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Wheat genotypes differ in Zn efficiency when grown in chelate-buffered nutrient solution

I. Growth

Zdenko Rengel and Robin D. Graham
Department of Plant Science, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond S.A. 5064, Australia

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Abstract

Ten *Triticum aestivum* and two *Triticum turgidum* conv. *durum* genotypes were grown in chelate-buffered nutrient solution at Zn supplies ranging from deficient to sufficient (free Zn activities from 2 to 200 p*M*, pZn from 11.7 to 9.7). The critical level of Zn ion activity in solution for healthy growth of wheat plants was around 40 p*M*. Genotypes differed in the growth response: those classified as Zn-efficient suffered less reduction of shoot growth and did not change the rate of root growth at a Zn supply quite deficient for Zn-inefficient genotypes. Root growth of Zn-inefficient genotypes increased at deficient Zn supply. The shoot/root ratio was the most sensitive parameter of Zn efficiency; Zn-efficient genotypes showed less reduction in the ratio when grown at deficient compared to sufficient Zn supply. Classification of wheat genotypes into Zn-efficient and Zn-inefficient groups after screening in chelate-buffered nutrient solution corresponded well with classification obtained in field experiments on Zn-deficient soil.

Introduction

Soils with low plant-available Zn (=Zn-deficient soils) are common in tropical and temperate climates, but are most widespread in regions with the Mediterranean type of climate, including the cropping areas of Western and South Australia (Sillanpää and Vlek, 1985). Genotypes which grow and yield well in soils too deficient in Zn for a standard genotype are considered Zn efficient (=tolerant to Zn-deficient soils) (Graham et al., 1992).

Relatively slow progress in deciphering the genetics, physiology and biochemistry behind the mechanisms of Zn efficiency has hampered the development of genotypes of superior Zn efficiency through conscious breeding efforts geared specifically toward that purpose. A reliable, inexpensive and fast screening technique to differentiate genotypes with respect to their Zn efficiency would be an essential component of any breeding effort; such a technique is presently unavailable (Graham and Rengel, 1993).

The advent of chelate-buffered nutrient solutions (Bell et al., 1991; Chaney et al. 1989) represents a major step forward in studying plant-micronutrient relationships at realistically low micronutrient activities which can constantly be maintained around plant roots, thus mimicking the situation occurring in a soil. Chelate-buffered nutrient solutions may be used to study micronutrient deficiencies because the micronutrient stress of varying severity can be imposed predictably and reproducibly (Parker et al., 1992). In the case of Zn, the critical levels of free ion activity for healthy plant growth in chelate-buffered nutrient solutions are 10-160 p*M* for *Hordeum vulgare* (Bell et al., 1991; Laurie et al., 1991; Norvell and Welch, 1993), between 10-60 p*M* for *Triticum aestivum*, *Zea mays*, *Elytrigia pontica* (tall wheatgrass), *Medicago sativa*, and *Glycine max* (Parker, 1993), and above 25

* FAX no: + 6183037109

p*M* for *Lycopersicum esculentum* (Parker et al., 1992; Parker, 1993) and *Oryza sativa* (Yang et al., 1994). These values correspond well with estimated activities of Zn^{2+} in soil solutions of alkaline soils in which crops commonly show Zn deficiency (Norvell et al., 1987), indicating that chelate-buffered nutrient solutions with appropriately low activities of Zn may be useful in evaluating genotypes for differential Zn efficiency as shown in the field.

No work has been reported yet on using chelate-buffered nutrient solutions to screen genotypes of crop plants differing in Zn efficiency. The aim of the present study was to assess the suitability of chelate-buffered solutions containing a wide range of Zn activities as a screening medium for differential Zn efficiency by evaluating 12 wheat genotypes of known field-observed Zn efficiency.

Materials and methods

Seed of 10 genotypes of *Triticum aestivum* L. (Aroona, BT-Schomburgk, Excalibur, Gatcher, Halberd, Molineux, Schomburgk, Songlen, Warigal, and Warigal-5R) and two genotypes of *T. turgidum* L. conv. *durum* (Desf.) MacKey (Durati and Kamilaroi) was sieved (passed through a 3-mm mesh, retained on a 2.5-mm mesh), surface-sterilised by soaking in 70% ethanol (v/v) for 1 min followed by sodium hypochlorite (3% active chlorine, v/v) for 5 min, thoroughly rinsed in high-purity double-deionised water (18 MOhm resistivity, DD water), left submerged in DD water for 3 h with hourly changes of water and occasional shaking in between, and germinated on the DD water pre-soaked Whatman No. 42 ashless filter paper at 20±1 °C in the dark for 24 h.

