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

Yusuf Genc
B. Sc., M. Sc. (Agriculture)
Department of Agronomy
University of Ankara, Ankara, Turkey

Submitted to the University of Adelaide
for the degree of Doctor of Philosophy

Department of Plant Science
Faculty of Agriculture and Natural Resource Sciences
University of Adelaide, Adelaide, South Australia

June 1999
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Tables</td>
<td>vii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>x</td>
</tr>
<tr>
<td>List of Plates</td>
<td>xii</td>
</tr>
<tr>
<td>List of Appendices</td>
<td>xiii</td>
</tr>
<tr>
<td>Declaration</td>
<td>xiv</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>xv</td>
</tr>
<tr>
<td>Summary</td>
<td>xvii</td>
</tr>
</tbody>
</table>

## CHAPTER 1. General Introduction

## CHAPTER 2. Literature Review

### 2.1. Introduction

### 2.2. Chemistry of zinc

### 2.3. Zinc in soils

#### 2.3.1. Availability of soil zinc to plants

##### 2.3.1.1. pH

##### 2.3.1.2. Clays

##### 2.3.1.3. Temperature and moisture

##### 2.3.1.4. Organic matter

#### 2.3.2. Transport of zinc to the root surface

#### 2.3.3. Uptake, translocation and distribution of zinc

#### 2.3.4. Interactions between zinc and other nutrients

##### 2.3.4.1. P-Zn interactions

##### 2.3.4.2. N-Zn interactions

##### 2.3.4.3. Interactions of Zn with other macronutrients

##### 2.3.4.4. Zn-micronutrient interactions

### 2.4. Zinc in plants

#### 2.4.1. Zinc and plant metabolism

##### 2.4.1.1. Carbohydrate metabolism

##### 2.4.1.2. Protein metabolism

##### 2.4.1.3. Membrane integrity

##### 2.4.1.4. Auxin metabolism

##### 2.4.1.5. Defence mechanism

##### 2.4.1.6. Reproduction

#### 2.4.2. Zinc deficiency in plants

##### 2.4.2.1. Symptoms of zinc deficiency

##### 2.4.2.2. Diagnosing zinc deficiency
Plant tissue analysis ................................................................. 26
Soil analysis ............................................................................ 28
2.4.3. Role of seed reserves .................................................. 29
2.5. Breeding for zinc efficiency ............................................. 29
  2.5.1. Genotypic variation in sensitivity to Zn deficiency ....... 30
  2.5.2. Screening methodologies for assessment of genotypic variation in response to Zn deficiency .............. 32
    2.5.2.1. Field testing ...................................................... 32
    2.5.2.2. Soil culture ..................................................... 34
    2.5.2.3. Solution culture .............................................. 35
  2.5.3. Selection criteria for tolerance to Zn deficiency ........... 35
  2.5.4. Sound and economic reasons for pursuing a breeding solution to Zn deficiency ........................................... 36
  2.5.5. Understanding of genetic control of tolerance to Zn deficiency ................................................................. 37
2.6. Mechanisms of tolerance to zinc deficiency .................... 38
  2.6.1. Release of phytosiderophores .................................... 38
  2.6.2. Root morphology .................................................... 40
  2.6.3. Microbial activity .................................................... 41
2.7. Conclusion ....................................................................... 42

CHAPTER 3. Effect of seed Zn content on early growth of barley under low and adequate zinc supply ...................... 44
3.1. Introduction .................................................................... 44
3.2. Materials and methods .................................................. 45
3.3. Results .......................................................................... 48
  3.3.1. Experiment 1 ......................................................... 48
    3.3.1.1. Visual symptoms .............................................. 48
    3.3.1.2. Shoot and root dry matter ................................ 48
    3.3.1.3. Zn concentration and content of shoots and roots 52
    3.3.1.4. Zn concentration in youngest expanded leaves 53
  3.3.2. Experiment 2 .......................................................... 54
    3.3.2.1. Visual symptoms .............................................. 54
    3.3.2.2. Shoot and root dry matter ................................ 57
    3.3.2.3. Zn concentration and content of shoots and roots 59
    3.3.2.4. Zn concentration in youngest expanded leaves 61
3.4. Discussion ...................................................................... 62
3.5. Conclusion ..................................................................... 66
CHAPTER 4. Screening for zinc efficiency and determining critical zinc deficiency concentration in Laffer sand under controlled conditions ................................................................. 67
  4.1. Introduction .................................................................................................. 67
  4.2. Materials and methods ............................................................................. 69
    4.2.1. Experiment 1 ...................................................................................... 69
    4.2.2. Experiment 2 ...................................................................................... 71
  4.3. Results ........................................................................................................ 73
    4.3.1. Experiment 1 ...................................................................................... 73
      4.3.1.1. Visual symptoms ......................................................................... 73
      4.3.1.2. Shoot and root dry matter ......................................................... 73
      4.3.1.3. Root:shoot dry weight ratio ...................................................... 75
      4.3.1.4. Zn concentration and content of shoots and roots ..................... 75
      4.3.1.5. Zn concentration in youngest expanded leaves and critical concentration .................................................. 79
    4.3.2. Experiment 2 ...................................................................................... 82
      4.3.2.1. Visual symptoms ......................................................................... 82
      4.3.2.2. Shoot and root dry matter ......................................................... 82
      4.3.2.3. Root:shoot dry weight ratio ...................................................... 84
      4.3.2.4. Concentration and content of Zn in shoots and roots of selected genotypes .................................................. 84
      4.3.2.5. Zn concentration in youngest expanded leaves of selected genotypes .................................................. 84
  4.4. Discussion ................................................................................................... 86
  4.5. Conclusion .................................................................................................. 90

CHAPTER 5. Screening for tolerance to zinc deficiency in Lancelin sand under glasshouse conditions ................................................................. 91
  5.1. Introduction ................................................................................................ 91
  5.2. Materials and methods ............................................................................. 92
    5.2.1. Experiment 1 ...................................................................................... 92
    5.2.2. Experiment 2 ...................................................................................... 94
    5.2.3. Experiment 3 ...................................................................................... 96
  5.3. Results ........................................................................................................ 98
    5.3.1. Experiment 1 ...................................................................................... 98
      5.3.1.1. Visual symptoms ......................................................................... 98
      5.3.1.2. Shoot and root dry matter ......................................................... 98
      5.3.1.3. Root:shoot dry weight ratio ...................................................... 100
    5.3.2. Experiment 2 ...................................................................................... 100
      5.3.2.1. Visual symptoms ......................................................................... 100
      5.3.2.2. Shoot and root dry matter ......................................................... 100
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 μg/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 F2 population for genetic analysis of tolerance to Zn deficiency. The parents, and the F2 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.