7.3.2.1 Visual symptoms

At day 15, typical Zn deficiency symptoms, as described earlier (7.7.1.1), appeared first in plants of Forrest and Amagi Nijo grown from small and medium seed when fertilized with 0.02 mg Zn/kg soil. At day 16, Forrest and Amagi Nijo grown from large seed, WI-2868 from small seed, Schooner and Skiff from medium seed exhibited deficiency symptoms at 0.02 mg Zn/kg soil. Tantangara was the last genotype to show Zn deficiency symptoms and even then, symptoms were mild compared to those observed in other genotypes.

There was a tendency that symptoms first appeared in plants grown from small seed then medium and finally large seed. However, at harvest, these differences in deficiency symptoms resulting from different seed sizes were negligible in all genotypes. Based on a scale of 1 (green leaves) to 6 (dead growing points), regardless of seed size, genotypes ranked, from the most to least severe Zn-deficiency symptoms, Forrest> Amagi Nijo > Schooner > WI-2868 > Skiff > Tantangara (Table 7.4; Plate 7.2.

Table 7.4. The expression of Zn deficiency symptoms (visual scores) in barley genotypes fertilized with 0.02 mg Zn/kg soil at 21 DAS in Experiment 2[a].

Genotype	Score
Forrest	4.5
Skiff	2.5
Amagi Nijo	3.8
Tantangara	2.0
Schooner	3.0
WI-2868	2.8
Tukey's $HSD_{0.05}$	1.1

[a]Average of three seed size

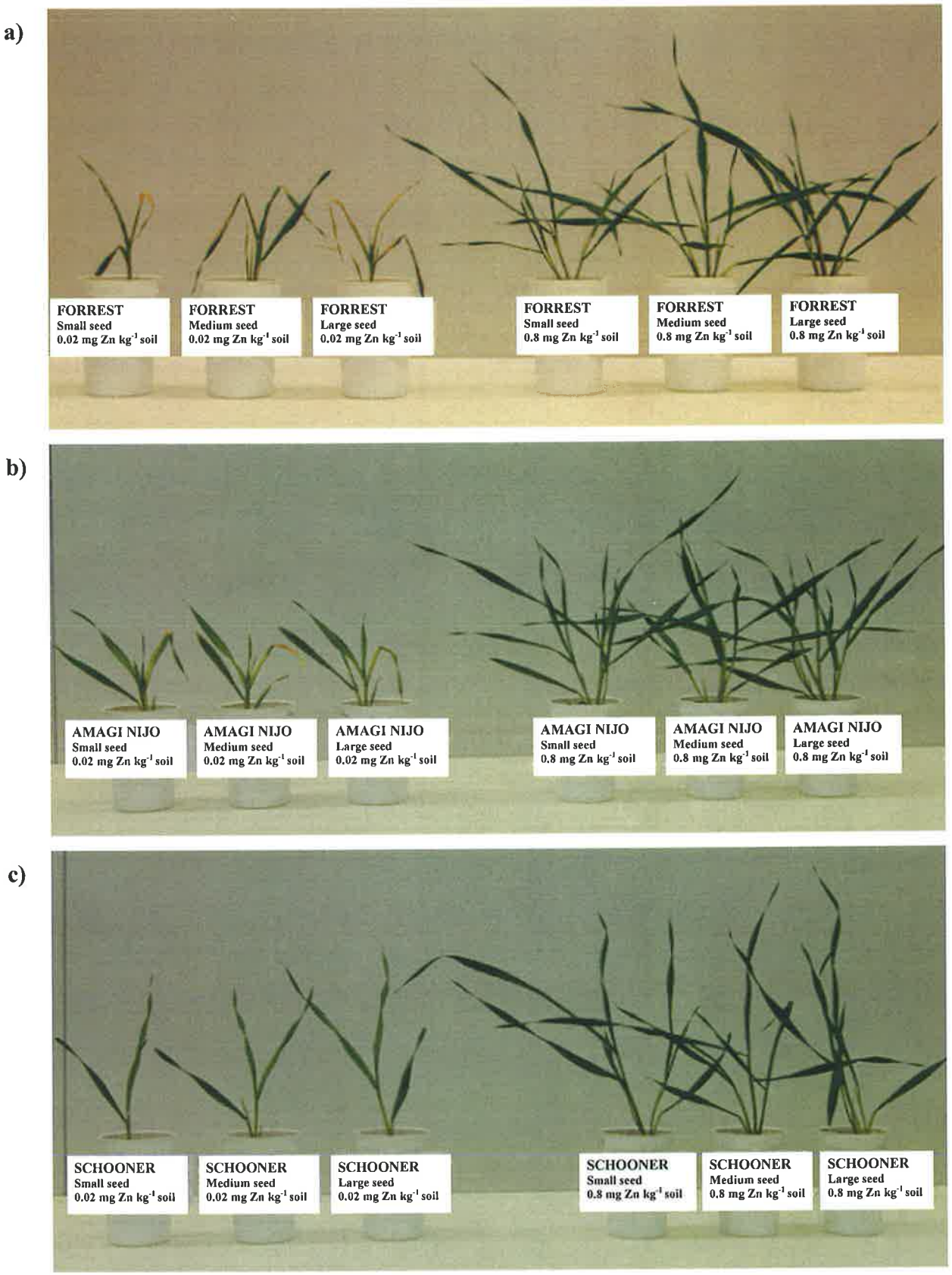
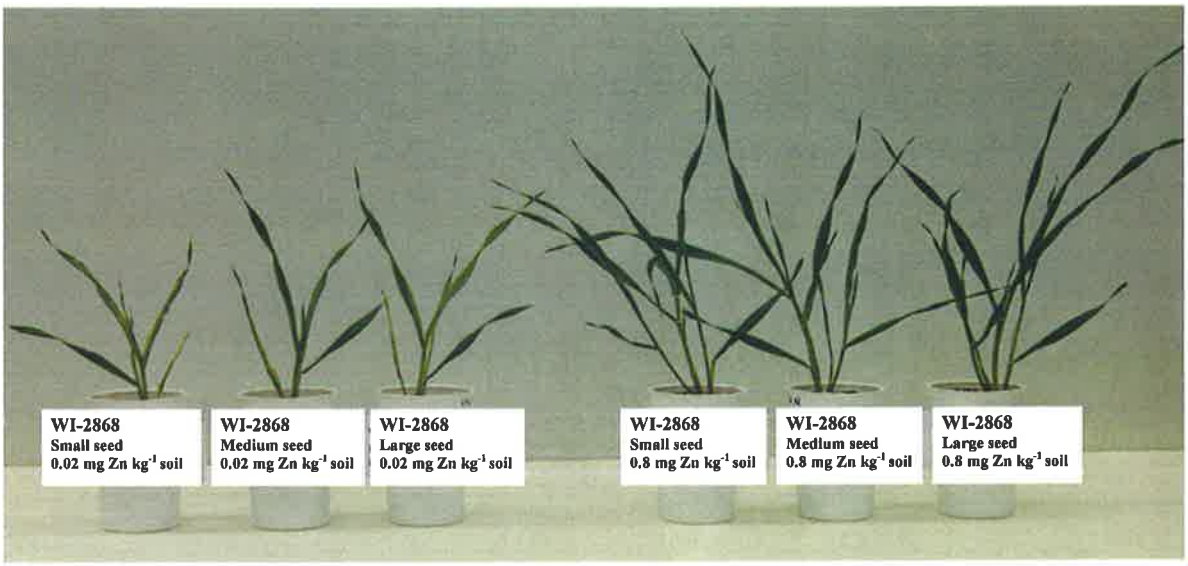


Plate 7.2. Effects of seed size and Zn fertilization on expression of Zn deficiency symptoms in barley genotypes (a, Forrest; b, Amagi Nijo; c, Schooner) at 21 DAS in Experiment 2.

d)



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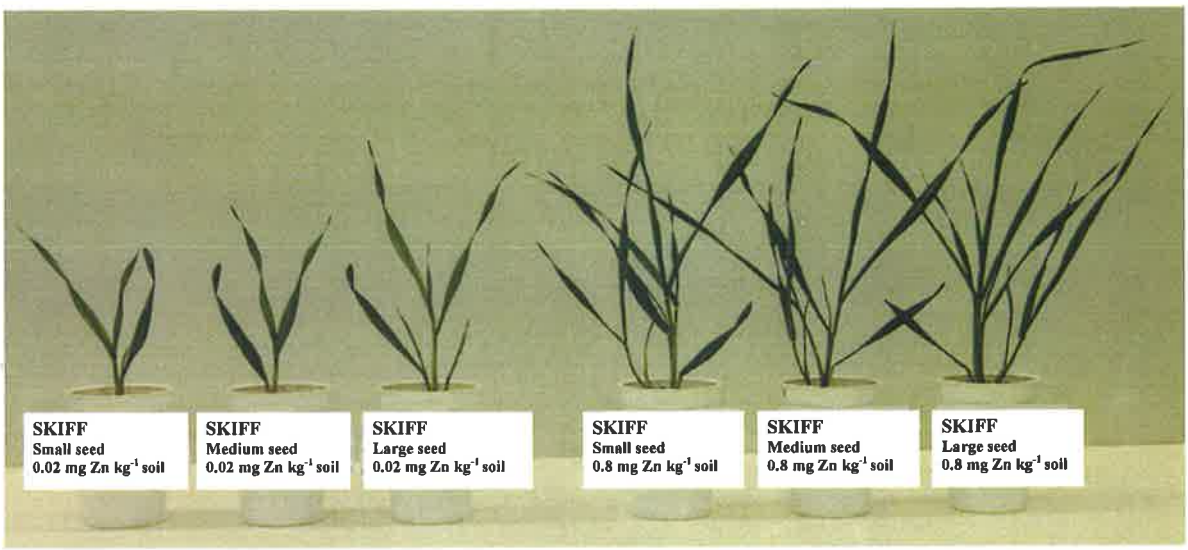

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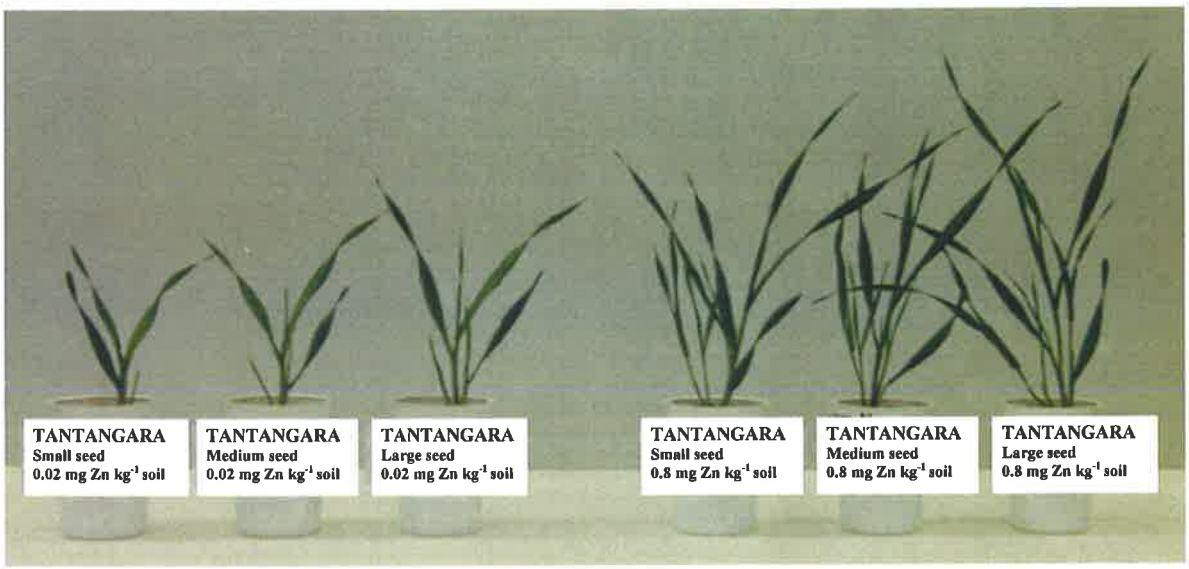


Plate 7.2. continued (d, WI-2868; e, Skiff; f, Tantangara).

7.3.2.2 Shoot and root dry matter

Seed size had a significant effect on root and shoot dry matter production at harvest (Tables 7.5b and 7.6b) On average, plants grown from large and medium seed produced 20 and 10% more shoot dry matter than those grown from small seed, respectively. The corresponding values for root dry matter were 40 and 20%, respectively. However, these responses were affected by Zn supply and genotype. Under Zn deficiency, Skiff, Tantangara and Schooner grown from large seed accumulated greater shoot dry matter than when grown from medium and small seed, while Forrest, Amagi Nijo and WI-2868 remained unaffected by seed size (Table 7.5a), but the differences were significant only in Tantangara. Under sufficient Zn supply, seed size effect was still evident. Most of the genotypes produced more shoot dry matter when grown from large seed compared with small (Forrest, Skiff, Amagi Nijo, Tantangara and WI-2868) and medium seed (Amagi Nijo and WI-2868) but only Forrest and Amagi Nijo grown from large seed had significantly higher shoot dry matter compared with small seed (Table 7.5a). In contrast to shoot dry matter, Genotype x Seed size x Zn fertilization interaction was found to be non-significant for root dry matter. It was only when Zn supply was adequate that WI-2868 had slightly greater root dry matter than Skiff, Tantangara and Schooner (Table 7.6a). Overall, root dry matter was greater when supplied with adequate than inadequate Zn (Table 7.6a).

Significant genetic differences in Zn efficiency occurred only when genotypes were grown from large seed: Tantangara had greater Zn efficiency than Amagi Nijo, Forrest and WI-2868 (Figure 7.5).

Table 7.5. The effects of Zn fertilization (mg/kg soil) and seed size on shoot dry matter (g/plant) of barley genotypes at 21 DAS in Experiment 2.

(a) G x Zn fert. x Seed size

	Zn fertilization					
	0.02			0.8		
Genotype	Small	Medium	Large	Small	Medium	Large
Forrest	0.11	0.15	0.15	0.34	0.44	0.48
Skiff	0.12	0.15	0.20	0.42	0.46	0.49
Amagi Nijo	0.14	0.16	0.16	0.43	0.47	0.56
Tantangara	0.12	0.16	0.23	0.37	0.40	0.45
Schooner	0.10	0.13	0.17	0.40	0.41	0.42
WI-2868	0.16	0.19	0.20	0.48	0.48	0.55
Tukey's $HSD_{0.05}$			0.10			

(b) Seed size

	Seed size		
	Small	Medium	Large
	0.27	0.30	0.34
Tukey's $HSD_{0.05}$		0.02	

Table 7.6. The effects of Zn fertilization (mg/kg soil) and seed size on root dry matter (g/plant) of barley genotypes at 21 DAS in Experiment 2.

(a) Genotype x Zn fert.

	Zn fertilization	
Genotype	0.02	0.8
Forrest	0.08	0.16
Skiff	0.10	0.15
Amagi Nijo	0.08	0.18
Tantangara	0.11	0.15
Schooner	0.08	0.15
WI-2868	0.11	0.19
Tukey's $HSD_{0.05}$	0.04	

(b) Seed size

	Seed size		
	Small	Medium	Large
	0.11	0.13	0.15
Tukey's $HSD_{0.05}$		0.01	

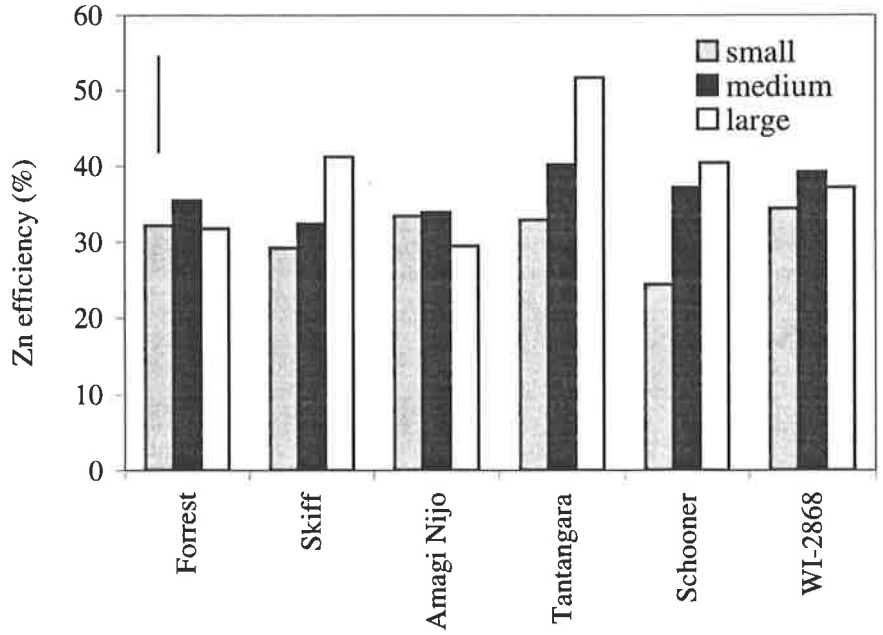


Figure 7.5. Effects of seed size on the expression of Zn efficiency (the ratio of shoot dry matter at 0.02 to that at 0.8 mg Zn/kg soil, %) in barley genotypes at 21 DAS in Experiment 2.The vertical bar represents Tukey's $HSD_{0.05}$ value for the genotype x seed size interaction.