Uniformly germinated seed with radicle emerging was sown in plastic cups (bottoms severed and replaced with a black 2-mm mesh) and covered with black polyethylene beads to exclude light; these cups were inserted through the holes drilled in the black polyethylene lids tightly fitting over 1.1-L pots made of the same material (all plastic- and glassware was thoroughly washed, soaked in 10% HNO_3 (v/v) for at least 24 h and rinsed extensively with DD water). Pots were filled with nutrient solution prepared using the method described by Norvell and Welch (1993); this solution contained (in μM): $Ca(NO_3)_2$ 2000, $MgSO_4$ 500, KNO_3 1500, KCl 100, MES-KOH 2000, $NH_4H_2PO_4$ 100, H_3BO_3 10, Na_2MoO_4 0.1, K_3-(N-(2-hydroxyethyl)ethylenedinitrilotriacetic acid) (HEDTA) 25, FeHEDTA 100, MnHEDTA 1, CuHEDTA 0.5, and NiHEDTA 0.1. Four Zn treatments were established by adding ZnHEDTA in total concentrations of 0.1, 0.5, 2 or 10 μM. Activities of all ions were calculated (Table 1) by the GEOCHEM-PC computer program (Parker et al., 1995) using the same formation constants for metal-HEDTA complexes and other thermodynamic constants as in the study by Norvell and Welch (1993).

Table 1. Free ion oncentrations and ionic activities of metals and ligands in the nutrient solution; calculations were done by the GEOCHEM-PC program

Ion	Concentration		Ion activity
	Nominal	Free ion	
	(μM)	(μM)	(μM)
NO_3^a	5800	5790	5210
Ca	2000	1890	1240
Mg	500	480	320
K	2864	2860	2570
NH_4	100	0.09	0.09
SO_4	502	41	27
Cl	100	10	9.0
Na	0.2	0.2	0.18
Fe	100	0.75	0.49
Mn	1	0.045	0.03
	(μM)	(pM)	(pM)
$B(OH)_4$	10	6980	6280
MoO_4	0.1	990	650
PO_4	100	5.6	2.2
Cu	0.5	0.03	0.02
Ni	0.1	0.01	0.006
$Zn_{0.1}$	0.1	3	2
$Zn_{0.5}$	0.5	15	10
Zn_2	2	60	40
Zn_{10}	10	302	200

[a] Concentration and activities of ions other than Zn correspond to the treatment containing 0.1 μM of total Zn ($Zn_{0.1}$).

Plants were initially grown in a half-strength nutrient solution (concentration of macro- and micronutrients 50% of what is shown in Table 1, except Zn, MES and K_3HEDTA which were at the same concentration as shown in Table 1). Nutrient solution was replaced with the fresh, full-strength one (Table 1) on days 10, 15, and 19 following the start of germination. The initial pH of all nutrient solutions was 6.0; following plant growth pH varied between 5.9 and 6.0 for

[Zn]=0.1 μM, 6.1 and 6.2 for [Zn]=0.5 μM, 6.1 and 6.3 for [Zn]=2 μM, and from 6.2 to 6.4 for [Zn]=10 μM depending on plant age and genotype (brackets indicate the total concentration). Solutions were continuously aerated.

Plants were grown in a growth chamber set up to operate at 15/10 °C day/night temperature, a 10-h photoperiod and the photosynthetic active radiation at plant tops of approximately 300 μmol m^{-2} s^{-1}.

At the onset of the experiment, four cups containing eight seedlings each were grown per pot. Two cups per pot were removed for first harvest and one cup each for the following two harvests. Harvests were done on days 10, 15 and 22 after the commencement of germination; root and shoot samples were gently washed in 3 lots of deionised water (about 5 s each), followed by a brief rinse in DD water for about 3 s. Samples were then blotted and dried at 80 °C for 48 h. Dry plant material was pre-digested in 70% (v/v) HNO_3 overnight and then heated at 126 °C until complete digestion occurred and volume of acid was reduced to about 1 mL (up to 8 h). Digests were cooled to the room temperature, made up to volume with 1% (v/v) HNO_3, decanted and analysed by an inductively coupled plasma emission spectrometer (ICP). A standard set of plant materials differing widely in Zn concentration was digested and analysed in the same manner as experimental samples.

Critical values of Zn required for achieving 90% of the maximum growth were estimated from the hyperbolic curves fitted to the relationship between Zn concentration in shoots and the relative shoot yield. The relative yield was calculated for each genotype × Zn treatment separately, taking the maximum yield within a replicate as 100% (Bell et al., 1990).

The Zn-efficiency classes were constructed by finding a median value of the trait under consideration (an average between data for genotypes ranked 6th and 7th) and creating the medium-efficiency interval as median ± S.E of the genotype effect. Genotypes with data falling above or below that medium interval were classed as Zn-efficient or Zn-inefficient, respectively.

