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Barley Genotypes Differing in Zinc Efficiency When Grown in Various Soil Types

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Authors' contributions

This work was carried out in collaboration between all authors. Author BS designed the study, managed the experimental process and wrote the first draft of the manuscript. Author NS analyzed the data and managed the literature searches. Author ES helped in soil analysis, added some sections and revised the final manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Plant genotypes differ in their zinc (Zn) uptake; and accumulation of Zn by genotypes could be affected by soil types. In pot experiments in 5 different soil types, Zn uptake of a barley landrace (Sahara = Zn-efficient) was compared with a bred barley cultivar (Clipper = Zn-inefficient) at three Zn treatments (0.0, 0.02 and 0.8 mg Zn/Kg soil). At 0.8 Zn treatment, Zn concentration and content in shoot (at different growth stages) and seed of Sahara were significantly higher than those of Clipper. Higher concentration of Zn in the youngest leaf blades was found in Sahara grown in all soil types. The results indicated that Sahara landrace was more efficient in absorbing Zn from different soils than Clipper cultivar. It can be concluded that different soil types did not affect shoot and seed Zn concentration and content of Sahara, and these traits can be used in the assessment of barley genotypes, and may be useful criteria in screening large populations in various regions with different soil types.

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1. INTRODUCTION

Soils vary widely in their zinc (Zn) content and ability to supply enough Zn for optimal crop growth. Zinc deficiency is the most widespread micronutrient deficiency in world soils. Sillanpää [1] reported that Zn deficiency had a higher frequency than deficiencies of any of the other 6 micronutrients included in the experiments in 15 countries. It is estimated that approximately 50% of soils used for cereal production in the world, have low level of plant-available Zn [2]. Soil deficiency is also aggravated by introduction of high-yielding cultivars, imbalanced use of fertilizers and a lack of adequate manure application [3]. Total Zn level of soils are rarely indicative of plant availability because Zn availability depends on pH, soil organic matter content, crop species, adsorptive surfaces, and other physical, chemical and biological conditions in the rhizosphere soil [4,5].

Plant species and genotypes differ in their Zn requirement; some are inherently more efficient than the others. Zinc efficiency varies among cereal species including wheat [6,7], barley [6,8], and rice [9]]. Improving Zn uptake efficiency has recently become a major plant breeding task in several countries.

Genotypic variations in Zn efficiency have been associated with different mechanisms operating within a plant system and in the rhizosphere. Plant genotypes possess various mechanisms for efficient acquisition of Zn from soils low in Zn availability [10]. These include increased Zn bioavailability in the rhizosphere due to release of root exudates, higher Zn uptake by roots, and efficient utilization and (re)-translocation of Zn [11-13]. In wheat, release of Zn-mobilizing phytosiderophores from roots [14,15], Zn uptake capacity of roots [16], root growth enhancement [17], higher activity of carbonic anhydrase [18,19], and a greater amount of sulfhydryl groups in root-cell plasma membranes [20] are reported as responsible mechanisms for expression of Zn efficiency. In barley, Genc et al. [21] found the greater efficiency of genotypes may be attributed to their higher uptake of Zn from the soil and more efficient utilization at a cellular level. In field experiments on Zn-deficient calcareous soils, Zn efficiency was positively correlated with total amount (uptake) of Zn in shoots [22].

A differential response of plants to Zn deficiency indicates that genotypic variation could be exploited in the breeding programs to produce genotypes with higher Zn efficiency [23]. Development of Zn-efficient crops may provide a number of benefits, such as reduction in the use of fertilizers, improvements in seedling vigour, resistance to pathogens, increased yields and enhancement of grain nutritional quality in Zndeficient soils [2,23,24].