7.3.3 Experiment 3

7.3.3.1 Visual symptoms and plant development

The typical symptoms of Zn deficiency, such as stunted shoot growth and pale yellow linear stripes on young leaves became visible first in plants of the Zn-inefficient durum genotype Yallaroi grown at 0.1 mg/ Zn/kg, 3 weeks after sowing. At this stage, the Zn-efficient wheat genotype Excalibur and all the barley genotypes did not show any visual symptoms of Zn deficiency. As growth progressed, the effect of Zn deficiency on plant growth became more evident, which was manifested by stunted shoot growth.

By the awn-visible stage, linear, pale yellow stripes were visible on flag leaves of barley genotypes, WA28784, Onslow, SA93013, Franklin, Amagi Nijo, Forrest, Harrington and Clipper. Those that developed no symptoms included Skiff, Tarm, Kinukei 21 and

Chariot. Of the wheat genotypes, the Zn-inefficient Yallaroi, developed the most severe leaf symptoms, while Excalibur exhibited only stunted growth.

Zn deficiency also delayed development but the effect varied with genotype. In Zn-deficient plants of Amagi Nijo, Forrest, WA28784, Onslow, SA93013 and Franklin, awns were not visible in contrast to their Zn-sufficient counterparts, and maturity was delayed 2-3 weeks. The other genotypes remained unaffected in their timing of awn appearance. Generally, the effect of Zn deficiency was greatest in stems other than the main culms.

7.3.3.2 Total dry matter production

At the awn visible stage, both barley and wheat genotypes responded significantly to Zn fertilization (Figure 7.6a). Among barley genotypes, under both low and adequate soil Zn, Harrington, Cheri, Onslow, Tarm, SA93013 and Yagan had greater shoot dry matter than the other genotypes. Of wheat genotypes, Excalibur had considerably higher shoot dry matter compared with Yallaroi under both low and adequate soil Zn. Zn efficiency among barley genotypes ranged from 42% (Amagi Nijo) to 69% (Skiff) and was greater than wheat genotypes (Yallaroi, 9%; Excalibur, 39%) (Figure 7.7a).

At maturity, dry matter followed a different pattern to that observed at awn visible stage. Among barley genotypes, under low soil Zn, Skiff, Clipper, Harrington, Tarm, Kinukei 21, Chariot and WI-2976 had higher total top yield (straw + grain) than the other genotypes (Figure 7.6b). Of the wheat genotypes, Excalibur had significantly higher total top yield than Yallaroi only under low soil Zn. Both barley and wheat genotypes showed a similar range of Zn efficiency (37-64% for barley; 2-44% for wheat) to that observed at awn visible stage (Figure 7.7b).

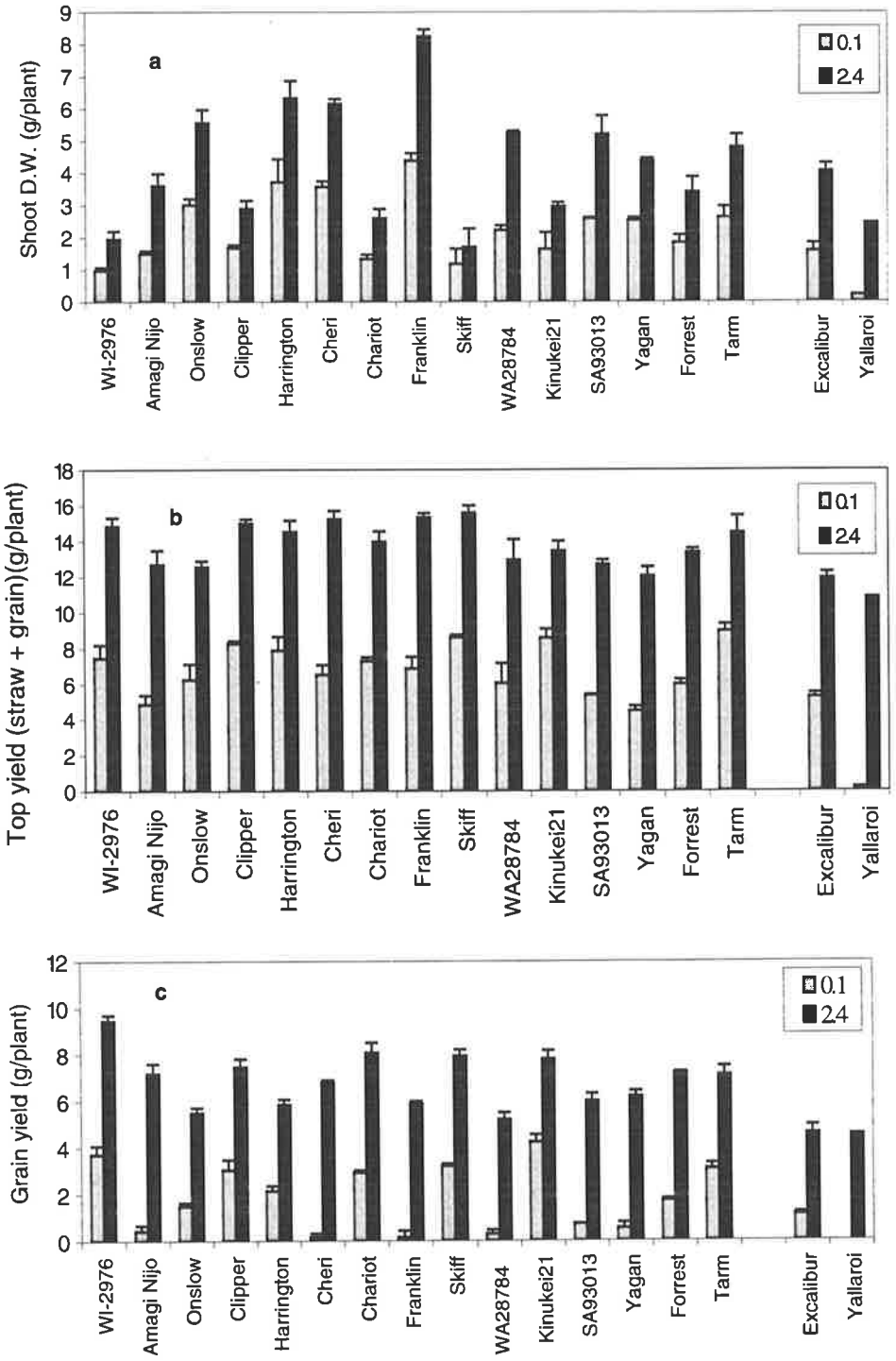


Figure 7.6. Effects of Zn fertilization (mg/kg soil) on top (a=awn visible stage; b=maturity) and grain yield (c) of barley and wheat genotypes grown in a growth room. The vertical bars represent standard errors based on three replicates.

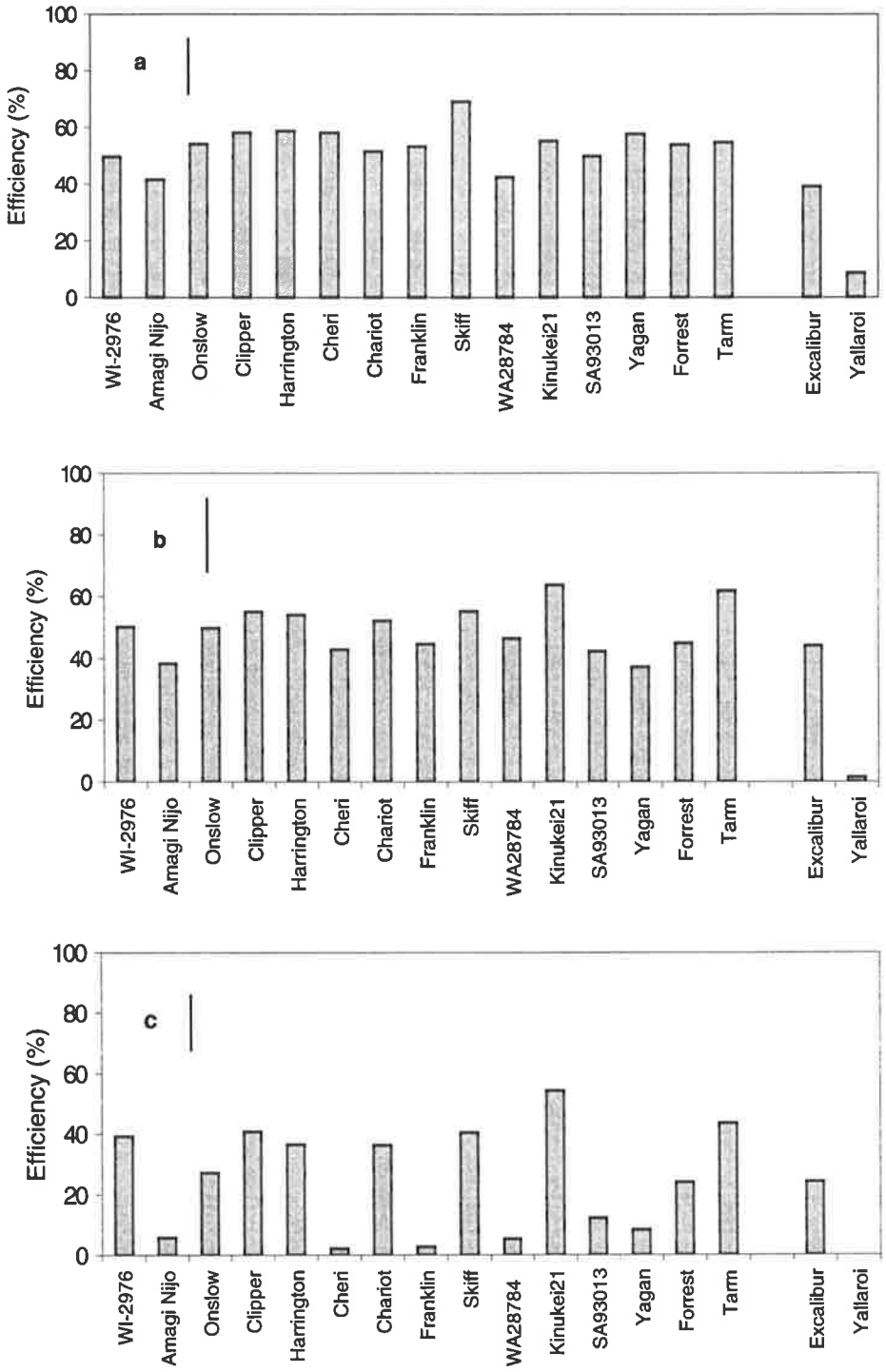


Figure 7.7. Zn efficiency of barley and wheat genotypes at awn visible stage (a) and maturity (b=total top yield; c=grain yield) grown in a growth room. The error bars represent Tukey's $HSD_{0.05}$ values for genotype effect.

7.3.3.3 Grain yield

In terms of absolute grain yield, genotypes differed significantly in their response to soil Zn (Plate 7.2). Under adequate soil Zn, Skiff, Kinukei 21, Chariot and WI-2976 had significantly higher grain yield than Harrington, Onslow, Franklin, Amagi Nijo, and WA28784 (Figure 7.6c). Under low soil Zn, Skiff, Kinukei 21, Chariot, WI-2976, Clipper, and Tarm had considerably higher grain yield than Yagan, Cheri, Franklin, SA93013, Amagi Nijo and WA28784. Of wheat genotypes, Yallaroi failed to produce grain at low soil Zn supply.

Zn efficiency based on grain yield, varied significantly among genotypes (Figure 7.7c). Zn efficiency ranged from 5% for WA28784 to 54% for Kinukei 21. In the two wheat genotypes, efficiency was recorded 24% for Excalibur and 0% (no grain production) for Yallaroi. Using Zn efficiency ranking based on grain yield, Cheri, Franklin, WA28784, Yagan, Amagi Nijo, Onslow, and SA93013 can be categorized as inefficient, while Kinukei 21, Tarm, Clipper, WI-2976, Skiff, Harrington and Chariot as efficient. Forrest seems to be intermediate in its Zn efficiency. According to this efficiency ranking, Excalibur seems to be intermediate in its efficiency, while Yallaroi can be categorized as Zn-inefficient.

The Zn-efficient genotypes generally produced greater numbers of grains per plant compared to Zn-inefficient genotypes under low soil Zn (Table 7.7). In addition, under low soil Zn, grains of Zn-efficient genotypes were much larger than those of Zn-inefficient genotypes (Plate 7.4; Table 7.7). However, the effect of Zn deficiency was more pronounced on stems other than main culms. In Zn-inefficient genotypes (e.g. Amagi Nijo), tillers other than main culms often failed to produce grain under low soil Zn, while Zn-efficient genotypes (e.g. Kinukei 21) produced considerable amount of grain (Plate 7.4).

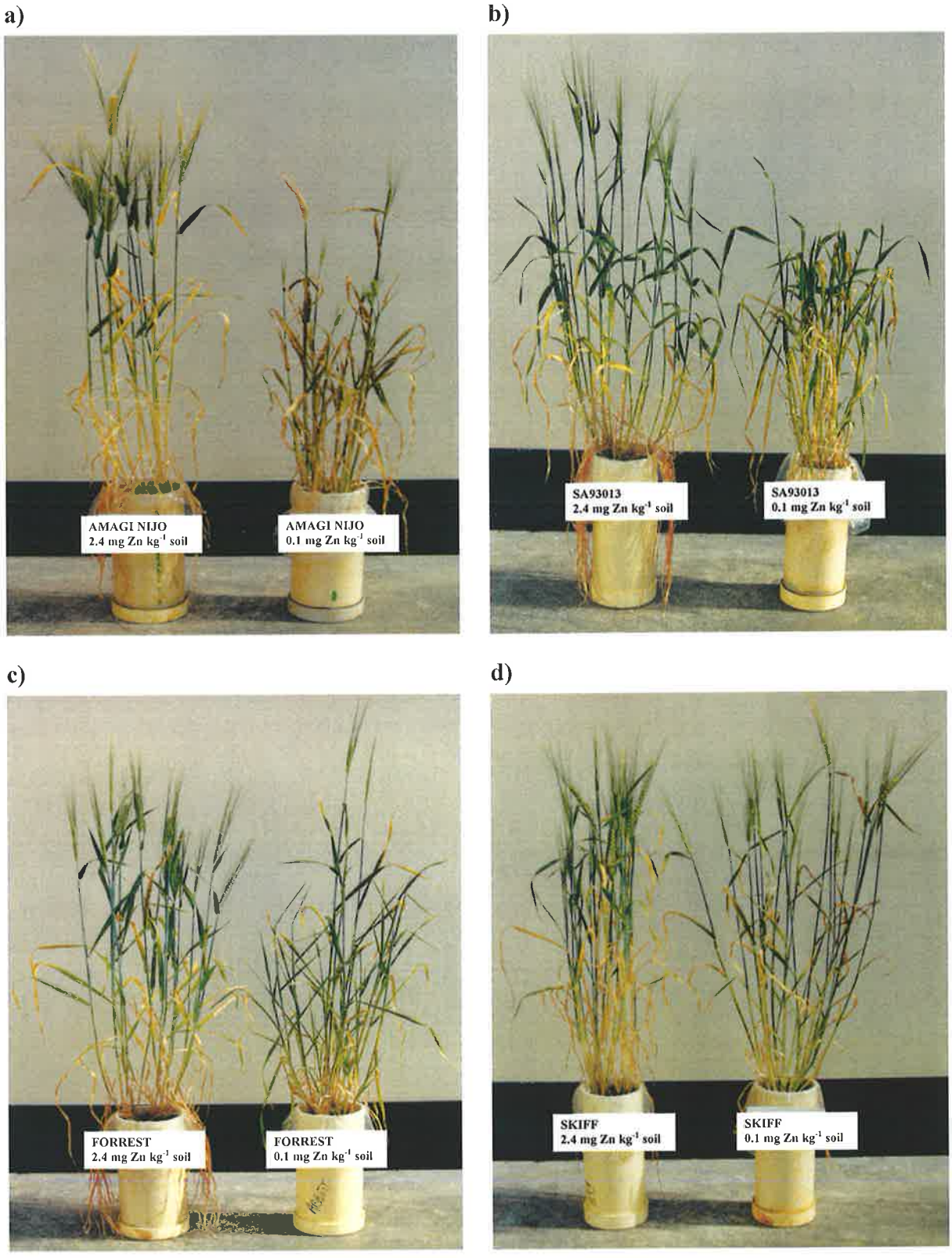


Plate 7.3. Effects of Zn fertilization on grain production of barley and wheat genotypes approaching maturity in Experiment 3 (a, Amagi Nijo; b, SA93013; c, Forrest; d, Skiff).