The experiment was set up in a completely randomised design with factorially arranged treatments (12 genotypes × 5 Zn treatments); three replicates were run over time. Data were analysed by analysis of variance using the routines of the GENSTAT 5 program (GENSTAT 5 Committee, 1989); Tukey's Honestly Significant Difference (HSD) at α=0.05 was used to assess the differences among pairs of treatment means as already described (Rengel, 1990). When data were calculated as ratios of results obtained at 2 and 200 pM Zn activity (hence only genotypes were compared, Figs. 5 and 7), analysis of variance was run with seed Zn content as a covariate because separate analysis showed that seed Zn content significantly differed among genotypes (Table 2). Conclusions about efficiency ranking of genotypes based on the analysis with the covariate included differed slightly from those based on the simple analysis of variance; however, Zn-efficient and Zn-inefficient genotypes were always classed as such irrespective of how statistical analyses were performed.

Table 2. Total concentration of Zn in the seed of 12 wheat genotypes used in this study. Data are presented as means ± S.E., n=3 replicates each containing around 100 seeds

Genotype	Seed Zn	
	(mg per kg D.W.)	(ng per seed)
Aroona	8.8±0.6	245±10
BT-Schomburgk	11.7±0.2	341±7
Durati	10.0±0.4	332±11
Excalibur	8.4±0.4	242±5
Halberd	8.5±0.2	252±4
Gatcher	9.5±0.2	258±18
Kamilaroi	8.7±0.1	268±8
Molineux	12.7±0.2	300±8
Schomburgk	7.9±0.1	224±6
Songlen	10.5±0.3	344±18
Warigal	12.0±0.5	353±22
Warigal-5R	12.4±0.5	308±5
Tukey's $HSD_{0.05}$	1.8	60

Results

Preliminary experiments were run to determine the optimal solution composition. Based on root and shoot growth and nutrient uptake in the preliminary experiments, solution concentration of P was reduced from 0.2 to 0.1 mM and that of Cu from 1 to 0.5μM. The MES was tested at 1, 2 and 3 mM; sufficient buffering was provided with 2 mM and no additional benefit was noted for 3 mM. In addition, root and shoot concentrations of all nutrients were relatively high prompting the use of 1/2-strength nutrient solution for the peri-

od between germination and the first harvest in the experiments reported here.

With 0.1 to 10 μM total Zn concentration and an excess of 25 μM HEDTA, Zn ion activity in solution varied from 2 to 200 pM. Activities of other metals remained constant across all Zn treatments (Table 1, see also Norvell and Welch, 1993).

Seed of 12 genotypes used was chosen from the field experiments performed on sites with soils containing low amounts of plant-available Zn (Graham et al., 1992). Seed differed in the Zn content (Table 2). In a separate experiment, greater seed Zn content was beneficial for early growth of wheat in Zn-deficient soil not fertilised with Zn (Rengel and Graham, 1995a). However, the influence of seed Zn content is expected to be smaller in the experiment described here (well-mixed nutrient solutions with no nil Zn treatment).

The genotypes tested showed varying degrees of reduction of shoot growth at 2 pM Zn activity compared to yields at 200 pM. Other visible symptoms of Zn deficiency were absent, except in the cvs. Durati and Kamilaroi which, after about 2 weeks of growth at 2 pM Zn and a week later when grown at 10 pM Zn activity, developed yellow chlorotic areas between the mid-vein and leaf margin of the second and subsequent leaves; these chlorotic areas would later assume a watery appearance, followed by necrosis of the central area of the leaf, while the tip, base and margins remained green (as typical for severe Zn-deficiency, *cf.* Grundon, 1987).

Wheat plants grown in solution containing low Zn activities for 15 or 22 days produced greater amounts of root and smaller amounts of shoot material than those grown in solutions containing higher Zn activities (Fig.1). The interaction genotype × Zn was significant ($p \leq 0.031$) only for root dry weight at the last harvest because an increase in solution Zn activity reduced root growth in all genotypes (to the greatest extent in Durati), except Warigal where no significant change occurred (Fig. 2). Shoot growth increased steeply when Zn activities increased to 40 pM and much less so for an aditional increase in Zn activity to 200 pM (Fig. 2).