Different screening methods have been used to evaluate Zn efficiency: nutrient solution culture [25], greenhouse soil bioassays [8,26] and field evaluations [27]. Field-based techniques are more laborious, and its results can be variable because the severity of nutrient deficiency varies between sites and years, due to the effects of other growth limiting factors like drought and disease. Lack of a suitable screening procedure to allow screening of a large number of genotypes in short time hampered breeding for Zn efficiency. Traits conferring Zn efficiency may be useful only in specific soil types, or may represent more general adaptations to low Zn availability. This information is essential in selecting and breeding more Zn efficient barley genotypes. In this study, a soil-based pot assays under controlled conditions was used as an alternative method to field trials to determine genotypic variation in the response of barley genotypes (Sahara 3771 and Clipper) to Zn deficiency in different soil types. Our objective was to determine if Zn efficiency in barley genotypes is limited to specific soil types.

2. MATERIALS AND METHODS

Two barley genotypes, Clipper (Zn-inefficient) and the Algerian land-race Sahara 3771 (Znefficient), used in this study were obtained from Australian Winter Cereal Collection. These two genotypes were the parents of Doubled Haploid lines (DHs). Clipper and Sahara seed Zn concentrations were 41 and 75 mg/kg dry matter, and seed Zn contents 2.1 ± 0.3 and 2.7 ± 0.3 µg/seed, respectively. Seeds were hand sorted to a uniform size.

After studying characteristics of different soil types in Western Australia, five different soil types with relatively neutral pH and Zn-deficient were collected as the top 10 cm from Lancelin, Kellerberrin-7, Wongan Hills, Kellerberrin-7A and Merredin areas in Western Australia. These soils were chosen based on McArthur [28] reference

book. Characteristics of all soils are given in Table 1. Soils were air-dried and sieved through a 2-mm stainless steel sieve. Two-kg samples of soils were placed into plastic bags. Basal nutrients (in mg/kg of dry soil) 91 KH₂PO₄, 145 K₂SO₄, 147 CaCl₂.2H₂O, 21 MgSO₄.7H₂O, 2 CuSO₄.5H₂O, 15 MnSO₄.H₂O, 0.7 H₃BO₃, 0.2 Na₂MoO₄.2H₂O, and 93 NH₄NO₃ together with three Zn treatments (0.0, 0.02 and 0.8 mg Zn/kg soil applied as ZnSO₄.7H₂O) were applied to the soil surface, allowed to dry and mixed well throughout. Then, 2 kg of mixed soils were placed into plastic-bag-lined milk cartons (9.5 x 9.5 x 21 cm) of two-litter volume.

Seed surface was sterilized by soaking in 70% (v/v) ethanol for 1 min, followed by 10% (v/v)sodium hypochlorite for 30 seconds, and rinsing in three changes of double-deionised (DDI) water (18 MOhms/cm resistivity). Seeds were placed in Petri dishes on ashless filter paper moistened with deionised water for germination at room temperature for 36 hours before sowing. Sixteen pre-germinated seeds of each genotype were sown in each pot. Seedlings were thinned for uniformity to 13 plants per pot at the two-leaf stage. Plants were grown in a growth room at 20 °C day/15 °C night temperature and 10 h photoperiod. Pots were rotated within a block daily to minimize the effect of microenvironments caused by uneven light distribution and/or airflow. Plants were watered with doubledeionized water daily by weight, keeping water content at 90% (w/w) of the field capacity. Field capacity for soils was: 10% Lancelin, 14% Kellerberrin-7, 18% Wongan 24% Hills, Kellerberrin-7A and 10% Merredin.

Several traits were measured during and at the end of experiment. Four, two and seven plants were harvested 53 and 84 days after sowing (DAS) and at maturity, respectively. Harvested plants were washed under running deionized water then dipped in three changes of DDI water. The youngest expanded leaf blades (YEBs) and flag leaves were taken from all harvested plants. Plant samples were oven dried at 70 °C for 72 hours. After weighting, dried samples were digested with a mixture of 70% (v/v) nitric acid (HNO_3) and concentrated perchloric acid $(HCIO_4)$ and analysed for Zn concentration by an atomic absorption spectrophotometer (AAS) as described elsewhere [29]. Total nutrient content was calculated by multiplying shoot dry weights with shoot Zn concentrations. Zinc uptake efficiency (ZUE) was calculated as a proportion of soil-applied Zn that accumulated in shoots.