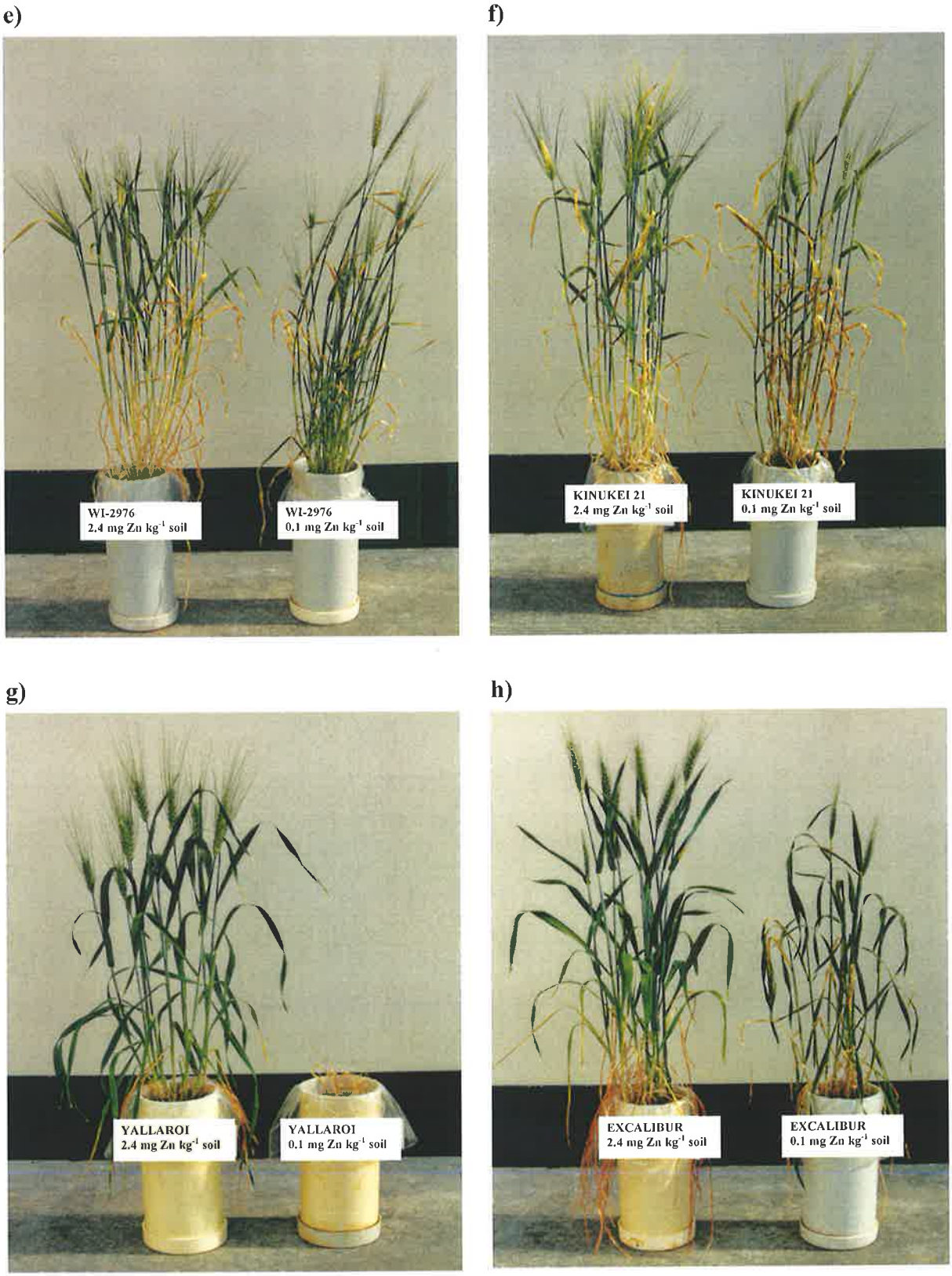


Plate 7.3. continued (e, WI-2976; f, Kinukei 21; g, Yallaroi; h, Excalibur).

Table 7.7. Effects of Zn fertilization (mg/kg soil) on number of tillers per plant, number of ears per plant, number of grains per ear, number of grains per plant, dry weight (D.W.) per grain (mg), and harvest index (HI).

Genotype	No.of tillers/plant Zn fert.		No.of ears/plant Zn fert.		No.of grains/ear Zn fert.		No.of grains/plant Zn fert.		D.W./grain (mg) Zn fert.		HI (%) Zn fert.	
	0.1	2.4	0.1	2.4	0.1	2.4	0.1	2.4	0.1	2.4	0.1	2.4
Barley												
Skiff	10.7	10.3	9.7	10.3	10.7	19.0	102.0	192.7	33	41	38	51
Clipper	8.7	8.3	6.0	8.3	15.3	20.0	90.0	160.7	35	47	37	50
Harrington	6.3	8.0	6.0	8.0	17.0	26.7	98.7	209.3	23	28	27	40
Tarm	7.0	7.0	6.3	7.0	13.7	23.3	74.0	158.3	42	45	35	49
Yagan	10.0	7.0	4.7	7.0	5.7	19.0	32.7	133.3	21	47	11	52
Cheri	8.0	7.7	4.0	7.7	4.0	23.7	11.7	176.7	9	39	2	45
Kinukei21	7.3	7.0	7.3	7.0	17.7	25.0	120.7	170.3	36	46	49	58
Forrest	6.3	6.3	6.0	6.3	10.3	22.0	60.0	130.7	29	56	29	54
Onslow	8.7	9.3	5.7	9.3	11.7	23.0	77.7	203.7	14	27	14	44
Franklin	10.0	8.3	3.0	8.0	2.7	28.7	11.7	218.7	9	27	2	38
Chariot	9.3	11.0	7.3	9.7	15.0	21.0	105.3	198.3	28	41	40	58
WI-2976	11.7	11.0	10.7	11.0	10.7	16.7	106.3	179.0	36	53	50	63
SA93013	7.7	9.0	5.0	8.7	11.3	21.0	53.7	179.3	14	34	14	47
Amagi Nijo	6.3	6.3	5.7	11.0	4.0	28.3	22.7	164.7	19	44	16	57
WA28784	6.3	7.3	3.7	7.3	2.0	25.0	14.7	174.7	6	30	4	40
Wheat												
Excalibur	8.0	7.7	3.7	4.3	14.3	35.3	48.0	151.3	24	31	21	39
Yallaroi	4.7	6.3	0.0	6.3	0.0	24.0	0.0	133.0	0	35	0	42
Tukey's $HSD_{0.05}$												
Genotype x Zn fert.	4.2		5.0		10.5		75.2		16		18	

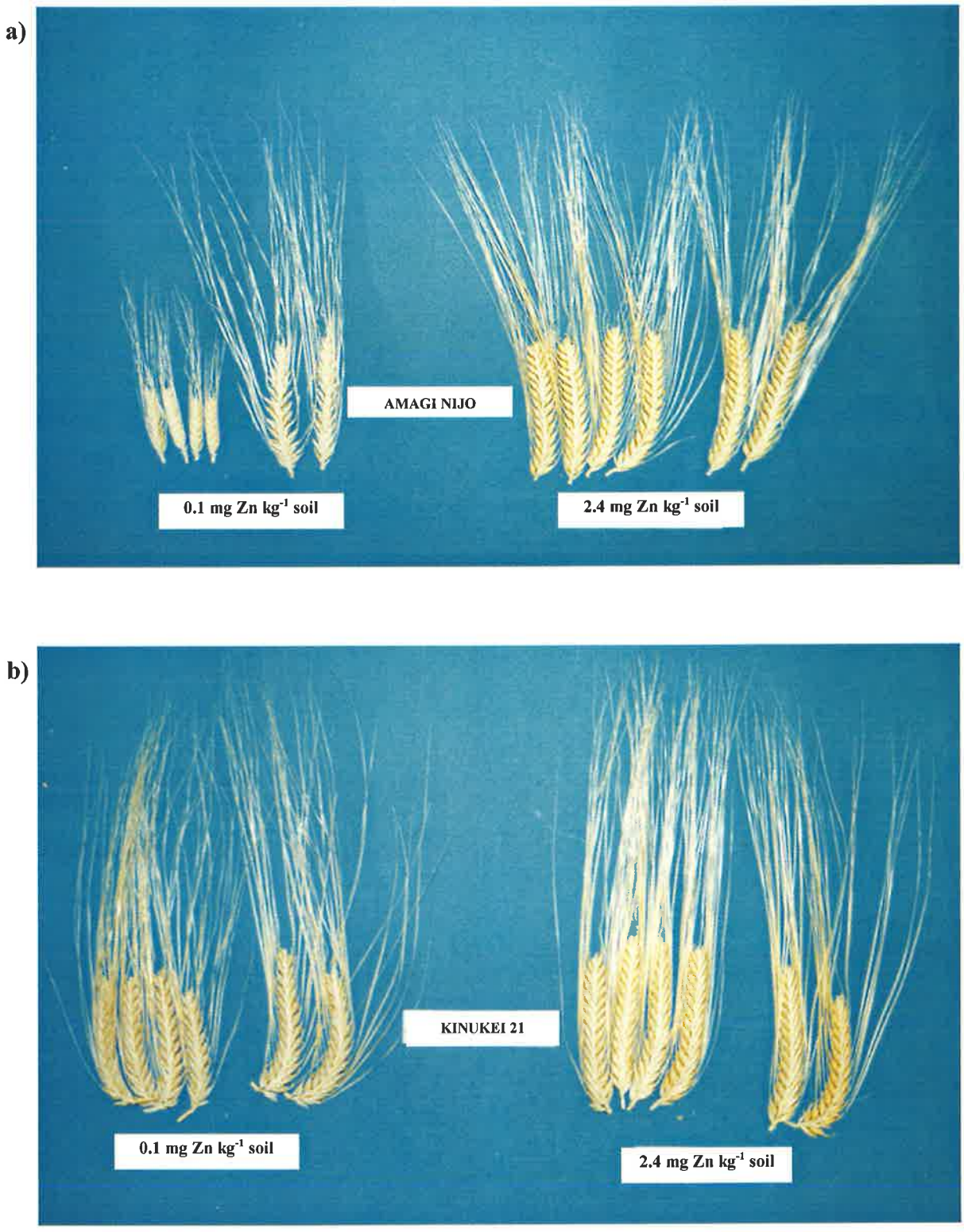


Plate 7.4. Effect of Zn fertilization on grain production in main (far right) and primary tillers (far left) of barley genotypes (a, Amagi Nijo; b, Kinukei 21) at maturity in Experiment 3.

7.3.3.4 Harvest index

Genotypes differed significantly in HI, depending on Zn status of soil. Under adequate soil Zn, Kinukei 21, WI-2976, Chariot and Amagi Nijo had higher HIs than Franklin, Harrington, and WA28784 (Table 7.7). In contrast, under low soil Zn, a slightly different pattern was observed: Skiff, Kinukei 21, Chariot and WI-2976 had significantly higher HIs than the other genotypes (Table 7.7). Wheat genotypes differed in HI only under low soil Zn. Overall, barley genotypes had greater HIs than wheat genotypes.

7.4 Discussion

7.4.1 Experiment 1

The results of the present study demonstrated that barley genotypes differed considerably in their tolerance to Zn deficiency, as indicated by the wide range in the severity of Zn deficiency symptoms as well as in the reduction in shoot growth. The differences in the severity of Zn deficiency symptoms and reduction in shoot growth are likely to be inherent and not confounded much by the variation in seed Zn content among genotypes since all the genotypes had reasonably similar Zn content in the seed. Of the control wheat genotypes of known Zn efficiency, Zn-inefficient Songlen and Yallaroi showed very severe Zn-deficiency symptoms, while Zn-efficient genotypes developed less severe symptoms (e.g. symptoms were slight in Trident and severe in Excalibur). The fact that in the present study, Excalibur, which is considered Zn-efficient showed symptoms of Zn deficiency provided evidence that Lancelin sand generated severe Zn deficiency stress. Previous studies with this genotype have been conducted in Laffer sand (Rengel and Graham, 1995a,b), which produces less severe Zn deficiency (Chapter 6).

Based on 56 barley and 4 wheat genotypes in the present study, the relationship between visual scores and reduction in shoot dry matter generally appeared to be linear (Figure 7.8), suggesting the possibility exists to use visual scores as a parameter for assessing

genotypes in their tolerance to Zn deficiency. This suggestion is supported by Cakmak *et al.* (1998) who studied a wide range of cereals, (*Secale cereale*, Triticale, *Hordeum vulgare*, *Triticum aestivum*, *Triticum durum* and *Avena sativa)* in response to Zn deficiency, and found that the effect of Zn deficiency on shoot dry matter production was very similar to the effect on leaf symptoms. Their results also show a linear relationship between reduction in shoot dry matter and the severity of leaf symptoms. However, both in the present study and in the study of Cakmak *et al.* (1998), there was a range in dry matter responses within each class of visual symptoms. For example, in the present study, Kinukei 21 had a greater reduction (72%) in shoot dry matter than Proctor (49%) despite the two genotypes being similar in expression of leaf symptoms (score 3). This was also evident in the study of Cakmak *et al.* (1998): *S. cereale* cv. Aslim had greater reduction (24%) in shoot dry matter than a local rye variety (6%) although they both were given the same visual score (5=symptoms are very slight or absent). This departure from the general trend can be expected to a certain extent, when a large number of genotypes is considered, which indicates genetic differences on Zn deficiency symptoms (e.g. appearance and severity of Zn deficiency symptoms).

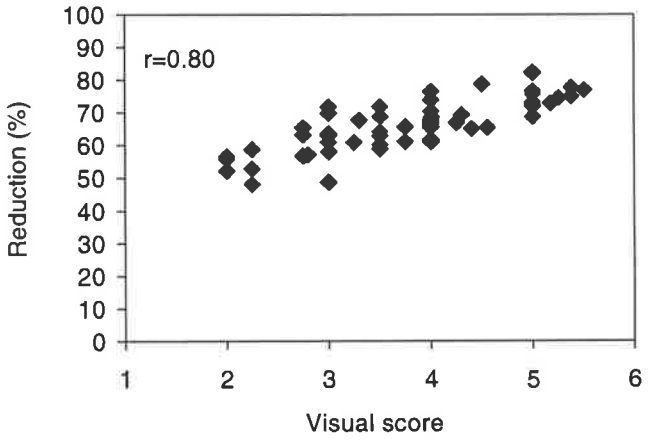


Figure 7.8. The relationship between Zn deficiency symptoms (visual scores; 1=green leaf, 6=dead growing points) and reduction in shoot dry matter of 56 barley (21 DAS) and 4 wheat (25 DAS) genotypes in Experiment 1. Data are means of four replicates.

The increase in root: shoot DM ratio under Zn deficiency (Cumbus, 1985; Loneragan *et al.*, 1987; Khan *et al.*, 1998a) generally occurs as an initial response to Zn deficiency. However, the nature of this phenomenon is not known well. In the literature, two different explanations have been found. According to Khan *et al.* (1998a), reduction in shoot growth at the expense of root growth may decrease metabolic demands of shoots, and increase relative surface area for ion absorption. In contrast, Cakmak *et al.* (1996b) consider it as a result of Zn deficiency-induced photo-oxidative damage in shoots which in turn results in a lower shoot growth. The present study did not investigate the factors responsible for the increase in root: shoot DM ratio under Zn deficiency. Therefore, the discussion will be restricted to a consideration of potential for root: shoot DM ratio as an additional parameter for assessing genotypes in tolerance to Zn deficiency. From the results reported here, it appears that root: shoot DM ratio is not useful in distinguishing between Zn-efficient and Zn-inefficient genotypes since it does not correlate with sensitivity of genotypes to Zn deficiency.

The Zn content of the shoots was better correlated with sensitivity of genotypes to Zn deficiency than Zn concentration in the shoot (Figure 7.9). Generally, genotypes with less severe deficiency symptoms contained more Zn in their shoots than those with more severe deficiency symptoms. This result is in accordance with the published results of other investigators who found that in field (Graham *et al.*, 1992), greenhouse (Cakmak *et al.*, 1996b) and nutrient solution studies (Rengel and Graham 1995), Zn-efficient bread wheat genotypes accumulated more Zn in the shoots that Zn-inefficient durum wheat genotypes. Cakmak *et al.* (1996b) suggested that the greater Zn accumulation in bread wheat genotypes than in durum wheat genotypes at deficient Zn supply could be due to higher Zn uptake and higher root-to-shoot transport capacity for Zn. Although experimental evidence is lacking in this study, from earlier results with Zn-efficient Tarm and Zn-inefficient Hamidiye (Chapter 4), it can be argued that the greater uptake and

root-to-shoot translocation of Zn at low Zn supply can also markedly contribute to tolerance to Zn deficiency in barley.