The shoot/root ratio increased with an increase in solution Zn activity for all three harvests; differences between genotypes in their response to Zn appeared only at the 15-d and 22-d harvests (the interaction genotype × Zn significant at $p \leq 0.001$) (Fig. 3). The shoot/root ratio of Durati plants grown at 2 pM Zn activity decreased between 10 and 15 d of growth and increased slightly afterwards (Fig. 4). In contrast,

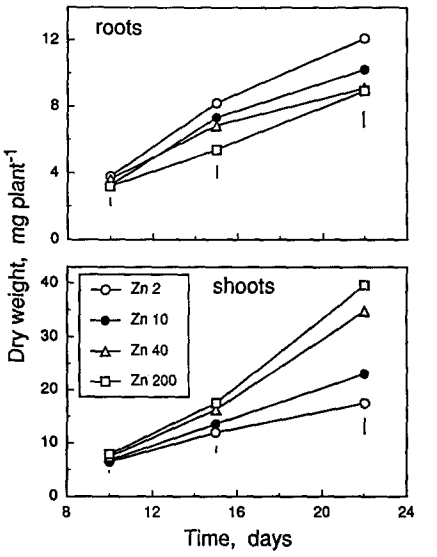

Fig. 1. Effects of duration of the Zn treatment on root and shoot dry matter accumulation in wheat plants. The data were averaged over all 12 genotypes tested because the interaction genotype×Zn was not significant (from $p \leq 0.21$ to $p \leq 0.99$ depending on the harvest time and the plant part), except for root dry weight at the 22-d harvest (see Fig. 2). Vertical bars represent the $HSD_{0.05}$ values for the Zn treatment effect at each harvest.

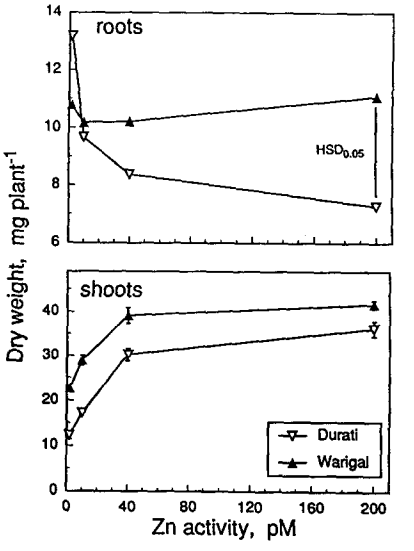

Fig. 2. Effects of the Zn treatment on root and shoot dry weight of two wheat genotypes grown in chelate-buffered nutrient solution for 22 days. For roots, the vertical bar represents the $HSD_{0.05}$ value for the interaction genotype × Zn. For shoots, vertical bars represent ±S.E. of corresponding means.

Warigal plants grown at 2 pM Zn activity had a relatively constant shoot/root ratio over time. For all three harvests Durati plants grown at 2 pM Zn activity had

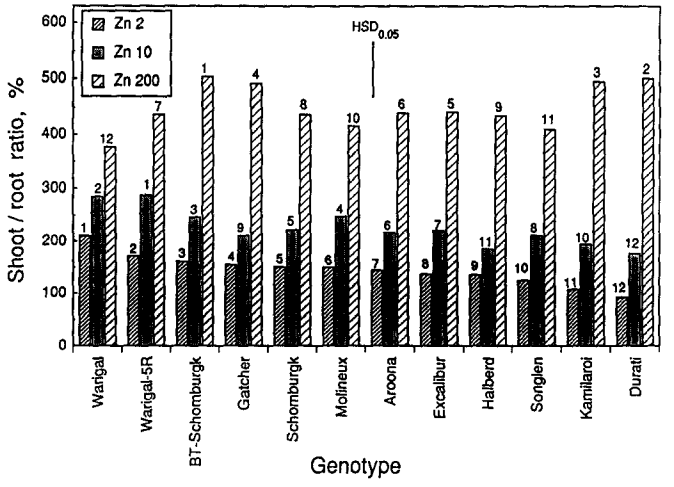

Fig. 3. Effects of the Zn treatment on the shoot/root ratio of 12 wheat genotypes grown in the chelate-buffered nutrient solution for 22 days. The data obtained at solution Zn activity of 40 p*M* were not included because they were almost identical to those observed at 200 p*M* Zn activity. The vertical bar represents the $HSD_{0.05}$ value for the interaction genotype × Zn. Numbers above columns represent a ranking for genotypes within each Zn treatment.

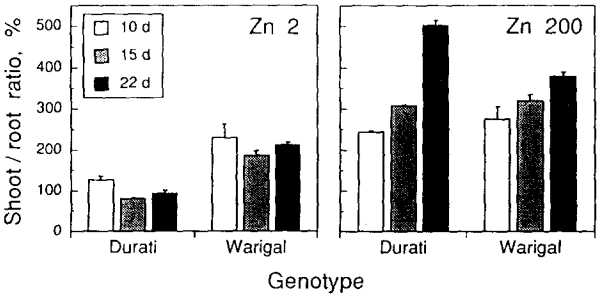

Fig. 4. Effects of solution Zn activities and duration of the Zn treatment on the shoot/root ratio of two wheat genotypes grown at 2 or 200 p*M* Zn activities. The data obtained for the two other Zn treatments were intermediate. The $HSD_{0.05}$ values for comparisons within growth periods were 51%, 15% and 107% for harvests after 10, 15 and 22 d, respectively. No strict statistical comparison between the harvests can be done; however, S.E. values of the means (vertical bars) were included for an approximate comparison.

the smallest and Warigal the largest shoot/root ratio of all genotypes tested (partly shown in Fig. 3).