The experiment was conducted in a completely randomized design (2 genotypes x 3 Zn levels x 2 replicates x 5 soils). Results were analysed by the GENSTAT statistical package. Duncan's Multiple Range Test at $\alpha = 0.05$ was employed in pairwise comparisons. Because of considerable variation in seed Zn concentration of Clipper and Sahara potentially affecting interpretation of the results, covariance analysis was performed using seed Zn as the covariate to eliminate that portion of experimental error.

3. RESULTS AND DISCUSSION

The soils utilized in this experiment did not impose visual Zn deficiency symptoms such as development of chlorotic areas on leaves in Clipper and Sahara genotypes. In Kellerberrin-7 soil, a shoot dry matter decease under Zn deficiency was highly pronounced for Clipper, whereas Sahara dry matter was similar at all Zn levels. At Zn supply of 0.8 mg/kg, Clipper had greater shoot dry matter than Sahara, but both genotypes achieved similar dry matter when Zn supplies were 0.0 and 0.02 mg/kg. However, Zn efficiency (the ratio of shoot growth at low compared to the adequate Zn) of Clipper (87%) was less than Sahara (97%). In other soils, there was no significant difference between genotypes for shoot dry matter.

In Lancelin, Kellerberrin-7, Wongan Hills and Kellerberrin-7A soils, shoot Zn concentration and content of both genotypes were much higher in plants under sufficient (0.8 mg/kg) than deficient Zn conditions (0.0 and 0.02 mg/kg). At 0.0 and 0.02 mg applied Zn/kg soil, Zn concentration and content in the shoots of Sahara tended to be higher than those in Clipper, but these differences between genotypes were not significant. Compared with control. both concentration and content of Zn were considerably increased when plants were fertilized with 0.8 mg Zn/kg soil (Figs. 1 and 2). Significant genotypic differences in shoot Zn concentration and content occurred between two genotypes at 0.8 mg Zn/kg, with Sahara accumulating more Zn in the shoots than Clipper. Zinc uptake efficiency (the percentage of applied Zn that accumulated in shoot) was higher in Sahara than in Clipper at 0.02 mg Zn/kg in Lancelin, Kellerberrin-7 and Wongan Hills soils. The genotypes did not differ for Zn uptake efficiency at 0.8 mg Zn/kg in any soil types (Table 2).

Soil type	Organic	Organic	NH ^{3 -} -N	NH ^{4 -} -N	Ρ	К	DTPA-	DTPA-	DTPA-	DTPA-	Total	рН	рН	EC
	carbon	matter	mg/kg	mg/kg	mg/kg	mg/kg	Cu	Zn	Mn	Fe	Zn	(CaCl ₂)	(H ₂ O)	µs/Cm
	(%)	(%)					mg/kg	mg/kg	mg/kg	mg/kg	mg/kg			(25°C)
Lancelin	0.7	1.2	1.5	3	3.5	13.5	0.17	0.16	1.2	25.3	3	6.2	6.7	15
Kellerberrin-7	0.5	1.0	6.5	3	7.0	170	0.44	0.14	4.4	9.7	9	6.0	6.8	1.6
Wongan Hills	0.7	1.3	2	3	3.5	63	0.20	0.16	5.2	31.3	5	5.5	6.1	14.9
Kellerberrin-7A	0.9	1.7	2	1	4.5	241	0.55	0.16	5.6	9.1	10	6.5	6.9	9.6
Merredin	0.4	0.7	3	1	3.5	93	0.34	0.12	3.9	8.9	5	6.2	6.7	9.1

Table 1. Characteristics of five collected soil types used to grow two barley genotypes, Sahara (Zn-efficient) and Clipper (Zn-inefficient) at various Zn treatments

Table 2. Zn uptake efficiency (± SE) (proportion of soil-applied Zn accumulated in shoots, %) of two barley genotypes grown at two Zn levels in the first harvest (53 days after sowing)