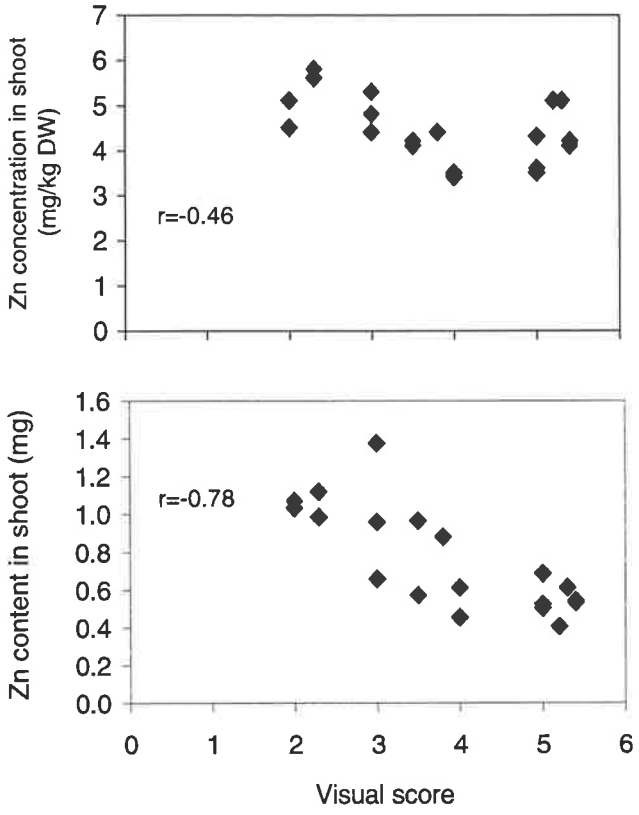


Figure 7.9. The relationship between Zn deficiency symptoms (Visual scores; 1=green leaf, 6=dead growing points) and Zn concentration (a) and content (b) of shoots of 18 barley (21 DAS) and 2 wheat genotypes (25 DAS) in Experiment 1. Data are means of three replicates (r values for Zn concentration and content are significant at 5% and 1%, respectively.)

High accumulation of Fe in shoots of Zn-deficient plants is a well-known phenomena (Cakmak *et al.*, 1994; Walter *et al.*, 1994). In this study as well as in previous studies (Chapters 3, 4, 5 and 6), the concentrations of Fe in shoot dry matter were markedly higher in Zn-deficient plants than in Zn-sufficient plants. This increase in Fe concentration under Zn deficiency is beyond the "concentration effect" which is a result of reduced shoot growth. Although the actual cause of the high Fe accumulation under Zn

deficiency is not known, greater passive uptake of Fe caused by increased root cell membrane permeability has been suggested previously as a possibility (see discussion in Chapter 6). In their study with bread and durum wheats, Cakmak *et al.* (1996c) reported that higher levels of phytosiderophores in Zn-deficient bread wheats over durum wheats may be involved in enhanced Fe translocation from roots to shoots. In the present study, the pattern of Fe accumulation was not consistent with sensitivity to Zn deficiency across the genotypes, indicating that phytosiderphores may not be involved in Fe accumulation in shoots of Zn-deficient barley plants. The research into mechanism(s) of high Fe accumulation under Zn deficiency is warranted. In contrast to Fe, increase in concentration of P and Mn can be attributed to "a concentration effect".

In conclusion, the present results reported here demonstrate that the current screening method offers potential, and visual scores can be used as a parameter in rapidly assessing barley genotypes in their tolerance to Zn deficiency. The results also indicate that leaf symptoms often do not become visible until very severe reductions (50-60%) in growth occur (Figure 7.8), therefore, the use of leaf symptoms as a selection criterion to distinguish between Zn-efficient and Zn-inefficient genotypes would rely on a severe Zn-deficient growth medium. The reduction of up to 50-60% without showing any visible symptoms, "hidden hunger", highlights once again the need to develop alternative assays to diagnose Zn deficiency before reductions of such magnitude occur. The results also suggest that considerable variation exists in the tolerance of barley to Zn deficiency, at least at the seedling stage, which could be exploited in a breeding programme to improve genotypes with higher tolerance to low available Zn.

7.4.2 Experiment 2

The results reported here indicate that seed size affects plant growth, and the greatest growth can be achieved when the largest seeds of a genotype are used. This is not

surprising since large seeds generally have greater nutrient reserves than medium and small seeds (Appendix 7.2). This finding confirms previous results in barley that seed size influences plant growth (Pinthus and Osher, 1966) and grain yield (Kaufmann and McFadden, 1963; Demirlicakmak *et al.*, 1963; Pinthus and Osher, 1966). Also in wheat, similar results were reported (Mousavi-Nik, 1998). The evidence here and elsewhere has conclusively led to the suggestion that for sound comparisons, seeds of comparable sizes need to be used. However, it should be borne in mind that seed size does not always correlate well with seed weight and thus mineral nutrient content, and it is seed weight but not seed size that should be considered in estimates of total nutrient reserves. In the study here, within each seed size category, Forrest and WI-2868 had approximately 10-15 % higher seed weight than other genotypes, but did not follow the same pattern for nutrient content (e.g. Forrest had greater Zn content in medium and small seed category than all the other genotypes within these seed size categories (Table 7.1) due to its greater seed Zn concentration over the other genotypes, Appendix 7.3). The finding that seed size, seed weight and Zn concentration in the seeds tended to vary with some genotypes can be explained by the fact that these three factors can be influenced by genetic and environmental factors. Also in his study with 10 wheat genotypes, (Mousavi-Nik, 1998) concluded that the origin of the seed from within the crop and/or its position on the mother plant may have an important effect on seed size and its nutrient composition. These conclusions once again demonstrate the difficulty of obtaining seed of similar nutritional content for screening studies.

In the present study, visual scores allowed a greater separation in terms of sensitivity to Zn deficiency among genotypes than relative shoot dry matter, and they were not influenced by seed size. Although tissue nutrient analyses were not performed in this study, an explanation for this may be that in plants grown from large seeds, Zn concentration in the shoots is presumably diluted to levels similar to those grown from

medium and small seeds, consequently, Zn deficiency symptoms of similar severity develop despite the differences in shoot dry matter.

Relative shoot dry matter, termed Zn efficiency, was affected by seed size, and significant genetic differences occurred when genotypes were grown from large seeds. It appears that a large proportion of these differences in Zn efficiency can be attributed to genetic differences in utilization of increased seed nutrient reserves in large seeds, in particular Zn. However, differences in seed Zn content among genotypes within each seed size category (Table 7.1) indicates that seed grading may not always ensure comparable nutrient contents for screening studies since genotypes may differ in seed weight as well as nutrient reserves in the seeds (Appendix 7.3).

In conclusion, screening is currently based on visual scores and involves seed selection based on seed weight rather than seed size for nutritional reasons given in the introduction. This study, however, demonstrated that in instances where expression of Zn deficiency symptoms is used as a selection criterion, and seed Zn content does not vary much among seed sizes, grading seed can be useful in screening studies since a small range in seed Zn content among seed sizes did not affect ranking of genotypes based on deficiency symptoms. However, further evaluation of the influence of seed size on expression of deficiency symptoms and plant growth in a large number of barley genotypes may be useful for better understanding of the relationship between seed size and either Zn deficiency symptoms or dry matter production.

7.4.3 Experiment 3

The present results demonstrated that grain yield reduction under Zn deficiency was mainly due to inhibition of flower buds leading to fewer grains. Despite setting fewer grains, the grain of Zn-deficient plants is generally less well filled than with adequate Zn. There was little effect of Zn on number of tillers or ears (Table 7.7). The reduction in

grain yield under Zn deficiency is often attributed to (1) enhanced formation of abscissic acid in the plant, causing premature abscission of leaves and flower buds; (2) disruption of the development and physiology of anthers and pollen grains (Brown *et al.*, 1993). However, it is not clear why the grain yield in the present study was drastically reduced in genotypes such as Franklin, Cheri, WA28784 compared with some other genotypes such as Kinukei 21, Skiff and WI-2976. The explanation may simply be genetic differences in the requirement of Zn for formation of flowers and grain filling. In their study with wheat, Rengel and Graham (1995b) also suggested that Zn deficiency may affect the ability of plants to initiate flower buds and set more seed. The effect was more evident in the Zn-inefficient than in the Zn-efficient wheat genotypes, and they reported that the difference between the two genotypes in grain yield was likely to be a consequence rather than the mechanism of Zn efficiency.

According to the agronomic definition of nutrient efficiency (Graham, 1984), when comparisons are based on grain yield, Kinukei 21, Tarm, Skiff, Clipper, WI-2976, Harrington and Chariot can be classified as more Zn-efficient than Forrest, Onslow, Amagi Nijo, SA93013, Yagan and Cheri (Figure 7.7c). However, the same genotypes did not differ distinctively when comparisons were based on top yields (e.g. vegetative growth at awn visible stage, top yield at maturity) (Figure 7.7a,b). Part of this lack of correlation between grain yield at maturity and top yield at the awn visible stage may have been due to genetic differences in partitioning of dry matter between straw and grain especially under low soil Zn. For example, under Zn deficiency, Franklin and Cheri achieved higher vegetative yield (top yield at awn visible stage, and straw yield at maturity), but had considerably lower grain yield than Chariot and WI-2976, indicating that Zn deficiency does not always reduce the vegetative and grain yield by similar proportions. In other words, ranking of genotypes can differ depending on measures of Zn efficiency (e.g. vegetative or grain yield) (Table 7.8). Although the pattern of

distribution of Zn within the plant for the barley genotypes studied in this experiment is not known, it can be speculated that genotypes that had greater grain yield under Zn deficiency may have remobilized Zn more to the ear, the pollen and grain than those that had lower grain yield. Harry (1982) showed conclusively that like Cu efficiency, Zn efficiency in triticale and rye was due to greater absorption of these nutrients and their retranslocation to the shoot, especially to the ear, the pollen and grain.

Table 7.8. Zn efficiency ranking of genotypes based on shoot dry matter at awn visible stage and grain yield at maturity in Experiment 3.

Genotype	Awn visible stage	Maturity
Skiff	1	4
Harrington	2	6
Clipper	3	3
Cheri	4	16
Yagan	5	12
Kinukei 21	6	1
Tarm	7	2
Onslow	8	8
Forrest	9	9
Franklin	10	15
Chariot	11	7
SA93013	12	11
WI-2976	13	5
WA28774	14	14
Amagi Nijo	15	13
Excalibur	16	9
Yallaroi	17	17

The present results also indicate that a proportion of genetic differences in Zn efficiency (relative grain yield) among barley genotypes, can be explained by differences in HI of these genotypes (Table 7.7). For instance, when grown under sufficient Zn supply, Franklin, Cheri and WA28784 had lower HIs than WI-2976, Chariot and, Kinukei 21, therefore, the lower HIs may have contributed at least partly to the lower grain yield of these genotypes when grown under low Zn supply. However, the significant differences

in Zn efficiency when genotypes did not differ significantly for HI, must be associated with the differential ability of the genotypes to tolerate Zn deficiency stress. Clipper, Skiff, and Tarm had greater Zn efficiency than Cheri, Franklin,WA28784, SA93013 and Yagan, but similar HIs.

7.5 General conclusion

Seedling screening at about 21 DAS can be useful in selecting genotypes with greater tolerance to low soil Zn as indicated by the large variation in deficiency symptoms as well as reduction in shoot dry matter among genotypes tested in this study. It involves a parameter, visual scores, which is straightforward to measure and also a good measure of tolerance to Zn deficiency at the seedling stage. With this current method, a large number of genotypes can be screened quickly and economically at a single level of Zn fertilization (3 weeks).

Based on 15 barley and 2 wheat genotypes grown under controlled conditions in the present study, relative grain yield at maturity (Experiment 3) correlated better with visual scores (r=0.68) than relative shoot growth (r=0.58) at the early stage (Experiment 1) (Table 7.9). The stronger correlation between visual scores at the early growth stage and relative grain yield at maturity, further suggests that screening for tolerance to Zn deficiency using visual scores at the early growth stage can be useful. However, in instances where HI differs considerably among genotypes, and it is not positively correlated with short term studies (e.g. early growth stage), vegetative growth is not a reliable indicator of economic yield (Woodend and Glass, 1993), and should only be used to discard the very worst genotypes rather than as a reliable screening procedure for nutrient-use efficiency (Schettini *et al.*, 1987).

Table 7.9. Linear correlation of coefficients between parameters measured at the early growth stage (Experiment 1) and relative grain yield at maturity (Experiment 3).

	Maturity
Early growth stage	Relative grain yield
Visual score	0.68**
Relative shoot growth	0.58*
Root:shoot DM ratio	0.16 ns
Zinc concentration in shoot	0.70**
Zn content in shoot	0.66**

*,** Significant at the 5% and 1% level, respectively.
ns; non-significant.

The differences in efficiency ranking between early growth and awn visible stages and the differences in their correlations with grain yield responses could be due to differential mechanisms of Zn efficiency operating at different stress levels and growth stages. The level of deficiency stress at the early stage in Experiment 1 (0.02 mg Zn/kg soil) was severe as indicated by Zn efficiency less than 30% in majority of genotypes, compared with moderate Zn deficiency stress at awn visible stage in Experiment 2 (0.1 mg Zn/kg soil) (range of Zn efficiency for most genotypes was 50-60%). Based on the results from a preliminary experiment (see 7.2.3), even the more Zn-efficient genotypes, classified based on grain yield in this study, probably would not have produced grain had they been grown at a severe level of deficiency stress such as 0.02 mg Zn/kg soil in Experiment 1. The results of these experiments indicate the difficulty of maintaining a similar level of Zn deficiency stress throughout the growth cycle in a single experiment and therefore, the need to test genotypes at different levels of deficiency stress depending on developmental stage. In the pot system, Zn deficiency stress generally becomes more severe as plant growth progresses, which is probably due to higher Zn requirements of plants at later stages. A differential ranking of Zn efficiency depending on the level of Zn deficiency

stress was also noted in medics by Streeter (1998). In agreement with suggestion by Streeter (1998), the present results suggest that screening should be carried out at a level where the greatest genotypic variation occurs (e.g. 0.02 and 0.1 mg Zn/kg soil for screening at the early growth stage and maturity, repectively).

The fact that vegetative growth at awn visible stage did not always correlate with grain yield may indicate different mechanisms of Zn efficiency at different stages of plants growth. Although experimental evidence is lacking, it can be assumed that genotypes that had greater Zn efficiency at the early vegetative stage and maturity (WI-2976, Tarm, Skiff, Harrington and Chariot) may have possessed more than one mechanism (e.g. greater uptake of Zn from soil at the vegetative stage and greater remobilization of Zn to reproductive organs at maturity). Further research is required to test this hypothesis of differential mechanisms of Zn efficiency operating at different developmental stages.

Another advantage of the newly developed seedling screening method used in Experiment 1 is that it can be used to further understanding of inheritance of tolerance to Zn deficiency which often involves screening of very large number of individual plants. This type of screening can be done successfully using small pots under controlled conditions. An example of this is the study of Longnecker *et al.* (1990) who investigated inheritance of tolerance to manganese efficiency in barley. In their study with single-level screening using small pots, they conclusively showed that chlorosis score was useful in selecting Mn efficiency in F_1, F_2 and F_3 populations derived from a Mn-efficient by a Mn-inefficient cross.

Grading seed into specific groups such as small, medium and large, does not always ensure comparable seed Zn content because seed Zn content within a certain seed size can differ with the genotype due to variability in seed weight and Zn concentration, both of which determine seed Zn content. Although it is cumbersome, until a screening

method independent of seed Zn content is developed, selection based on seed weight as opposed to seed size appears to be the most practical approach for achieving similar seed Zn content for screening studies.

CHAPTER 8

Inheritance of tolerance to Zn deficiency in barley

8.1 Introduction

The use of zinc-based fertilizer in conjunction with Zn-efficient genotypes is considered the most practical approach for cropping Zn-deficient soils, and a breeding effort for this has been justified previously (Graham, 1984; Graham and Welch, 1996). However, progress towards producing genotypes with greater Zn efficiency has been slow due to the lack of a rapid and reliable screening technique and understanding of the genetic control of tolerance to Zn deficiency (e.g. the number of genes, and their mode of expression). Currently, very little is known of the genetics of tolerance to Zn deficiency in higher plants except that multigenic control has been proposed for tolerance in rice (Mahadevappa *et al.*, 1981), soybean (Hartwig *et al.*, 1991), rye (Graham, 1984; Cakmak *et al.*, 1997c; Schlegel *et al.*, 1998) and *Haynaldia* (Schlegel *et al.*, 1998).

The studies described in Chapter 7 showed that considerable variation exists in tolerance to Zn deficiency among barley genotypes and visual scores at the seedling stage and grain yield were highly correlated. Moreover, visual scores were not affected by seed size and therefore they could be used as a robust method of assessing Zn efficiency. It follows that visual scores could be used in screening segregating populations to select for tolerance to low soil Zn. Similarly, studies on Mn efficiency in barley have demonstrated that Mn-deficiency symptoms in the growth cabinet correlate well with grain yield in the field, and symptom expression can be used successfully to screen F_2 and F_3 populations to determine the genetics of Mn efficiency (McCarthy *et al.*, 1988; Longnecker *et al.*, 1990). Given that there is a good correlation between

visual scores and grain yield (Chapter 7), an F_2 population from a cross between Skiff (moderately tolerant) and Forrest (sensitive) was screened using visual scores. Analysis was restricted to the F_2 population because there was insufficient time to verify the F_2 classification in the F_3 population.