Three different expressions of Zn efficiency of wheat genotypes have been compared: better relative shoot growth at 2 p*M* Zn than at 200 p*M* Zn activity (Fig. 5), greater shoot growth at 2 p*M* Zn activity in solution (Fig. 6), and smaller decrease in the shoot/root ratio due to reduction in solution Zn activities (Fig. 7). The genotype Warigal was the only one ranked as Zn-efficient by all three indicators. Genotypes Schomburgk, Aroona, Gatcher, and Excalibur

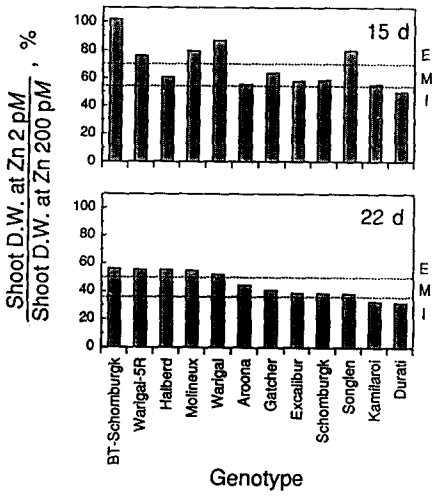

Fig. 5. The Zn efficiency ranking (E=efficient, M=medium, I=inefficient) of 12 wheat genotypes grown in chelate-buffered nutrient solution for 15 or 22 days. The ranking was based on the ratio of shoot dry matter production at deficient (2 p*M*) and sufficient Zn supply (200 p*M* Zn activity). The data were adjusted for the covariate (seed Zn content). The mid-point of the medium-efficiency interval corresponds to the median value of the parameter given on the Y axis; the upper and lower boundaries of the medium-efficiency interval were constructed by adding to or subtracting from the median point the value of the standard error of the genotype effect.

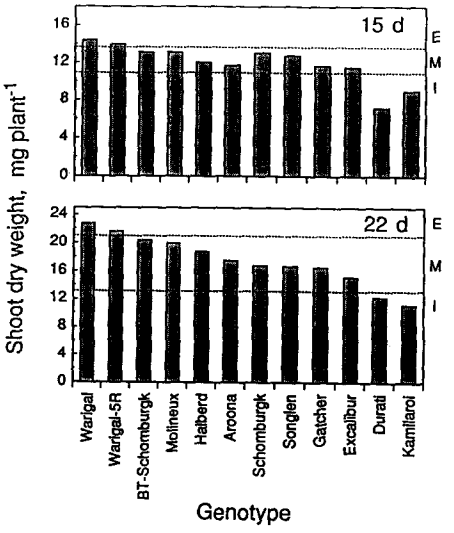

Fig. 6. The Zn efficiency ranking of 12 wheat genotypes grown in chelate-buffered nutrient solution for 15 or 22 days. The ranking was based on shoot dry matter accumulation at deficient Zn supply (2 p*M* Zn activity). No differences were detected among genotypes after 10 d of growth ($p \leq 0.21$). For the construction of efficiency intervals and explanations of symbols refer to Figure 5.

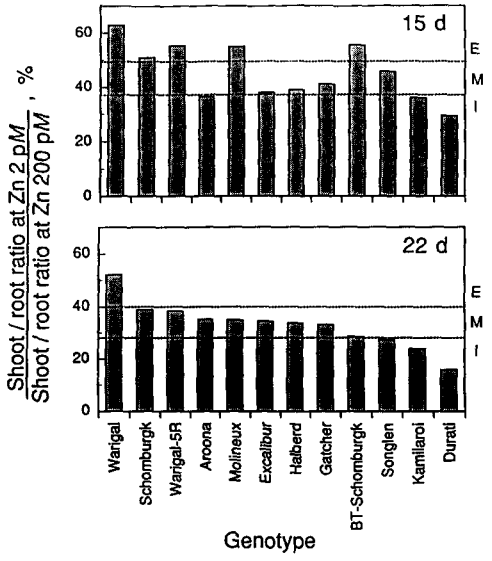

Fig. 7. The Zn efficiency ranking of 12 wheat genotypes grown in chelate-buffered nutrient solution for 15 or 22 days. The ranking was based on the quotient of shoot/root ratios at deficient (2 p*M*) and sufficient Zn supply (200 p*M* Zn activity). No differences were detected among genotypes after 10 d of growth ($p \leq 0.58$). For the construction of efficiency intervals and explanations of symbols refer to Figure 5.