Soil type	Zn fertilization (mg/kg soil)								
		0.02	0.8						
	Sahara	Clipper	Sahara	Clipper					
Lancelin	26 (±0.7)	17 (±0.7)	4.1 (±0.7)	2.1 (±0.7)					
Kellerberrin-7	50 (±1.1)	41 (±1.1)	3.5 (±1.1)	2.6 (±1.1)					
Wongan Hills	27 (±0.8)	19 (±0.8)	2.2 (±0.8)	0.7 (±0.8)					
Kellerberrin-7A	28 (±1.4)	26 (±1.4)	3.9 (±1.4)	2.0 (±1.4)					
Merredin	26 (±1.3)	26 (±1.3)	3.0 (±1.3)	2.4 (±1.3)					



Applied Zn level (mg/kg soil)

Fig. 1. Effects of Zn fertilization rates and four soil types on Zn concentration in the shoots of barley genotypes, Sahara (Zn-efficient) and Clipper (Zn-inefficient) at the first harvest (53 days after sowing)



Means with the same letter within a graph are not significantly different at P = 0.05. [Merredin soil data were not included here since they were not significant]

Applied Zn level (mg/kg soil)

Fig. 2. Effects of Zn fertilization rates and four soil types on Zn content in the shoots of barley genotypes, Sahara (Zn-efficient) and Clipper (Zn-inefficient) at the first harvest (53 days after sowing)

Means with the same letter are not significantly different at P = 0.05. [Merredin soil data were not included here since they were not significant] Zn concentration in the youngest expanded leaf blades (YEBs) of Sahara plants grown at 0.0 and 0.02 mg Zn/kg were significantly lower than that at 0.8 mg Zn/kg in Lancelin, Kellerberrin-7, Wongan Hills, and Kellerberrin-7A soils (Fig. 3). In Clipper, there was no significant difference for YEBs Zn concentration between 0.0 and 0.8 mg/kg applied Zn levels in Lancelin and Wongan Hills soils. Zinc concentration in the YEBs of Clipper significantly decreased when no Zn was applied compared to 0.8 mg Zn/kg in Kellerberrin-7 and Kellerberrin-7A soils. Under deficient conditions (0.0 and 0.02 mg Zn/kg), the concentration of Zn in YEBs did not differ significantly between genotypes, although it was slightly higher in Sahara. At 0.8 mg Zn/kg, the genotypes differed significantly, with more Zn accumulated in YEBs by Sahara compared with Clipper.

At the second harvest, in both genotypes, shoot dry matter increased with increasing soil Zn application, but showed no significant Zn treatment effect in all soils. Significant genotypic differences in shoot dry matter occurred between two genotypes only in Lancelin soil at 0.8 mg Zn/kg, with Clipper having considerably greater dry matter than Sahara. However, Zn efficiency value of Sahara (89%) was slightly higher than Clipper (82%).

At the second harvest, shoot Zn concentrations increased with increasing Zn fertilization and showed a significant Zn treatment effect in all soils. Shoot Zn concentration reduced considerably under low Zn supply in both genotypes in Lancelin, Kellerberrin-7 and Kellerberrin-7A soils (Fig. 4). Shoot Zn concentration of Clipper did not differ among Zn treatments in Wongan Hills soil. Significantly more Zn was accumulated in shot tissues by Sahara than Clipper when Lancelin, Kellerberrin-7, Wongan Hills and Kellerberrin-7A soils were fertilized at 0.8 mg Zn/kg. Although Sahara had higher concentration of Zn at 0.0 and 0.02 mg/kg soil applied Zn, there was no considerable difference between genotypes. Zn content was greater in Sahara than Clipper only at the sufficient level of applied Zn in Wongan Hills soil: in other soils at all Zn levels there were no significant genotypic differences in Zn content.







Means with the same letter are not significantly different at P = 0.05. [Merredin soil data were not included here since they were not significant] The amount of Zn accumulation in the youngest expanded leaf blades (YEBs) increased with Zn fertilization, showing a significant (P<0.01) Zn treatment effect in Lancelin, Kellerberrin-7, Wongan Hills, Kellerberrin-7A and Merredin soil types (Fig. 5). Zinc concentration in Sahara YEBs significantly differed with increasing applied Zn in all five soils. In contrast, Clipper had similar YEBs Zn concentration at all applied Zn levels in all soils. Sahara had significantly greater Zn concentration in YEBs than Clipper when fertilized at 0.8 mg Zn/kg. YEBs Zn concentrations were similar between genotypes at 0.0 and 0.02 mg Zn/kg levels in all soils.