8.2 Materials and methods

8.2.1 Selection of parents

Skiff and Forrest were chosen as parents because of their differences in severity of Zn deficiency symptoms (Experiment 2, Chapter 5): Skiff showed slight symptoms and Forrest expressed very severe symptoms when grown in soil low in Zn. Although a later experiment found larger genetic differences among the other genotypes than that between Skiff and Forrest (Experiment 3, Chapter 7), at the time when the initial cross was made, these two genotypes were considered representative of the variation available within barley. Nonetheless, Skiff has consistently been found to be more efficient than Forrest in all experiments conducted with these two genotypes.

8.2.2 Production of F_1 and F_2 seed

Crosses were made in a growth room (20/15 °C (day/night) and 14 h photoperiod) in June 1998 by hand transfer of pollen from the male parent (Forrest) to the emasculated female parent (Skiff). The resultant F_1 seed was planted in the same growth room in August 1998 under the same conditions to produce F_2 seed. Both the parents and F_1 plants were grown at a marginally low level of Zn nutrition (0.2 mg/kg soil) in Laffer sand to produce both parental and F_2 seeds with a consistently low Zn concentration (~12 mg Zn/kg D.W.). Earlier experiments (Chapter 3) had shown that seed Zn content can influence screening tests, and Laffer sand with marginal Zn supply (0.2 mg/kg soil) can be useful for producing seed with a relatively low Zn concentration (~12-13 mg/kg

D.W.). Seed was threshed by hand and stored at 4 °C for three weeks prior to starting the screening study.

Table 8.1. Average seed weight (mg/seed D.W.), Zn concentration (mg/kg D.W.) and content (μg/seed) of parental (Skiff, Forrest) and F_2 seeds in the present study. Standard errors are based on three replicates each containing 10 seeds.

Genotype	Weight	Zn concentration	Zn content
Forrest	47.6 ± 0.2	11.9 ± 0.2	0.57 ± 0.01
Skiff	48.3 ± 0.3	12.9 ± 0.1	0.62 ± 0.01
F_2 seed	47.9 ± 0.7	11.0 ± 0.2	0.53 ± 0.01

8.2.3 Soil preparation and nutrient treatments for screening F_2 population

A Zn-deficient soil, Lancelin sand, was used in this study and the seedlings grown at a single rate of Zn fertilization (0.02 mg/kg soil). Pots contained 400 g of soil, to which basal nutrients and Zn (in solution) were added, as described in Chapters 5, 6 and 7. This level of Zn fertilization gave the best discrimination between the parent genotypes in a preliminary experiment with five Zn levels (0, 0.02, 0.04, 0.08, and 0.8 mg/kg soil). The results from this preliminary experiment also indicated that deficiency symptoms gave a greater separation between the parents than relative shoot growth, Zn concentration and Zn content in the shoot, confirming the results from the earlier screening experiments (Chapter 7). Therefore, deficiency symptoms were used as the only criterion for tolerance to Zn deficiency.

8.2.4 Plant growth and assessment of Zn deficiency

A total of 185 F_2 seeds, plus 16 seeds of each parent (Skiff and Forrest) with similar Zn content (Table 8.1) were used in this study. Seed germination, sowing and thinning were carried out as described previously (Chapters 5, 6 and 7). The parents and F_2 individuals were harvested at 21 days after sowing (DAS), and were scored for Zn

deficiency symptoms using a scale of 1 to 8 (see below), which was modified from the scale of 1 to 6 used previously (Chapters 6 and 7). The need for this modified scale arose because more classes of deficiency symptoms occurred in the F_2 population than were seen between the parents in the present study and observed in previous experiments (Chapters 6 and 7). The modified scale was as follows (see Figure 8.2):

1=dark green, healthy leaves,

2=pale green leaves,

3=linear chlorotic areas appearing on young leaves,

4=chlorotic areas extending across the young leaves,

5=large chlorotic areas on young leaves,

6=young leaves collapsing in the centre,

7=linear chlorotic areas visible on old leaves,

8=both young and old leaves turn pale yellow.

8.2.5 Genetic analysis

The response of F_1 hybrids compared to the parents could not be measured in this study due to limited availability of F_1 seed. Instead, genetic analysis was restricted to the F_2 generation and segregation pattern was examined using visual scores (deficiency symptoms).

Chi-squared analysis was used for testing the goodness of fit of the observed segregation ratios to frequencies expected for monogenic (1 sensitive: 3 intermediate-tolerant or 1 sensitive: 2 intermediate: 1 tolerant) or digenic (1 sensitive: 15 tolerant) segregations. Chi-squared value (χ^2) was calculated using the following formula (Gomez and Gomez, 1984):

$$\chi^2 = \sum_{i=1}^{p} \frac{(n_i - E_i)^2}{Ei}$$

where p is the number of classes, ni is the observed number of units falling into class i, and Ei is the number of units expected to fall into class i.

8.3 Results and discussion

The parental lines and the F_2 individuals tested in this study exhibited a wide range of visual scores (Figure 8.1; Plates 8.1 and 8.2). Based on a score of ≤3 and ≥7 considered as the cutoff point for the parents, the F_2 segregating population can be classified into three categories: tolerant (score ≤3; 39 F_2 plants), intermediate (score 4-6; 103 F_2 plants) and sensitive (score ≥7: 43 F_2 plants). Chi-squared analysis shows that this fits a 1:2:1 ratio (χ^2=2.52, n.s, *df*=2) and supports the hypothesis that tolerance to Zn deficiency at the seedling stage in Skiff is controlled by a single gene with no dominance.

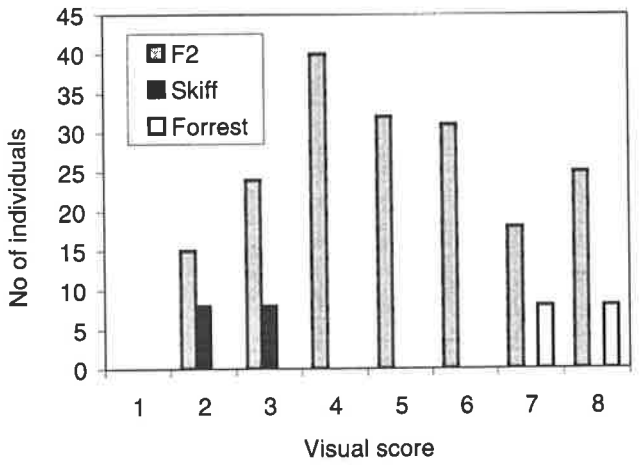


Figure 8.1. Distribution of visual scores of parents and F_2 progeny from a cross between Skiff (moderately tolerant) and Forrest (sensitive).

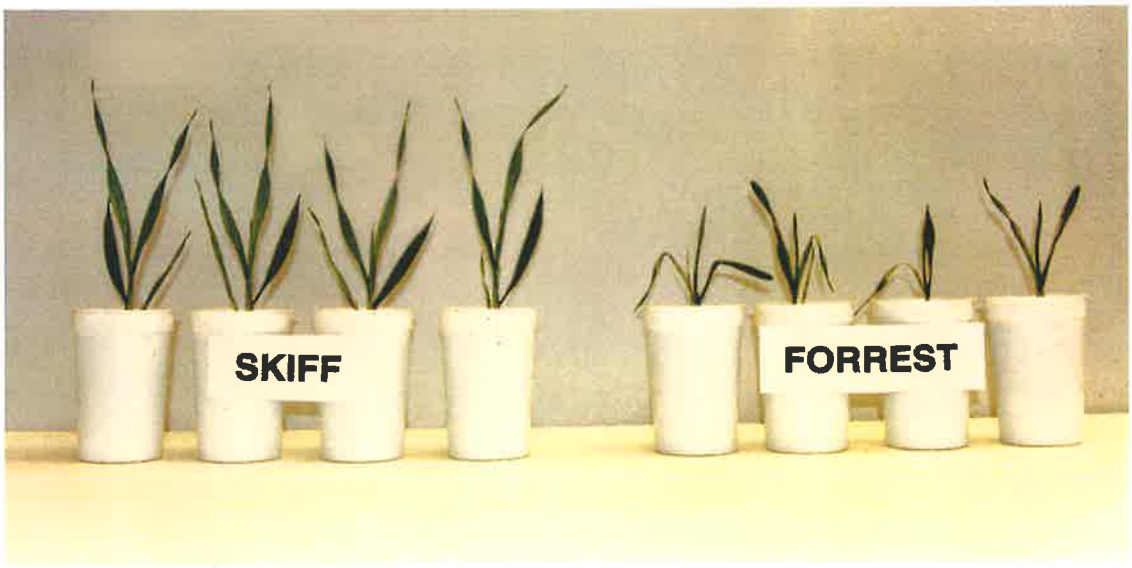


Plate 8.1. Variation in severity of Zn deficiency symptoms (visual scores) between the two parents, Skiff (score: 2-3) and Forrest (score: 7-8).

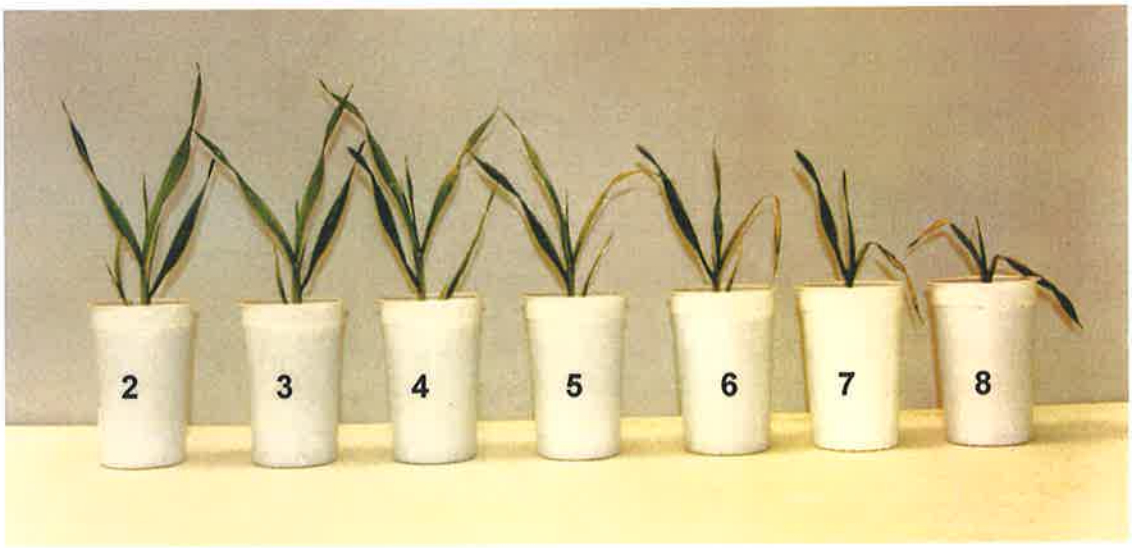


Plate 8.2. Variation in severity of Zn deficiency symptoms (visual scores) within the F_2 population from the cross between Skiff and Forrest (for description of visual scores, see section 8.2.).

In contrast to this finding, other studies in rye (Graham, 1984; Cakmak *et al.*, 1997; Schlegel *et al.*, 1998), rice (Mahadevappa *et al.*, 1981) and soybean (Hartwig *et al.*, 1991) have suggested that Zn efficiency may be under polygenic control. The differences between the results of this and other studies could be due to (i) differences in genotype, (ii) seed Zn content and (iii) selection criteria used. For example, in this study, moderately tolerant Skiff and sensitive Forrest appeared to differ by only one gene, and it is possible that other genotypes with greater tolerance to Zn deficiency than Skiff may carry more than one gene. This possibility is supported by the studies on Mn efficiency. It has been reported that Mn efficiency is controlled by one gene in barley (Longnecker *et al.*, 1990; McCarthy *et al.*, 1988), but two genes in durum wheat (Khabaz-Saberi *et al.*, 1999) and soybean (Graham *et al.*, 1995). Similarly, inheritance of Fe deficiency has been reported to be controlled by a single gene in soybean (Weiss, 1943) but two genes in dry beans (Coyne *et al.*, 1982). Seed Zn content can affect interpretation of the studies on Zn efficiency, as indicated in Chapter 3. With respect to the effect of selection criteria, parent genotypes and distribution pattern in the F_2 or F_3 generation, and consequently, interpretation of analysis of the genetics of tolerance to Zn deficiency may differ depending on the selection criteria used: in this study, visual scores allowed a greater separation between parents than shoot dry matter. Although shoot dry matter was not measured in this study, the distribution pattern seen in the F_2 generation may have differed had shoot dry matter been used since there were more classes in deficiency symptoms than shoot dry matter (Plate 8.2).

Plant material from this screening was used for a Bulk Segregant Analysis by Mr. Paul Bogacki and Dr. Kevin Williams of the South Australian Research and Development Institute, in conjunction with a DNA fingerprinting technique, amplified fragment length polymorphism (AFLP), developed by Vos *et al.* 1995. Two sets of 15 F_2 plants

representing the two extremes in visual symptoms were selected, of which 8 from each set were screened for polymorphism at the DNA level with 180 AFLP primer combinations in an effort to identify molecular markers linked to genes encoding tolerance to Zn deficiency. The results of this AFLP analysis are given in Table 8.2. The close association (94%) between $AFLP_1$ and Zn efficiency indicates that this marker may be close to the locus conditioning Zn efficiency. However, this result needs further confirmation in a validation population. If the same linkage can be detected with this marker in the validation population, then this marker can be used in a breeding program (i) to select for Zn efficiency, and (ii) accelerate the introgression of the Zn efficiency gene(s) into breeding lines. Research into verification of the present results is currently underway (Kevin Williams, pers. commun.).

Table 8.2. Distribution of AFLP markers among individual F_2 plants differing in severity of deficiency symptoms from the cross Skiff x Forrest (K. Williams *et al.*, 1999, unpublished).

	Linked markers			
Plant number*	$AFLP_1$	$AFLP_2$	$AFLP_3$	$AFLP_4$
MT_1	-	-	+	+
MT_2	-	-	+	+
MT_3	-	-	+	+
MT_4	-	-	+	+
MT_5	-	-	+	+
MT_6	-	-	+	+
MT_7	-	-	+	+
MT_8	-	-	+	+
S_1	+	-	+	-
S_2	+	+	-	-
S_3	+	+	-	-
S_4	+	-	-	-
S_5	+	+	-	-
S_6	-	-	-	-
S_7	+	+	+	+
S_8	+	+	+	+
Marker association (%)	94	81	81	81

* MT=Moderately tolerant, S=Sensitive.

8.4 Conclusion

The results of this preliminary study indicated that Zn efficiency in Skiff was controlled by a single gene with no dominance, compared to Zn-inefficient Forrest, and visual scores were useful for the analysis of the inheritance of Zn efficiency, especially in segregating populations, such as F_2. The results of this study at the molecular level indicated that the $AFLP_1$ may be a putative marker for Zn efficiency which would improve and simplify the screening for Zn efficiency in the future.

Chapter 9

General Discussion

9.1 Introduction

Zinc deficiency is considered to be the most widespread micronutrient deficiency in cereals worldwide, causing severe reductions in grain yield and quality (Graham *et al.*, 1992; Cakmak *et al.*, 1996a). A recent soil survey found that 49% of samples from a survey of 190 soils from 25 countries were Zn-deficient (Sillanpää, 1990). In Western Australia, 8 Mha of arable land have been reported to be Zn deficient, which is the largest contiguous area of known Zn deficiency. In other parts of Australia, a large proportion of Australia's wheat belt has also been reported to be low in Zn (Graham and Welch, 1996). Similarly, 4.5 Mha of Central Anatolian soils, which represents 50% of the wheat production area of Turkey, have been reported to be Zn-deficient (Cakmak *et al.*, 1996a).

Zn deficiency can be alleviated by applying Zn fertiliser or by breeding more Zn efficient genotypes: however, fertilizers are costly, not always effective and often require repeated applications. There is considerable genetic variability for tolerance to Zn deficiency among cereals and genotypes within a given species (Graham *et al.*, 1992; Cakmak *et al.*, 1996a), indicating there is potential for selecting and/or breeding Zn-efficient varieties for Zn-deficient soils. The use of small amounts of Zn fertilizer, in conjunction with Zn efficient genotypes, is a cost effective and environmentally friendly approach for improving crop production on Zn deficient soils (Graham and Welch, 1996; Cakmak *et al.*, 1999).

There has been some work on selecting for tolerance to Zn deficiency in barley, and genetic variability for this trait has been reported by several investigators (Pathak *et al.*, 1979; Takkar *et al.*, 1983; Graham *et al.*, 1992; Yilmaz *et al.*, 1996). Field screening has been the basis of selection, but the number of genotypes tested has been small, and considerable variation in the field results across the sites and over years has been observed (Graham *et al.*, 1992; Yilmaz *et al.*, 1996). In a study with nine barley genotypes grown at two Zn-deficient sites in South Australia, Lameroo and Yeelanna (DTPA-extractable Zn=0.2 mg/kg for both sites), Graham *et al.* (1992) reported that ranking of genotypes for Zn efficiency differed considerably between the two sites. They attributed the differences to the variation in the severity of Zn deficiency and the amount of rainfall at each site. Similarly, from the field study of Yilmaz *et al.* (1996), it appears that ranking of ten genotypes of barley for Zn efficiency was influenced markedly by soil type and environmental conditions. In their study, Zn deficiency stress and range in Zn efficiency was greater at Comakli (DTPA-Zn, 0.08 mg/kg; efficiency, 34-64%) than at the other two sites, Konya (DTPA-Zn, 0.11 mg/kg; efficiency, 60-85%) and Eskisehir (DTPA-Zn, 0.13 mg/kg; efficiency 80-99%), which could be partly attributed to lower DTPA-extractable Zn at Comakli. In addition to the problems associated with environmental variability, field sites are often located far from research institutions, are not easily accessible, and involve a high maintenance cost. Therefore, it is necessary to develop an alternative method to field screening, which is easy, simple, cost effective and able to predict field performance.