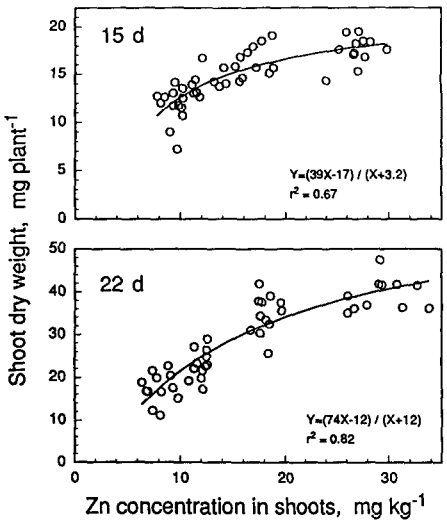

Fig. 8. The relationship between Zn concentration in shoots and production of shoot dry matter for 12 wheat genotypes grown in chelate-buffered nutrient solution for 15 or 22 days. The data for all genotypes were combined because the genotype effect was non-significant for Zn concentration in shoots ($p \leq 0.18$ and $p \leq 0.29$ for 15- and 22-d harvests, respectively).

were always ranked as medium-effcient; genotypes Durati and Kamilaroi were consistently ranked as Zn-inefficient (Figs. 5-7). To a certain extent, ranking depended on the duration of the Zn-deficiency stress. Ranking after 10 d showed clear differences only between Warigal as the most efficient and Durati or Kamilaroi as the most inefficient genotypes (data not shown); more precise ranking of genotypes in between these two extremes of Zn efficiency required between 15 and 22 d of exposure to the Zn-deficiency stress.

The proportionally greatest difference (over 3-fold) between the most efficient and the least efficient genotype was achieved by comparing shoot/root ratios at 2 p*M* with those at 200 p*M* Zn activity after 22 d of growth (Fig. 7). This indicator of Zn efficiency relies on the observed decrease in the shoot/root ratio (due to reduced shoot and increased root growth) as a consequence of increased severity of the Zn-deficiency stress; such a decrease in the shoot/root ratio was greater for the Zn-inefficient genotypes (Fig. 3).

If the efficiency classes were constructed for the shoot/root ratio of plants grown at 2 p*M* activity for 22 d in a way similar to efficiency classes based on other indicators (the medium-efficiency interval between values for shoot/root ratios of 117 and 177% - median value ± S.E., calculated from the data presented in Fig. 3), Warigal was the only genotype classed as Zn-efficient, while Durati and Kamilaroi were the only inefficient genotypes. Such a ranking corresponds well with ranking obtained with other indicators (see above); the shoot/root ratio for plants grown at deficient Zn supply can also be a useful indicator of Zn efficiency of wheat genotypes as tested here.

Net uptake of Zn was sufficient to sustain shoot dry matter production without a 'dilution effect'; plants with greater shoot weight also had increased Zn concentrations in shoot (Fig. 8). Such a relationship was not significant for 10-d-old plants (not shown). Critical concentrations of Zn in shoot tissue (corresponding to 90% of the maximum shoot yield) were estimated to be 15, 16 and 21 mg Zn (kg shoot D.W.)$^{-1}$ for 10-, 15- and 22-d-old plants, respectively (data not shown).

Discussion

Stunted growth and other Zn deficiency symptoms appeared on plants after about 2 weeks of growth at 2 p*M* Zn activity in chelate-buffered nutrient solutions in the present study, observations consistent with reports on wheat grown in conventional nutrient solutions with no Zn added (cf. Cakmak and Marschner, 1988; Webb and Loneragan, 1990) as well as with studies on barley

grown in chelate-buffered nutrient solution (Norvell and Welch, 1993). While most reports indicate reduced root growth as a consequence of Zn deficiency for a number of different plant species grown in conventional nutrient solutions (Cakmak and Marschner, 1988; Cakmak et al., 1989), results for wheat grown under similar conditions indicate no reduction (Cakmak and Marschner, 1988) or even a slight increase in root growth after 18 d at nil Zn supply (Webb and Loneragan, 1990). These studies are consistent with observations reported here on unchanged (Zn-efficient genotypes) or increased root growth (Zn-inefficient genotypes) for wheat plants (Figs. 1 and 2) as well as with reports on unchanged root growth of barley (Norvell and Welch, 1993; Welch and Norvell, 1993) grown in chelate-buffered nutrient solution at low solution Zn activities.