At maturity, shoot dry weight and seed yield of two genotypes were reduced under low Zn supply in all soils, but the reduction was significant only in Merredin soil, where Clipper had higher seed yield than Sahara at 0.8 mg Zn/kg soil. Zinc deficiency resulted in a significant decline in shoot Zn concentration in both genotypes in all soil types. Shoot Zn concentration of Sahara was higher than that of Clipper at 0.8 Zn level in all soils except Wongan Hills. This trait did not differ between genotypes at 0 and 0.02 mg Zn/kg treatments (Fig. 6). At adequate Zn fertilization, there was a significant difference in shoot Zn content in the two cultivars grown in Lancelin, Kellerberrin-7A and Merredin soils Fig. 7. Flag leaf Zn concentration was not affected by Zn fertilization and genotype, except in Merredin soil, where Sahara had higher concentration than Clipper at 0.8 mg Zn/kg soil.

Zinc fertilization significantly influenced seed Zn concentration and content of genotypes in all soil types. Seed Zn concentration was greater in Zn-efficient Sahara than Zn-inefficient Clipper at 0.8 mg Zn/kg soil (Fig. 8). However, seed Zn concentration in Sahara was higher in 0 and 0.02 mg Zn/kg treatments. Two genotypes significantly differed only at 0.02 mg Zn/kg in Wongan Hills and Kellerberrin-7A soils.

Two barley genotypes did not develop any Zn deficiency symptoms when no Zn was applied. No symptoms of Zn deficiency in early stages of growing in this study may be attributed to high seed Zn content as well as the amount of plant available soil Zn concentration. Other studies on barley [30] confirmed that with increasing seed Zn content the severity of deficiency symptoms decreased and eventually were not observed at all. Similarly, wheat plants derived from the high seed Zn content also had better seedling vigour than those originating from low-Zn seed [31].



Applied Zn level (mg/kg soil)



Means with the same letter are not significantly different at P = 0.05. [Merredin soil data were not included here since they were not significant]



Applied Zn level (mg/kg soil)



Means with the same letter are not significantly different at P = 0.01. [Merredin soil data were not included here]



Fig. 6. Effects of Zn fertilization rates and four soil types on Zn concentration in the shoots of barley genotypes, Sahara (Zn-efficient) and Clipper (Zn-inefficient) at maturity Means with the same letter are not significantly different at P = 0.05. [Wongan Hills soil data were not included here since they were not significant]



Fig. 7. Effects of Zn fertilization rates and three soil types on Zn content in the shoots of barley genotypes, Sahara (Zn-efficient) and Clipper (Zn-inefficient) at maturity Means with the same letter are not significantly different at P = 0.05.

[Wongan Hills soil data were not included here since they were not significant]

DTPA (diethylenetriamine pentaacetic acid) extractable Zn concentrations were almost similar in all soils (Table 1). The critical Zn deficiency level was 0.2 mg/kg DTPA-extractable Zn for wheat plants grown in calcareous soils in the field and glasshouse experiments in Turkey DTPA-extractable [32]. The critical Zn concentration for wheat growing in acidic soils in Australia was 0.25 mg/kg [33]. On the other hand, severity of visual Zn deficiency symptoms and corresponding decreases in shoot dry matter production were higher in wheat than barley [22,34]. Therefore, the absence of visible Zn deficiency symptoms in this experiment may be related not just to the amount of plant-available Zn in soils and higher seed Zn content of genotypes, but also to the relatively high capacity of barley genotypes to adapt to Zn-deficient soil conditions.

Clipper and Sahara differed significantly in YEBs Zn concentration at the vegetative stage. The concentrations of Zn in YEBs (in mg Zn/kg dry mater) were 13-22 for Sahara and 10-17 for Clipper when no Zn was applied (Fig. 3). Zinc concentrations in YEBs of less than 10 mg/kg might indicate Zn deficiency, inducing visual symptoms on common bean leaves [3,35]. However, for barley, the critical deficiency concentration of Zn in YEBs was estimated to be 20 mg Zn/kg dry matter [21].