Graham (1984) reviewed screening procedures for selecting for improved micronutrient efficiency, and reported that screening in potted soil generally ranked genotypes in the same order as field studies. Screening in potted soil also is simpler, faster and less expensive than field work, particularly if it is based on a seedling test,

and may overcome some of the limitations associated with field tests; at least, it may supplement field work. It can also be useful in accurately testing genotypes once populations are reduced to a manageable size, or in assessing large numbers of segregants from crosses of nutrient efficient x nutrient inefficient parents (Graham, 1991). The present study was conducted to develop a soil-based pot assay, to assess its potential as an alternative to field tests and, using this method, to determine the extent of genotypic variation for tolerance to Zn deficiency in barley.

The purpose of this chapter is to discuss factors affecting the efficiency of a soil-based pot assay, the appropriate selection criteria for screening for Zn efficiency, and to discuss genetic control of Zn efficiency with emphasis on the present study.

9.2 Effects of soil and seed source on the efficiency of a soil-based pot assay for screening for Zn efficiency

9.2.1 Soil source

As reported in Chapter 4, in South Australia Laffer sand has been the basis for screening for Zn efficiency in various crops including wheat, chickpea, rapeseed and medics. The soil has a low DTPA-extractable Zn (0.06-0.12 mg/kg soil), which is desirable when screening for Zn efficiency. Following the success with these crops, Laffer sand was used initially as a Zn-deficient growth medium in the present study; however, considerable variation in Zn responsiveness was observed when two different batches of this sand were used (Chapter 4), which was explained by the differences in DTPA-extractable Zn of the two batches. At present, it is not known why soil Zn content between the two batches of Laffer sand differed considerably, although the two batches were collected from the same virgin site, but at different times. This result has important implications for future studies using potted soil. Firstly, if a series of

experiments is to be carried out, soil from the same batch should be used throughout the experiments. This practice would help minimize variation in soil Zn content from batch to batch. Secondly, the Zn status of the soil to be used needs to be characterized. There is generally a good correlation between micronutrient status of soils and plant growth, which forms the basis of the concept of 'critical concentration of soil extractable micronutrients; below this concentration a growth response is expected to occur. It follows that determination of the critical DTPA-extractable Zn of the soil used for screening, and DTPA-extractable Zn for each batch from that soil, would give some indication of the likely response of plants grown in that soil. In this study, there were considerable responses to Zn in Lancelin sand at a DTPA-extractable Zn (0.12 mg Zn/kg) lower than the critical value (0.14 mg Zn/kg; Brennan, 1993), suggesting that the critical value in conjunction with DTPA-extractable Zn can be used to estimate Zn responsiveness before the start of the experiments. The critical value used in this study was initially determined for wheat but was applicable to barley; however, it should be kept in mind that the critical DTPA-extractable Zn may differ with crop species since it is derived from the growth response to several rates of Zn on each soil, which may also differ depending on crop species.

Soil properties other than Zn can influence the availability of Zn and consequently, the degree of Zn deficiency stress. For example, in the present study, despite having similar DTPA-extractable Zn, Lancelin sand generated a greater Zn deficiency stress than Laffer sand. The analysis of soil properties in the two soils indicated that this may be due to greater organic matter content in Lancelin sand (0.63%) than in Laffer sand (0.07%). It is possible that a proportion of Zn in Lancelin sand may have been strongly bound onto organic matter. The fact that the two soils had differential deficiency stress

at similar DTPA-extractable Zn further suggested that critical DTPA-extractable Zn differs with soil type; therefore, it should be determined for each soil type separately.

9.2.2 Seed source

9.2.2.1 Seed Zn content

It has been well established that seed nutrient content can markedly influence the growth of plants, especially when the nutrient in question is limiting plant growth (Graham, 1984). The results from Chapter 3 have conclusively shown that seed Zn content affects vegetative growth of barley grown under Zn deficient conditions. Studies with wheat (Rengel and Graham, 1995a,b; Yilmaz *et al.*, 1998) and rapeseed (Grewal *et al.*, 1996) have reported similar results for both early growth and grain yield.

An important outcome of the present study was that seed Zn content influenced Zn efficiency ranking among barley genotypes, which bears practical implications for studies of comparisons of Zn efficiency: the efficiency of barley genotypes grown from seed with large differences in Zn content can not be properly compared. Therefore, it is suggested that in routine screening, seed with similar Zn content should be used. The Zn content should preferably be low because high seed Zn content can mask genetic expression of Zn efficiency at the seedling stage (e.g. lack of symptoms). Similar Zn contents can be achieved by selecting seed of exactly the same weight and similar Zn concentration. However, producing seed with similar Zn concentrations for a large number of genotypes can be a difficult task (if not practically impossible) since genotypes differ in their ability to load Zn into their seeds (White *et al.*, 1981; Longnecker and Robson, 1993). This practical difficulty emphasizes the need to develop a screening method independent of seed Zn content (e.g. biochemical/

molecular) in the future. In the meantime, seed with low Zn content (e.g. 0.5-0.9 µg/seed) can be produced either in Zn-deficient plots in the field (see 5.4) or in Zn-deficient soil under controlled conditions (see 3.2). Seed produced under fertile conditions in glasshouses can contain very large quantities of Zn, which would reduce the ability to distinguish between Zn-efficient and Zn-inefficient genotypes. With seed produced in the field, care must be taken to ensure that seed is pure.

9.2.2.2 Seed weight vs seed size

Seed selection based on seed weight is commonly used to obtain seed with similar Zn content, but it is cumbersome when a large number of genotypes is considered. An alternative is to use mechanically graded seed which sorts seed according to size. It was found in Chapter 7 that seed Zn content did not change consistently with seed size (e.g. Forrest: 0.8, 1.0, 0.9 µg/seed; Schooner: 0.6, 0.7, 0.7 g/seed, from small, medium and large seed, respectively) and Zn efficiency was not consistently affected by seed size. The expression of visual symptoms was unaffected by seed size, which was attributed to the small range in seed Zn content. This result suggests that seed grading may be useful in instances where seed Zn content does not vary much among the genotypes. As observed in Chapter 5, genotypes differ in seed weight and seed Zn concentration, therefore, seed selection based on seed weight is still considered the better method of achieving seed with similar Zn content for screening studies until a screening method independent of seed Zn content is developed.

9.3 Selection criteria for Zn efficiency

At the start of this project (1995), grain yield was the primary selection criterion for Zn efficiency in wheat (Graham, 1992) and barley (Pathak *et al.*, 1979; Takkar *et al.*, 1983; Yilmaz *et al.*, 1996). However, as reported earlier (2.5.2.1), some authors have

raised concerns over the use of grain yield response as a selection index since it is complex and almost everything in the genome is likely to contribute to final grain yield (Graham, 1984; Blum, 1988). Graham (1984) suggested that it would be ideal if nutrient efficiency were expressed, and therefore measurable, at the seedling stage. Following Graham's suggestion, an attempt was made to evaluate Zn efficiency at the seedling stage by studying differences in visual symptoms and physiological responses (reduction in shoot dry matter, concentration and content of Zn in shoot, and root: shoot dry weight ratio) between barley genotypes differing in Zn efficiency in the field (Chapter 4) and to see whether Zn efficiency measured at the seedling stage correlated with grain yield at maturity.

9.3.1 Visual scores vs shoot dry matter at the early stage (21 DAS)

Barley genotypes tested in this study exhibited a wide range of visual deficiency symptoms and reduction in shoot dry matter at the seedling stage. A simple scoring system based on severity of deficiency symptoms was devised and a significant positive relationship between visual scores and reduction in shoot dry matter (Chapter 7) was demonstrated. This suggested that visual scores could be used as a parameter for assessing barley genotypes for Zn efficiency. However, both in the present study (Chapter 7) and elsewhere (Cakmak *et al.*, 1998), there was a range in dry matter responses within each class of visual symptoms, which can be expected to a certain extent, when a large number of genotypes is considered. This phenomenon may indicate differential mechanisms involved in expression of visual symptoms and reduction in shoot dry matter, which warrants further study.

Visual symptoms are useful particularly for genetic studies (e.g. Chapter 8). When screening large numbers of plants in segregating populations, testing individual plants

at more than one level of Zn to estimate reductions in dry matter is not possible and a visual score allows the level of genetic variation to be assessed rapidly and non-destructively. However, it should be kept in mind that in screening germplasm and segregating populations, development of visual symptoms often requires severe deficiency stress, as described in this study and elsewhere (Hannam *et al.*, 1987). Symptoms do not become apparent until reductions of up to 50-60% in shoot growth occur. Therefore, in screening of germplasm, unless reductions in shoot growth of this magnitude can be generated, differences among genotypes may not be detected if selection is based on visual scores alone. In such instances, reduction in shoot dry matter or relative shoot dry matter may be more useful.

9.3.2 Root:shoot dry weight ratio at 21 DAS

When a diverse range in germplasm was screened (Chapter 7), it appeared that the root:shoot ratio generally increased in most genotypes when grown under Zn deficiency, a phenomenon which occurs as the initial response to Zn deficiency (Cumbus, 1985; Loneragan *et al.*, 1987; Khan *et al.*, 1998a). However, the root:shoot ratio under Zn deficiency did not always correlate well with the sensitivity of genotypes to Zn deficiency. Tantangara, Skiff and Galleon developed less severe symptoms (Figure 7.1) but had greater root:shoot ratios (ratio: 0.64-0.65; Figure 7.4.) than WA28774, Kinukei 19 and Gairdner (the ratio: 0.45-0.47). In contrast, despite their significant differences in severity of deficiency symptoms, both Skiff and Forrest had similar root:shoot ratios (0.64). In some cases, the sensitive genotypes (Natasha, 0.73) had greater ratios than less sensitive genotypes (Tarm, 0.54), suggesting that the root:shoot dry weight ratio may not be useful as a selection index for tolerance to Zn deficiency. The present study did not investigate the cause of genetic differences in root:shoot ratio under Zn deficiency and discussion was limited to only a consideration

of potential for root:shoot DM ratio as a additional parameter in the assessment of genotypic variation in tolerance to Zn deficiency.

9.3.3 Zn concentration in the shoot at 21 DAS

Tissue Zn concentration is used widely to characterise plant Zn status and this study showed that critical Zn concentration (YEBs) in barley was ~20 mg/kg D.W. (Chapter 4). This value is more appropriate than the adequate range of published values (15-70 mg/kg D.W.; Weir and Cresswell, 1994). However, under severe Zn deficiency, tissue Zn concentration does not appear to be useful criterion for selecting for Zn efficiency: genotypes had similar Zn concentration in shoots despite showing large differences in severity of Zn deficiency symptoms (Chapters 3, 4, 5 and 7). The differential expression of Zn deficiency symptoms at similar concentration of Zn in YEBs and shoots in the present study indicated a differential utilization or compartmentalization of Zn in leaf cells. This was suggested as one of the mechanisms of Zn efficiency in cereals (Graham and Rengel, 1993) and demonstrated in recent studies on wheat (Rengel, 1995; Cakmak *et al.*, 1998) and rye (Cakmak *et al.*, 1998). These studies found that activities of some enzymes, such as carbonic anhydrase (Rengel, 1995) and Cu/Zn-superoxide dismutase (Cakmak *et al.*, 1998), were greater in Zn-efficient than Zn-inefficient genotypes, despite having similar concentrations of Zn in the tissues. The relationship between enzyme activity and Zn concentration in barley is yet to be demonstrated.

9.3.4 Zn content in the shoot

The Zn content in the shoot was better correlated with sensitivity of genotypes to Zn deficiency than Zn concentration in the shoot. Genotypes with less severe symptoms had more Zn in the shoot than those with more severe symptoms. This finding agrees

with the published results of other investigators who found that in the field (Graham *et al.*, 1992), greenhouse (Cakmak *et al.*, 1996a) or using solution culture (Rengel and Graham, 1995), Zn-efficient bread wheats accumulated more Zn in the shoot than Zn-inefficient durum wheats. The greater Zn accumulation in bread wheats was attributed to higher Zn uptake as well as greater root-to-shoot transport capacity of Zn (Cakmak *et al.*, 1996). Although this study did not investigate mechanisms of tolerance to Zn deficiency specifically, based on earlier results with two genotypes (Zn-efficient Tarm and Zn-inefficient Hamidiye in Chapter 4), it can be argued that the greater uptake and root-to-shoot transport of Zn at low Zn supply may also markedly contribute to tolerance to Zn deficiency in barley. However, further research using a wide range of genotypes is required before this can be generalized as a mechanism of tolerance to Zn deficiency in barley.

9.3.5 Grain yield vs vegetative growth

Genetic differences in Zn efficiency were greater in grain yield than in vegetative growth measured at awn visible stage (Chapter 7), suggesting a differential ability among genotypes to partition dry matter between straw and grain. Despite the absence of tissue analysis in this study, it can be assumed that genotypes with higher grain yield under Zn deficiency may have mobilized more Zn to ear, the pollen and grain than those with lower grain yield. This is in accordance with the results of Harry (1982) who conclusively showed that like Cu efficiency, Zn efficiency in triticale and rye was due to greater absorption of these nutrients and their translocation to the shoot, especially to the ear, pollen and grain. The results also indicate that a proportion of the differences in Zn efficiency can be attributed to differences in HI; therefore, genotypes differing in HI can not properly be compared for Zn efficiency.

This study found that relative grain yield at maturity correlated with visual scores ($r=0.68$, $P<0.01$) and relative shoot growth ($r=0.58$, $P<0.05$) of seedlings at 21 DAS, suggesting that screening for tolerance to Zn deficiency using visual scores and/or relative shoot growth at 21 DAS can be useful. The stronger correlation of grain yield with visual scores than relative shoot growth favors the use of visual scores as a selection index over relative shoot growth in breeding programs in which simple parameters, such as visual scores, are needed. Using shoot growth as a parameter, it is important that genotypes do not differ considerably in HI. In instances where HI differs significantly among genotypes; shoot growth is not an reliable indicator of grain yield (Woodened and Glass, 1993), therefore, relative shoot growth should only be used to discard the very worst genotypes rather than as a reliable screening method for nutrient-use efficiency (Schettini *et al.*, 1987). In addition, growth habit and maturity status should be considered when diverse germplasm is to be screened. For example, a genotype with higher growth rate may develop deficiency symptoms earlier than a genotype with slower growth rate. Therefore, in screening for Zn efficiency, it is recommended that genotypes be compared at a specified growth stage rather than a certain period of time after sowing.

9.3.6 Pot assay vs field screening using grain yield as a selection criterion

Some of the barley genotypes used in the present study were also tested in the field at three sites in South Australia (Lameroo, Titinara and Birchip in 1998), as part of ongoing field screening for Zn efficiency (R. D. Graham, unpubl.). As expected, large genotype x environment interactions occurred in the field. Of the field sites, Birchip was the most Zn responsive. The barley genotypes, Amagi Nijo and SA93013, that showed marked responses to Zn in growth room experiments (Chapter 7) also showed considerable visual responses to Zn fertilization at Birchip prior to anthesis (Appendix

9.1). However, this site suffered severe drought stress after anthesis and thus, the yield response of these genotypes to Zn fertilization could not be measured properly at maturity.

Values of Zn efficiency were greater in the field than in the pot assay, and rankings of genotypes also differed (Table 9.1). This could be attributed to variation in Zn deficiency stress and growing conditions. Zn deficiency stress, expressed as DTPA-extractable Zn, was greater in the pot assay (0.12 mg/kg soil) than that in the field (0.18 mg/kg soil), which was reflected in Zn efficiency: in the pot assay, efficiency ranged from 3% to 41%, whereas in the field, genotypes achieved efficiency greater than 84% except for SA93013 which had the lowest efficiency (63%). This differential Zn deficiency stress between field and pot experiments was more evident in the durum wheat genotype used as control in the present study, Yallaroi: it had a Zn efficiency of 70% in the field, but failed to produce grain under controlled conditions (Chapter 7). The greater degree of Zn deficiency in the pot assay than in the field allowed a better discrimination in Zn efficiency among genotypes, and allowed the identification of 2 genotypes, Skiff and Forrest, for later genctic studies. That these 2 genotypes differed little in their Zn efficiency in the field (Table 9.1.) was probably associated with the less severe Zn stress at Birchip compared to the pot assay. There is consistent evidence in all chapters that increasing the level of soil Zn increases Zn efficiency within a genotype, and at high levels of soil Zn, there is a reduction in the difference between efficient and inefficient genotypes (Chapter 4).