Zinc activity in solution appears to be a powerful determinant of the shoot/root ratio in wheat plants. After 10 d of growth at 2 p*M* Zn activity, absolute amounts of root and shoot dry matter barely changed (Fig. 1) but the shoot/root ratio decreased when compared to plants grown at 200 p*M* (Fig. 4). Such a result is consistent with other studies using conventional nutrient solution cultures where Zn deficiency reduced the shoot/root ratio in wheat (Cakmak and Marschner, 1988) and *Phaseolus vulgaris* plants (Cakmak et al., 1989) even though no change in the shoot/root ratio was recorded for *Lycopersicum esculentum* and *Gossypium hirsutum* plants (Cakmak and Marschner, 1988). In addition, barley plants grown in chelate-buffered nutrient solution similar to the one used in the present study also showed a tendency to increase root weight while having shoot growth severely reduced with a decrease in solution Zn activities (Norvell and Welch, 1993). It is interesting to note that toxic Zn levels also affect shoot growth more than root growth, thus resulting in a decreased shoot/root ratio in some plant species (e.g. *Pisum sativum*, Paivoke, 1983).

Generally, the shoot/root ratio is controlled by a mineral supply without apparent involvement of a hormonal regulation (Jackson, 1993). A shortage of a nutrient in the outside medium causes reduction in the amount transported to shoots which then experience nutrient deficiency and reduced growth; this reduced growth causes changes in assimilate partitioning (greater amounts being available for transport to roots) (see Freeden et al., 1989 for phosphorus). A decrease in the shoot/root ratio under Zn deficiency observed here (Figs. 2-4) may be a compensatory mechanism geared toward greater acquisition of a scarce resource from the environment by maintaining or increasing root growth at the expense of shoot growth. Such compensatory mechanism is less obvious for Zn-efficient genotypes which are either better capable of extracting Zn from deficient environments or more efficient in utilising Zn taken up, thus reducing or even obviating a need for increased root growth at the expense of shoot growth.

Wheat genotypes differed in the extent of reduction of shoot/root ratio as a consequence of low solution Zn activity (Figs. 3 and 4). Genotypes least tolerant of Zn deficiency (Kamilaroi and Durati) showed the largest decrease in the shoot/root ratio (Fig. 3). While Zn-efficient genotypes (like Warigal) maintained almost the constant shoot/root ratio over the course of 22 days of growth in solution with 2 p*M* Zn activity, Zn-inefficient Durati under the same conditions (Fig. 4) showed a decrease in the shoot/root ratio with the duration of the Zn-deficiency stress. This observation is likely to correspond to a gradual depletion of seed Zn reserves and building up of a sufficient mass of roots required to support growth of a unit of shoot. Zinc-efficient genotypes were apparently faster in adapting to environments with low Zn activity.

While genotypic differences in the shoot/root ratio between Zn-efficient Warigal and Zn-inefficient Durati were obvious at 2 p*M* Zn for all three harvests, no differences among these two genotypes were observed at 200 p*M* Zn activity for the first two harvests (Fig. 4). Such a result, coupled with a trend towards reversal of ranking of Warigal and Durati with respect to the shoot/root ratio when grown at the two extremes of solution Zn activities (see Fig. 3), indicates that changes in the shoot/root ratio were not due to general differences among genotypes tested (not being isogenic lines, genotypes differ in a number of traits); the observed differences in the shoot/root ratio were induced by Zn deficiency, *i.e.* they manifested the genotype × Zn interaction.

The interaction genotype × Zn for shoot dry weight was non-significant at all three harvests (and for root dry weight after 10 and 15 d of growth), indicating that shoot growth of all genotypes responded to the Zn treatment in a similar manner even though not at the same magnitude (see Fig. 2). The shoot growth response started to level off at 40 p*M* Zn activity (more so in Zn-inefficient Durati than in Zn-efficient Warigal, Fig. 2). This Zn activity in solution can therefore be considered a critical level for healthy growth of wheat plants, at least those more Zn-efficient. This critical level is within a range reported for other crops grown

in chelate-buffered nutrient solutions (Bell et al., 1991; Laurie et al., 1991; Parker et al., 1992; Norvell and Welch, 1993; Yang et al., 1994).

Critical concentration of Zn in shoot tissue for attaining 90% of the maximum yield was between 15 and 21 mg kg^{-1} depending on plant age but irrespective of genotype (see Results). These levels are in general agreement with other published critical levels (Dang et al., 1993; Reuter and Robinson, 1986) for plants not grown in chelate-buffered nutrient solution.