Zinc efficiency mechanisms might be related to mobility and distribution of Zn within the leaves. Results of this study showed that under Zndeficient conditions, YEBs Zn concentration were similar between Zn-efficient Sahara and Zninefficient Clipper genotypes. This result is corroborated by other studies showing a lack of correlation between leaf Zn concentration and Zn efficiency [3,36]. Hence, it can be concluded that Zn translocation and accumulation in leaves are probably not involved in expression of high Zn efficiency in barley.

An increase in Zn fertilization resulted in an increase in both shoot Zn concentration and content of genotypes at the vegetative stages and maturity. The response of genotypes to applied Zn was consistent in all soils. Sahara had greater Zn concentration and content in the shoots than Clipper when genotypes were fertilized with 0.8 mg Zn/kg soil. Higher Zn uptake capacity of Sahara over Clipper can be considered as one of the possible mechanisms for its higher Zn efficiency in this experiment.

Enhanced Zn uptake of plants under Zn deficient conditions was considered as an important factor determining Zn efficiency of genotypes of wheat in field and glasshouse [37,38] and barley in the glasshouse experiments [8,21]. Also in the nutrient solution trials, differences in Zn efficiency between wheat genotypes were closely related to the Zn uptake capacity of genotypes and Zn translocation to shoots [8,16].

Despite the lack of differences between Clipper and Sahara in seed Zn concentration or content after growing under Zn deficiency, the genotypes differed in response to high Zn application. At 0.8 Zn treatment, higher seed Zn concentration and content in Sahara than Clipper indicated that Zn enrichment trait is available within the barley genome, which could allow for substantial increase in seed Zn concentration and content. High micronutrient seed content (especially Zn) is a desirable quality factor for human nutrition where a high dependence on grains for food may result in Zn deficiencies in humans [39,40].

An important role of the seed is to supply the young seedling with minerals until it has developed a root system large enough to take over this role. In Zn-poor soils, there may not be enough Zn in seed reserves to last while the root system is developed to compensate for the low Zn supply. Plants derived from high Zn-seed, therefore, can produce more numerous and



Fig. 8. Effects of Zn fertilization rates and five soil types on Zn concentration in the seed of barley genotypes, Sahara (Zn-efficient) and Clipper (Zn-inefficient) at the third harvest. The letter above the columns represents mean of Zn concentration in mg kg - dry matter Means with the same letter are not significantly different at p = 0.05

bigger grains, especially under Zn-deficient soil conditions [41]. Adoption of Zn-efficient barley genotypes with Zn-dense seeds is likely to be attractive to farmers because of the potential for higher profits when cropping soils with low plant available Zn.

Soil types did not affect Zn uptake efficiency of genotypes significantly. In all types of soil, Sahara could absorb more Zn than Clipper. It means the differences of total amount of Zn or organic matter in different soils could not alter the behaviour of genotypes in acquisition of Zn from soil. In contrast to this study, Solaiman et al. [42] found that soil types influence phosphorus efficiency of wheat and canola genotypes. Therefore, due to little variation in soils selected for this study seems a better conclusion can be reached when the genotypes are tested in some soils vary in some characteristics such pH, total Zn and other soil Zn-fractions, organic matter, calcium carbonate and even clay.

4. CONCLUSIONS

In conclusion, under adequate Zn supply, Clipper and Sahara exhibited a differential response to Zn in various soil types. The greater efficiency of Sahara over Clipper under sufficient Zn supply may be attributed to its higher uptake of Zn from soil. Genetic differences in response to Zn deficiency reported previously in the field can be reproduced under controlled conditions using the soil-based pot screening method. The differences were expressed in shoot growth and Zn concentration and content at different vegetative stages as well as shoot and seed Zn concentration and content at maturity. It appears that soil-based pot assays under controlled conditions may offer potential improvements over field experiments in screening for tolerance to Zn deficiency. The results also indicate that shoot and seed Zn concentration and content can be used to diagnose the Zn status of barley genotypes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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