The field data obtained so far are insufficient to verify the usefulness of the proposed method to field screening. However, the consistent responses of wheat genotypes of known Zn efficiency (Zn-efficient Excalibur and Zn-inefficient Yallaroi) as well as

some of the inefficient barley genotypes (Amagi Nijo and SA93013) in both pot and field trials indicates that screening under controlled conditions can predict field response. However, this needs to be confirmed at a Zn responsive site using a wide range of genotypes.

Field screening has provided valuable information on the magnitude of genotype x environment interactions. It follows that in instances where yield potential is affected by growth limiting factors other than Zn, such as drought stress at Birchip, there is a risk that a genotype sensitive to drought stress would achieve erroneously high Zn efficiency due to its low yield at adequate Zn supply, or vice versa. An example of this is Franklin, which had 50% less grain yield than WI-2976 at low and adequate Zn, but both genotypes had similar efficiencies (Table 9.1). The lower grain yield in Franklin compared with WI-2976 can be explained partly by the maturity of the two genotypes: late maturing Franklin was affected by drought to a greater extent than early-medium maturing WI-2976. This result highlights once more the difficulty of assessing Zn

Table 9.1. Grain yields and Zn efficiencies of eight barley and two wheat genotypes grown under controlled and field conditions. Zn efficiency (E) is calculated as [(-Zn)/(+Zn)*100] (R. D. Graham, unpubl.).

Genotype	Birchip			Pot assay		
	Grain yield (t/ha)			Grain yield (g/plant)		
	(-Zn)	(+Zn)	E (%)	(-Zn)	(+Zn)	E (%)
Barley						
Skiff	1.07	1.22	88	3.21	7.94	40
Franklin	0.52	0.58	90	0.17	5.89	3
Clipper	0.95	1.00	95	3.04	7.46	41
Forrest	0.98	0.98	100	1.73	7.11	24
Amagi Nijo	0.87	1.03	84	0.42	7.18	6
WI-2976	1.14	1.26	90	3.72	9.44	39
Harrington	0.78	0.72	108	2.14	5.84	37
SA93013	0.63	1.01	63	0.73	5.99	12
Wheat						
Excalibur	1.14	1.50	76	1.11	4.61	24
Yallaroi	0.63	0.89	70	0	4.53	0

efficiency in the field independently of other stresses. Therefore, there is a need to develop alternative methods to field screening, which was one of the aims of this study.

9.4 Recommendations for screening method

The seedling screening method developed during this study shows potential for identifying Zn-efficient genotypes. Based on the discussion in previous sections the following recommendation on a screening method can now be made:

(i) Soil Zn concentration needs to be below the critical DTPA-extractable Zn. This may require the critical level to be determined for the soil and Zn concentration for each soil batch at the start of experiments may also need to be measured.

(ii) Seed with similar Zn contents should be used. The Zn content should preferably be low (e.g. 0.5-0.9 μg Zn/seed).

(iii) Genotypes of known Zn efficiency should be included as controls to indicate the most appropriate time of assessment.

(iv) When defining the Zn concentration(s) at which to test plants, soil volume and growth stage should be taken into consideration. A low soil Zn concentration in small pots is suitable at the early stage, while a marginal soil Zn concentration in larger pot is required if the plants are grown to later stages. Two levels of Zn (deficient and adequate) are suggested for routine screening work and a preliminary experiment is often useful to determine the test levels before a large scale of screening is performed. However, when screening segregating populations, it is not possible to use a number of Zn levels and a single level (deficient) is the only alternative.

(v) Under very severe deficiency both Zn-efficient and Zn-inefficient genotypes will eventually show deficiency symptoms. Therefore, comparisons should be

made after the appearance of Zn deficiency symptoms on the most sensitive genotypes but before the development of Zn deficiency symptoms on all genotypes. This allows the best discrimination between Zn-efficient and Zn-inefficient genotypes. The time of assessment may vary depending on the soil Zn concentration; in Lancelin sand symptoms first appeared 12-14 DAS and plants were harvested at 21 DAS.

(vi) A score based on expression of Zn deficiency symptoms in the seedlings can be used as a non-destructive method of assessing variation among genotypes.

9.5 Genetic analysis of Zn efficiency

The genetic analysis of the F_2 population from the cross between Skiff and Forrest using visual scores revealed that tolerance to Zn deficiency in Skiff relative to Forrest is probably controlled by a single gene with no dominance. However, this needs to be confirmed in further generations. Based on visual scores at the early stage, Skiff can be classified as moderately tolerant (score 2-3), and Forrest as sensitive (score 7-8). From the genetic analysis of other nutrients elsewhere, such as Mn efficiency (Longnecker *et al.*, 1988; McCarthy *et al.*, 1988; Khabaz-Saberi *et al.*, 1999; Graham *et al.*, 1995), it can be hypothesized that tolerance to Zn deficiency may be controlled by more than one gene in genotypes with greater tolerance to Zn deficiency than Skiff. This hypothesis of polygenic control can be tested following the identification of genotypes with greater tolerance to Zn deficiency than Skiff, and construction of F_2 or doubled haploid populations.

Screening of the two contrasting bulks of the F_2 population from the cross between Skiff and Forrest with AFLP markers revealed a close association (94%) between the Zn efficiency and an $AFLP_1$ marker (Chapter 8). This result is encouraging, but needs

further confirmation in a validation population. If the same marker is detected in the validation population, then it can be used as a marker in a breeding program (i) to select for tolerance to Zn deficiency, and (ii) accelerate the introgression of the Zn efficiency gene(s) into breeding lines.

9.6 Future work needed

(i) The correlation between pot assay developed during this study and field screening needs to be established using a large number of genotypes. For example, barley genotypes tested at the early stage and maturity in this study can be tested at Zn-deficient sites for their tolerance to Zn deficiency. Field screening of this type should be carried out for at least 2-3 years by measuring their performance at various growth stages. This would also provide some indication of the extent of genotype x environment interactions.

(ii) The recent studies with other cereals reported the greater Zn acquisition from soils and utilization of Zn in tissues as the main mechanisms of Zn efficiency (Rengel and Graham, 1995, 1996; Cakmak *et al.*, 1996b, 1998). The present study did not examine directly the involvement of these two factors as mechanisms of efficiency in barley; however, the results suggested that better utilization of Zn in tissues may also be one of the mechanisms for Zn efficiency in barley (Chapters 4 and 7). Therefore, further research is required to elucidate uptake, and root-to-shoot transport of Zn as factors responsible for Zn efficiency of barley using barley genotypes identified in this study.

(iii) The results of genetic analysis in this study needs to be confirmed in F_3 families as well as in other F_2 and F_2-derived F_3 families from crosses between Zn-efficient and Zn-inefficient genotypes, either determined in this study (Chapter

7) or to be identified in the future. Given the difficulties of screening segregating populations, such as being irreversible (if lost in the process) and non-replicable, stable populations (e.g. doubled haploid) should be constructed in the future.

(iv) Refining of the work initiated in this study to identify molecular markers linked to Zn efficiency gene(s) using F_2 or doubled haploid populations is needed, which would form the basis of future screening, marker-assisted selection.

Appendix 3.1. Effect of Zn fertilization (mg/kg soil) on Zn content (µg/plant) in shoots of barley genotypes at tillering (14 days after sowing) in Experiment 1.

Zn fertilization	Tantangara	Amagi Nijo
0	0.7 (0.54)[a]	0.7 (0.50)
0.04	1.8 (1.02)	1.8 (1.02)
0.2	6.6 (2.02)	6.8 (2.05)
0.8	16.3 (2.84)	17.5 (2.92)
3.2	47.2 (3.87)	43.0 (3.78)
Tukey's $HSD_{0.05}$		
Genotype x Zn fert.	ns	

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.
Ns;non-significant.

Appendix 3.2. Effect of Zn fertilization (mg/kg soil) on Zn content (µg/plant) in roots of barley genotypes at tillering (14 days after sowing) in Experiment 1.

Zn fertilization	Tantangara	Amagi Nijo
0	1.1 (0.75)[a]	0.9 (0.63)
0.04	1.2 (0.78)	1.2 (0.80)
0.2	2.4 (1.21)	2.7 (1.30)
0.8	7.6 (2.15)	9.1 (2.31)
3.2	48.8 (3.90)	39.3 (3.69)
Tukey's $HSD_{0.05}$[b]		
Genotype x Zn fert.	(0.18)	

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.
[b] The $HSD_{0.05}$ values are applicable to log-transformed data.

Appendix 3.3. Effect of Zn fertilization (mg/kg soil) on root and shoot dry weight (g/plant) of two barley genotypes at 26 DAS in Experiment 2.

Zn fertilization	Shoot dry wt.		Root dry wt.	
	Tantangara	Amagi Nijo	Tantangara	Amagi Nijo
0	0.39	0.41	0.22	0.26
0.04	0.51	0.58	0.27	0.32
0.8	0.69	0.85	0.26	0.39
Tukey's $HSD_{0.05}$				
Genotype x Zn fert.	0.08		0.06	

Appendix 3.4. Effect of seed Zn content on root and shoot dry weight (g/plant) of two barley genotypes at 26 DAS in Experiment 2.

	Shoot dry wt.		Root dry wt.	
Seed Zn content	Tantangara	Amagi Nijo	Tantangara	Amagi Nijo
Low	0.47	0.46	0.22	0.21
Medium	0.52	0.57	0.25	0.29
High	0.52	0.70	0.25	0.38
Very high	0.61	0.74	0.28	0.43
Tukey's $HSD_{0.05}$				
Genotype x Seed Zn	0.06		0.05	

Appendix 3.5. Effect of Zn fertilization (mg/kg soil) on Zn content (µg/plant) in shoots of barley genotypes at 26 DAS in Experiment 2.

Zn fertilization	Tantangara	Amagi Nijo
0	2.2 (1.13)[a]	2.2 (1.12)
0.04	3.8 (1.54)	3.3 (1.45)
0.8	29.7 (3.42)	32.5 (3.51)
Tukey's $HSD_{0.05}$[b]		
Genotype x Zn fert.	(0.06)	

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.
[b] The $HSD_{0.05}$ values are applicable to log-transformed data.

Appendix 3.6. Effect of seed Zn content on Zn content in shoots of two barley genotypes at 26 DAS in Experiment 2.

Seed Zn content	Tantangara	Amagi Nijo
Low	10.5 (1.83)[a]	10.5 (1.80)
Medium	11.1 (1.95)	11.8 (1.92)
High	11.7 (2.02)	14.0 (2.12)
Very high	14.2 (2.31)	14.4 (2.26)
Tukey's $HSD_{0.05}$[b]		
Genotype x Seed Zn	(0.06)	

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.
[b] The $HSD_{0.05}$ values are applicable to log-transformed data.

Appendix 3.7. Effects of Zn fertilization (mg/kg soil) and seed Zn content on Zn content in shoots (µg/plant) of barley at 26 DAS in Experiment 2.

Zn fertilization	Seed Zn content			
	Low	Medium	High	Very high
0	1.3 (0.82)[a]	1.8 (1.01)	2.2 (1.17)	3.5 (1.50)
0.04	2.6 (1.27)	3.0 (1.38)	3.6 (1.53)	5.0 (1.79)
0.8	27.7 (3.36)	29.5 (3.42)	32.7 (3.51)	34.5 (3.57)
Tukey's $HSD_{0.05}$[b]				
Seed Zn x Zn fert.			(0.09)	

[a] Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.
[b] The $HSD_{0.05}$ values are applicable to log-transformed data.

Appendix 3.8. Effects of Zn fertilization (mg/kg soil) and seed Zn content on Zn content (µg/plant) in roots of two barley genotypes at 26 DAS in Experiment 2.

Genotype	Zn	Seed Zn content			
		Low	Medium	High	Very high
Tantangara	0	1.7 (1.00)*	2.3 (1.19)	2.4 (1.28)	3.8 (1.57)
	0.04	2.6 (1.31)	3.2 (1.44)	3.3 (1.46)	4.5 (1.71)
	0.8	13.6 (2.68)	14.1 (2.72)	14.5 (2.74)	15.0 (2.77)
Amagi Nijo	0	1.2 (0.78)*	2.3 (1.18)	3.7 (1.40)	4.7 (1.70)
	0.04	2.9 (1.36)	3.1 (1.41)	3.6 (1.54)	5.3 (1.84)
	0.8	12.6 (2.61)	15.5 (2.80)	18.4 (2.88)	17.6 (2.88)

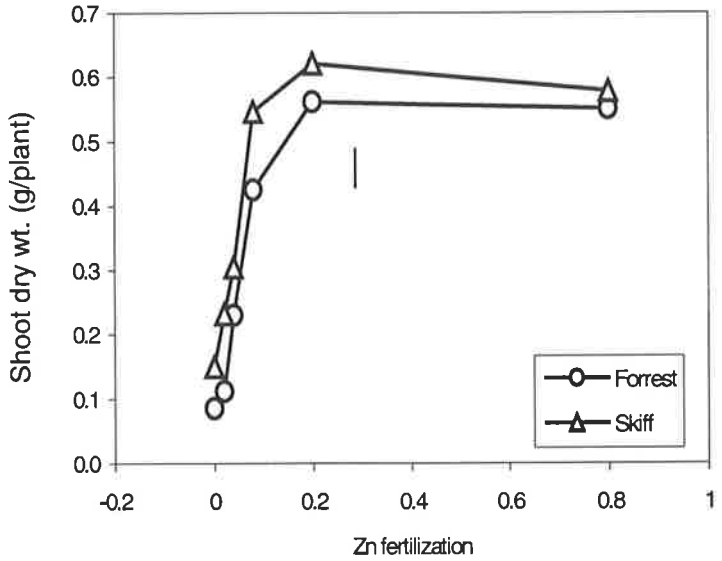
Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.
* The HSD values (log-transformed) are greater than one another at P=0.06.

Appendix 4.1. Effect of Zn fertilization (mg/kg soil) on root:shoot dry weight ratio of barley genotypes at 26 DAS in Experiment 2.

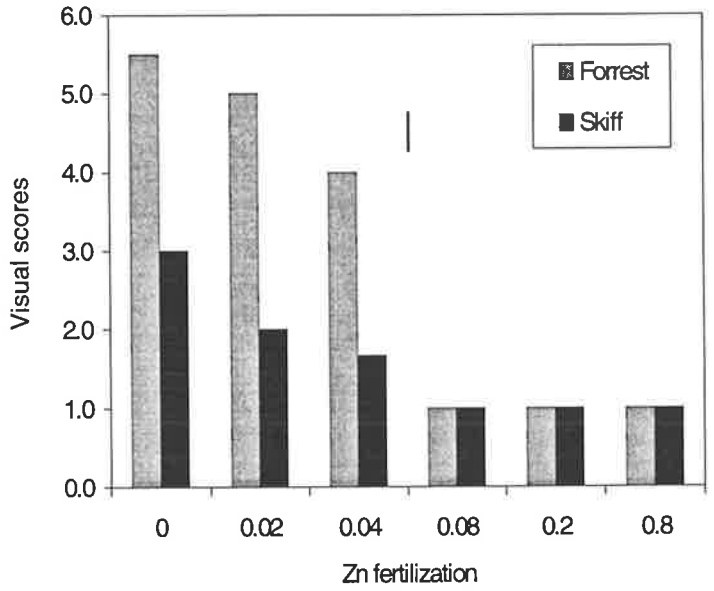
	Zn fertilization			
Genotype	0	0.04	0.8	Mean
Tarm	0.56	0.61	0.57	0.58
Hamidiye	0.41	0.43	0.41	0.42
Yesevi	0.57	0.57	0.55	0.56
Erginel	0.55	0.55	0.60	0.57
Galleon	0.57	0.47	0.47	0.50
WI-2875	0.51	0.47	0.45	0.48
Amagi Nijo	0.39	0.42	0.42	0.41
Yagan	0.52	0.45	0.50	0.49
WI-2597	0.49	0.44	0.41	0.45
Sahara	0.48	0.46	0.50	0.48
WI-2868	0.49	0.50	0.59	0.53
Harrington	0.60	0.52	0.52	0.55
Skiff	0.54	0.49	0.48	0.50
Clipper	0.63	0.58	0.49	0.57
Schooner	0.48	0.51	0.47	0.49
Tantangara	0.42	0.39	0.45	0.42
Forrest	0.59	0.60	0.55	0.58
CI3576	0.63	0.48	0.44	0.52
Haruna Nijo	0.59	0.48	0.53	0.53
Chebec	0.67	0.60	0.56	0.61
Mean	0.54	0.50	0.50	
Tukey's $HSD_{0.05}$				
Zn fert.		0.03		
Genotype		0.12		
G x Zn		ns		

ns; non-significant

a)