Wheat plants grown in the present study in solutions with 2 or 10 p*M* Zn activity accumulated P in their shoots (see Rengel and Graham, 1995b). Contrary to others who suggested that Zn deficiency and P toxicity symptoms are hard to distinguish in a number of species (Loneragan et al., 1982), including wheat grown in the conventional nutrient solution (Webb and Loneragan, 1988), we observed clear visual differences (according to criteria specified by Grundon, 1987) between Zn deficiency symptoms (on Durati and Kamilaroi) and P toxicity symptoms (only on Excalibur). Other genotypes did not exhibit either symptoms. Follow-up experiments with reduced solution P concentrations have shown that response to Zn deficiency and classification into appropriate Zn efficiency classes for several wheat genotypes were similar to those of the present study (data not shown). We therefore conclude that relatively high P accumulation in shoots (see Rengel and Graham, 1995b) had little, if any, influence on the Zn deficiency response and the Zn efficiency ranking of wheat genotypes (with exclusion of Excalibur which clearly suffered from P toxicity). In the case of Excalibur, further experiments with solution P concentration maintained at low levels by daily additions (5 to 10 μmol L^{-1} per day) showed that Excalibur is more Zn-efficient in chelate-buffered nutrient solutions than Gatcher and Durati, the ranking which corresponds to that observed in field experiments (Z Rengel and M S Wheal, in prep.).

Three different criteria of Zn efficiency used here (Figs. 5-7) resulted in a similar ranking of wheat genotypes. Shoot growth at the high degree of stress imposed (Fig. 6) has been suggested as a good indicator (MacNair, 1993) because it would mimic the natural course of evolution (as a selection of genotypes most able to sustain growth in a limiting environment). In contrast, for crop genotypes a two-level screening (limiting and non-limiting environment) (Fig. 5) was frequently used (Graham et al., 1992) to avoid selecting genotypes of superior performance in limiting environments but of relatively poor agronomic performance in non-limiting environments. The shoot/root ratio (Figs. 3 and 7) does not appear to have been used earlier to rank genotypes for nutrient efficiency. Much larger reduction in the shoot/ratio when Zn-deficient and Zn-sufficient plants were compared (Fig. 7) characterised Zn-inefficient genotypes. In contrast, Zn-efficient genotypes can sustain a relatively larger shoot growth per unit of root when subjected to Zn deficiency. Further work, especially with soil-grown plants, is needed for a proper assessment of suitability of the shoot/root ratio as a criterion for Zn efficiency of wheat genotypes.

A correlation exists between the Zn efficiency ranking of wheat genotypes grown in chelate-buffered nutrient solution (Figs. 5-7) and ranking of these genotypes based on grain yield in field experiments on Zn-deficient soil (Graham et al., 1992): (i) Durati, Kamilaroi and Songlen have consistently showed poor performance under Zn deficiency, and (ii) Warigal, Warigal-5R and Aroona have been among the most Zn-efficient genotypes. In contrast, genotypes Excalibur (considered Zn-efficient) and Gatcher (Zn-inefficient) (Graham et al., 1992; Nable and Webb, 1993) ranked equal (medium efficient) in the present study (Figs. 5-7); the reason for such an observation might have been apparent P toxicity Excalibur had suffered from (the only one of all genotypes tested). Overall, the correlation between Zn efficiency ranking in chelate-buffered nutrient solution and Zn-deficient soil appears to be sufficient to suggest that at least some mechanisms of Zn efficiency are expressed to a similar degree in both soil and solution environments. In general, good correlations between field and laboratory testing of a number of genotypes in their response to various deficiency and toxicity stresses is notoriously hard to achieve (Rengel and Jurkic, 1992, and references therein).

A mechanistic explanation of differential Zn efficiency among genotypes of crop plants is still lacking. It may, however, be safely assumed (Graham and Rengel, 1993) that more than one mechanism is responsible for the level of Zn efficiency in a particular genotype (such an assumption may have wide applicability in deficiency and toxicity stresses caused by various ions).

The expression of different Zn efficiency mechanisms may be related to the intensity of the Zn-deficiency stress or to other environmental conditions. Therefore, screening for differential expression of various Zn efficiency mechanisms may only be achieved under controlled conditions: in contrast, field testing (if done over a number of years and a number of rel-

evant locations and soil types) may give an estimate of the overall level of Zn efficiency as a net result of interactions among various efficiency mechanisms and the genotype × environment interaction. The study reported here and in the accompanying paper (Rengel and Graham, 1995b) represents the first step toward defining growth and nutrient accumulation parameters for evaluation of Zn-efficiency mechanisms under controlled conditions. It is expected that efficiency mechanisms, once identified, will serve as a basis for developing a well-targeted screening procedure for use in breeding programs aimed at producing genotypes of superior Zn efficiency for cropping Zn-deficient soils in a more sustainable way.

In summary, chelate-buffered nutrient solution appears to be a promising environment for studying genotype-Zn interactions with respect to differential Zn efficiency and (at least some) mechanisms behind the Zn-efficiency trait. Research conducted in our laboratory following the study presented here confirmed an expression of several possible mechanisms of Zn efficiency when plants were grown in chelate-buffered nutrient solution.

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