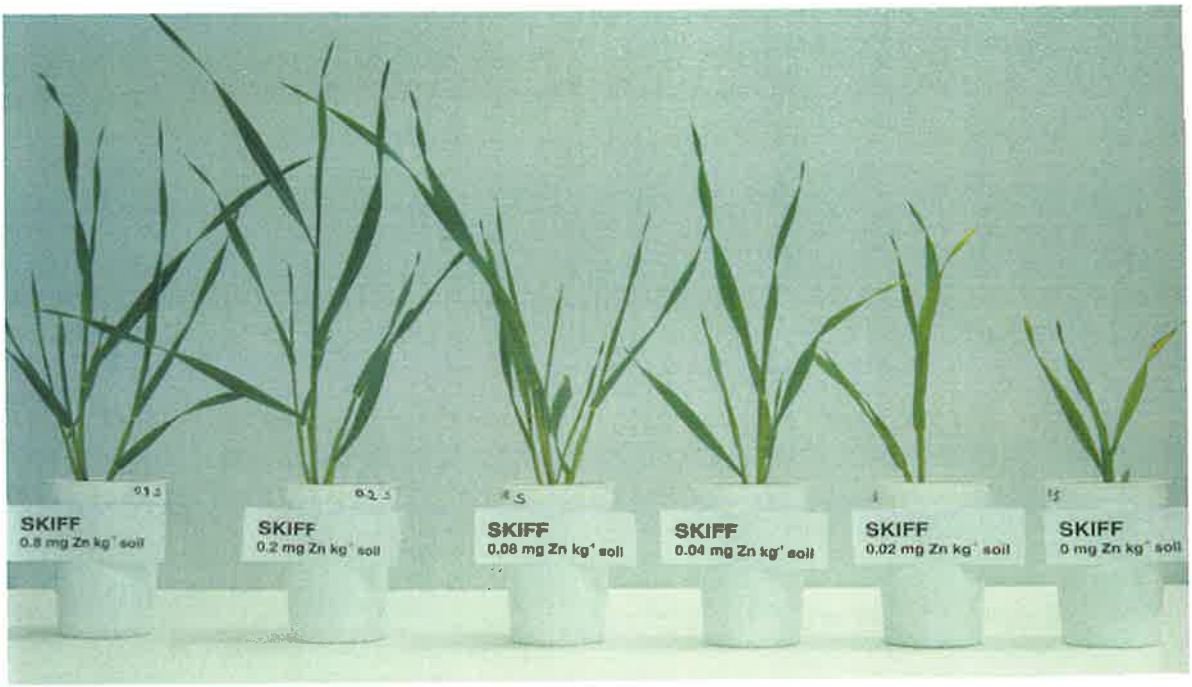
b)



Appendix 6.1. Effect of Zn fertilization on shoot growth (a) and expression of Zn deficiency symptoms (b) in barley genotypes in two barley genotypes at 21 DAS. Plants were scored on a scale of 1 to 6 (1, no symptoms; 6, very severe symptoms; for details see 5.2.2). The vertical bars represent Tukey's $HSD_{0.05}$ value for Genotype x Zn fertilization interaction.

a)

b)



Appendix 6.2. Effects of Zn fertilization on expression of Zn deficiency symptoms and plant growth in barley genotypes (a, Forrest; b, Skiff) at 21 DAS.

Appendix 7.1. Seed weight (mg/seed), Zn concentration (mg/kg D.W.), and Zn content (µg/seed) of barley and wheat genotypes used in Experiment 1. Standard errors are based on three replicates containing 10 seeds each.

	Seed weight	Zn concentration	Zn content
Barley			
Amagi Nijo	44 ± 2	16.1 ± 0.8	0.70 ± 0.03
Arapiles	43 ± 2	14.5 ± 1.0	0.62 ± 0.04
Bearpaw	43 ± 2	18.2 ± 1.2	0.78 ± 0.05
Blenheim	44 ± 2	20.4 ± 0.5	0.90 ± 0.02
Chariot	43 ± 2	15.2 ± 0.1	0.66 ± 0.01
Chebec	44 ± 2	16.0 ± 0.7	0.70 ± 0.03
Cheri	44 ± 2	14.2 ± 1.9	0.62 ± 0.08
Clipper	44 ± 2	15.3 ± 0.9	0.67 ± 0.04
Europa	45 ± 2	17.4 ± 1.8	0.78 ± 0.08
Fitzgerald	45 ± 2	15.8 ± 0.2	0.71 ± 0.01
Forrest	44 ± 2	17.9 ± 0.3	0.79 ± 0.01
Franklin	43 ± 2	16.5 ± 0.3	0.72 ± 0.01
Gairdner	46± 2	17.8 ± 0.9	0.82 ± 0.04
Galleon	43 ± 2	16.4 ± 0.6	0.71 ± 0.03
Hamidiye	43 ± 2	21.2 ± 0.5	0.91 ± 0.02
Harrington	43 ± 2	16.7 ± 0.4	0.72 ± 0.02
Haruna Nijo	44 ± 2	14.6 ± 1.0	0.64 ± 0.04
Kinukei 15	38 ± 2	15.8 ± 1.0	0.60 ± 0.04
Kinukei 19	43 ± 2	15.6 ± 0.8	0.68 ± 0.04
Kinukei 21	43 ± 2	15.0 ± 0.4	0.64 ± 0.02
Manley	41 ± 2	17.4 ± 1.6	0.72 ± 0.07
Molloy	44 ± 2	18.1 ± 0.7	0.79 ± 0.03
Mundah	44 ± 2	16.2 ± 1.3	0.71 ± 0.06
Namoi	44 ± 2	20.6 ± 1.5	0.90 ± 0.07
Natasha	41 ± 2	15.7 ± 1.3	0.64 ± 0.05
NSW WB 190R	44 ± 2	16.0 ± 0.3	0.70 ± 0.01
O'Connor	43 ± 2	17.2 ± 0.4	0.75 ± 0.02
Onslow	42 ± 2	17.0 ± 0.9	0.72 ± 0.04
Proctor	40 ± 2	14.9 ± 0.8	0.59 ± 0.03
SA 93013	43 ± 2	16.9 ± 0.5	0.73 ± 0.02
Schooner	43 ± 2	16.5 ± 0.5	0.72 ± 0.02
Skiff	44 ± 2	18.4 ± 0.6	0.80 ± 0.03
Steptoe	44 ± 2	20.5 ± 0.6	0.90 ± 0.03
Stirling	44 ± 2	18.8 ± 0.1	0.82 ± 0.00
Tantangara	43 ± 2	16.6 ± 0.8	0.72 ± 0.04
Tarm	44 ± 2	23.3 ± 1.1	1.03 ± 0.05
TR-306	46 ± 2	19.7 ± 1.7	0.90 ± 0.08
VIC 86045B	43 ± 2	16.0 ± 1.3	0.69 ± 0.05
VIC 9307	44 ± 2	15.4 ± 0.9	0.67 ± 0.04
VIC 9316/11	44 ± 2	14.0 ± 0.6	0.61 ± 0.03
VIC 9409	43 ± 2	16.6 ± 0.5	0.72 ± 0.02
VIC 9524	44 ± 2	16.6 ± 0.5	0.72 ± 0.02
WA 28776	44 ± 2	15.1 ± 1.1	0.66 ± 0.05
WA 28784	44 ± 2	15.1 ± 0.6	0.66 ± 0.03
WA 73S276	44 ± 2	18.9 ± 0.6	0.83 ± 0.03
Waveney	40 ± 2	17.3 ± 1.4	0.69 ± 0.05

Cont.

WI-2597	44 ± 2	14.7 ± 0.8	0.64 ± 0.03
WI-2868	44 ± 2	17.2 ± 0.4	0.75 ± 0.02
WI-2875-22	44 ± 2	15.8 ± 1.0	0.69 ± 0.04
WI-2976	44 ± 2	16.5 ± 0.5	0.72 ± 0.02
WI-2978	44 ± 2	16.3 ± 0.7	0.71 ± 0.03
WI-2986	44 ± 2	16.4 ± 0.4	0.72 ± 0.02
WI-3044	43 ± 2	17.0 ± 0.4	0.74 ± 0.02
WI-3050	43 ± 2	15.4 ± 0.9	0.67 ± 0.04
WI-3051	44 ± 2	17.0 ± 0.7	0.74 ± 0.03
Yagan	43 ± 2	17.0 ± 0.3	0.74 ± 0.01
Wheat			
Excalibur	43 ± 2	16.9 ± 1.1	0.73 ± 0.05
Songlen	43 ± 2	9.1 ± 0.2	0.39 ± 0.01
Trident	45 ± 2	16.4 ± 0.4	0.74 ± 0.02
Yallaroi	44 ± 2	16.7 ± 2.5	0.73 ± 0.11

Appendix 7.2. Mean nutrient concentration (mg/kg dry wt) in shoots of 18 barley and 2 wheat genotypes (selected out of 56 barley and 4 wheat genotypes) grown in Lancelin sand fertilized with two Zn levels (0.02 and 0.8 mg Zn/kg soil) in Experiment 1.

Genotype	Zn fert.	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
Barley															
Amagi Nijo	0.02	277	43	10.8	6.0	6.6	2.2	3.4	3.4	6870	2930	1000	47700	11700	6130
	0.8	71	24	8.2	9.4	9.4	0.6	0.9	23.0	7600	1930	1110	64000	5400	3600
Chariot	0.02	330	52	12.1	10.0	5.8	1.9	2.9	4.8	7970	2970	1410	54700	12200	6400
	0.8	84	19	7.0	9.5	11.7	0.7	1.0	26.0	7800	1930	1070	60300	5830	3730
Cheri	0.02	557	52	10.7	13.1	6.7	2.5	3.9	21.9	7400	3300	1090	45300	9700	7200
	0.8	76	18	5.8	8.3	8.6	0.6	0.9	4.1	6630	1690	980	59700	5570	3570
Clipper	0.02	303	49	11.6	10.9	5.8	2.6	4.1	26.0	6770	2830	1200	48300	11630	5270
	0.8	79	19	7.7	9.2	8.7	0.7	1.1	4.3	8770	1920	1240	63300	6500	3870
Forrest	0.02	303	45	11.6	9.0	6.8	2.3	3.6	21.4	7070	2770	890	56700	11970	7300
	0.8	74	16	5.9	7.5	8.1	0.6	0.9	3.5	6830	1650	1020	58300	4700	3730
Franklin	0.02	717	86	13.5	19.7	6.9	2.4	3.7	23.8	8830	3100	1380	46300	9000	7400
	0.8	75	19	7.1	9.8	11.0	0.6	0.9	4.2	8170	1580	1060	58700	5870	3230
Harrington	0.02	373	44	13.0	9.7	6.0	1.6	2.5	22.4	8270	2900	950	51700	9370	7170
	0.8	77	14	6.9	8.1	8.6	0.6	1.0	4.0	7330	1610	940	57000	5700	3023
Kinukei 21	0.02	290	42	9.4	5.5	4.3	2.3	3.6	4.4	7400	2730	920	43000	6700	5730
	0.8	59	22	7.4	9.0	7.4	0.7	1.0	25.1	8800	1870	1040	63700	5130	4100
Manley	0.02	433	68	12.4	17.7	6.6	1.8	2.7	4.4	6870	3070	1190	57700	9100	8170
	0.8	87	15	7.6	10.6	10.1	0.6	0.9	23.3	8000	1780	1000	61300	6300	3770
Onslow	0.02	287	40	13.1	7.9	5.4	2.6	4.1	4.1	7800	2630	1160	59700	13700	7730
	0.8	77	16	6.8	8.6	10.3	0.7	1.0	23.7	7430	1660	1020	63700	5800	3870
Proctor	0.02	257	46	9.8	9.4	8.8	1.4	2.1	5.3	7430	2700	1200	55000	7430	6500
	0.8	90	21	5.3	8.5	11.2	0.7	1.0	25.1	8100	1900	1360	60000	6130	3830
SA93013	0.02	290	52	13.4	9.8	7.0	2.3	3.6	3.6	6670	2830	750	38700	10600	6630
	0.8	67	17	8.0	7.5	9.2	0.6	0.9	19.3	6370	1680	990	53000	5500	3400
Skiff	0.02	453	60	10.5	13.5	5.2	1.8	2.7	5.6	7430	3000	1260	67700	10400	7000
	0.8	79	18	5.8	8.8	8.0	0.9	1.3	22.0	7900	1920	1100	68700	5800	4430
Tantangara	0.02	473	70	10.0	12.3	7.2	1.5	2.3	4.5	7930	3100	1640	74000	7630	7600
	0.8	99	20	6.1	10.0	8.2	0.7	1.1	25.2	7830	1990	1130	69300	6670	3930

Cont.

Tarm	0.02	357	32	14.2	10.8	5.6	1.7	2.6	5.1	5870	2370	1090	52700	8560	5200
	0.8	83	18	9.0	8.6	10.9	0.8	1.3	23.3	5830	1450	1070	68700	5930	4470
WA28784	0.02	390	52	13.5	10.0	7.5	2.7	4.1	4.2	7500	2700	1160	55300	16900	8430
	0.8	81	15	6.9	7.5	7.7	0.6	1.0	21.2	7570	1750	1090	62300	5500	3970
WI-2976	0.02	227	56	10.0	13.2	7.1	2.1	3.2	5.8	7270	2170	1200	69000	9270	6700
	0.8	88	20	6.6	8.2	4.9	1.1	1.6	21.1	7500	1480	1670	72000	5300	5070
Yagan	0.02	366	57	11.6	10.5	7.4	1.9	3.0	3.4	7130	2420	1020	51300	7800	6130
	0.8	83	14	6.6	8.7	8.3	0.7	1.1	21.5	6400	1620	1700	64300	5170	4230
Wheat															
Excalibur	0.02	183	148.3	12.8	11.7	7.1	3.5	5.3	5.1	4830	2830	50	46700	18570	7230
	0.8	96	43.0	7.7	10.2	10.5	0.8	1.2	29.0	5300	1900	100	58000	6430	4030
Songlen	0.02	211	122.7	13.0	10.5	7.6	5.6	8.6	5.1	5900	2830	70	43700	14270	8030
	0.8	109	36.0	7.6	9.4	5.9	0.9	1.4	32.6	5870	1760	100	50000	6300	4100
Genotype (G)		**	**	**	**	ns	**	**	**	**	**	**	**	**	**
Zn fert. (Zn)		**	**	**	**	**	**	**	**	ns	**	ns	**	**	**
G x Zn		**	**	ns	**	*	**	**	**	ns	*	**	**	**	**

ns; non-significant, * P≤ 0.05, **P≤0.01

Appendix 7.3. Seed size[a], seed weight (mg/seed dry wt) and mean nutrient concentration (mg/kg dry wt) in the seed of of barley genotypes in Experiment 2.

Genotype	Seed size	Seed wt.	Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S
	S	36	35	18	4.0	8.1	2.4	1.0	1.5	22	423	1413	230	4833	3200	1413
Forrest	M	47	33	17	4.2	7.3	2.6	0.7	1.1	22	400	1420	205	4600	3333	1440
	L	55	32	17	3.8	6.8	2.7	0.6	1.0	17	380	1360	175	4000	2933	1343
	S	32	35	15	3.9	5.4	2.8	1.1	1.7	19	387	1330	313	4900	3367	1493
Skiff	M	40	30	14	4.1	5.1	2.6	0.9	1.3	18	363	1307	310	4600	3167	1437
	L	46	30	15	3.3	5.3	2.9	0.8	1.2	19	367	1300	214	4267	3167	1400
	S	30	29	17	5.2	5.1	2.5	1.2	1.8	19	343	1427	217	4067	3233	1360
Amagi Nijo	M	39	26	17	5.1	4.8	2.8	0.9	1.4	17	323	1387	191	3900	3033	1310
	L	45	27	18	4.4	4.5	2.5	0.8	1.2	18	337	1420	150	3933	3333	1320
	S	32	29	16	3.6	5.4	3.3	1.1	1.7	18	450	1360	400	4833	3233	1457
Tantangara	M	39	29	15	2.7	5.0	3.0	0.9	1.3	18	430	1340	303	4433	3133	1430
	L	46	31	18	2.2	5.8	3.3	0.8	1.2	22	407	1440	174	4433	3700	1690
	S	33	34	14	3.4	5.2	2.5	1.0	1.6	18	327	1303	353	4833	3133	1437
Schooner	M	41	33	14	3.2	5.1	2.5	0.9	1.3	17	313	1307	320	4400	3067	1453
	L	45	35	15	3.0	4.9	2.8	0.8	1.2	16	313	1300	277	4400	2933	1483
	S	37	31	15	4.4	5.5	2.6	1.0	1.5	16	360	1233	327	4700	2900	1353
WI-2868	M	46	29	14	4.2	5.3	2.6	0.8	1.2	17	367	1217	280	4233	2767	1403
	L	52	30	17	3.4	5.4	3.1	0.7	1.0	18	390	1333	280	4500	3233	1513
Genotype (G)		**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Seed size (SS)		**	*	**	**	*	ns	**	**	ns	*	ns	**	**	ns	ns
G x SS		*	ns	*	ns	ns	ns	ns	ns	**	ns	ns	**	**	**	**

[a]Seed sizes were large, L>2.8, medium, 2.8>M>2.5 and small, 2.5>S>2.25 mm in diameter

ns; non-significant, * P≤ 0.05, **P≤0.01

a)

-Zn +Zn

b)

+Zn -Zn

Appendix 9.1. Effect of Zn fertilization (-Zn, +Zn) on plant growth and expression of Zn deficiency symptoms in barley genotypes Amagi Nijo (a) and SA93013 (b) at Birchip in 1998.

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