Indices to screen for grain yield and grain-zinc mass concentrations in aerobic rice at different soil-Zn levels

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Abstract

Zinc is an important micronutrient for both crop growth and human nutrition. In rice production, yields are often reduced and Zn mass concentrations in the grains are often low when Zn is in short supply to the crop. This may result in malnutrition of people dependent on a rice-based diet. Plant breeding to enhance low-Zn tolerance might result in higher yields and nutritional quality but requires effective selection criteria embedded in physiological insight into the Zn husbandry of the crop and applicable in field evaluation of advanced breeding material or in screening of existing varieties. Using existing and newly developed low-Zn tolerance indices, this study presents the results of screening experiments carried out in high- and low-Zn soils. Sixteen accessions of aerobic rice were grown under greenhouse conditions to conceptualize the indices and 14 under field conditions to validate the indices. As the differences in soil-Zn levels in these experiments did not result in differences in grain yield, literature data were used from experiments where the soil-Zn level did have an effect on grain yield, to further check the validity of the indices. Several indices were applied to evaluate the genotypic low-Zn tolerance performance in attaining (relatively) high grain yield, high grain-Zn mass concentration, or both. The results indicate that the grain-Zn mass concentration efficiency index is different from the grain yield efficiency index and that the low-Zn tolerance indices identified superior genotypes best. Amongst the indices tested, the low-Zn tolerance index for grain yield and the low-Zn tolerance index for grain-Zn mass concentration were closely correlated with grain yield and grain-Zn mass concentration, respectively. Therefore, the low-Zn tolerance index for grain yield was effective in screening for high stability and high potential of grain yield, and the low-Zn tolerance index for grain-Zn mass concentration was effective for grain-Zn mass concentration under low and high soil-Zn conditions.

Genotypic differences in yield and grain-Zn mass concentration were shown to be unrelated and therefore deserve separate attention in breeding programmes. Combining the low-Zn tolerance index for grain yield and the low-Zn tolerance index for grain-Zn mass concentration in a single low-Zn tolerance index was considered but did not appear to be superior to using the two indices separately.

Additional keywords: breeding, low-zinc tolerance, *Oryza sativa* L., yield index, zinc efficiency

Introduction

Zinc is an important micronutrient for both crop growth and human nutrition. In rice production, especially in aerobic rice production, when Zn is in short supply to the crop, yields are often reduced (Gao *et al.*, 2006) and Zn mass concentrations in the grains are often low (Jiang *et al.*, 2007; submitted a, b). This may result in Zn malnutrition of people who depend on a rice-based diet.

Micronutrient malnutrition – often called 'hidden hunger' – has been estimated to afflict over two billion people, especially resource-poor women and children in the developing world, and their numbers are increasing (Buyckx, 1993; McGuire, 1993; Yip & Scanlon, 1994; Hambidge, 2000; Von Braun *et al.*, 2005). Crop products constitute the primary source of all micronutrients for humans, especially in developing countries. For instance, in China 70–85% of the Zn intake is derived from plant sources (Yang *et al.*, 2000). Therefore, enhancing the Zn mass concentration in cereals destined for human consumption is being considered a sustainable long-term solution for combating Zn malnutrition (Graham, 1984; Graham & Welch, 1996; Rengel *et al.*, 1999; Frossard *et al.*, 2000; Von Braun *et al.*, 2005).

Zn mass concentration in cereals may be increased by applying Zn fertilizer to the soil or directly to the plants (Broadley *et al.*, 2007). Continued fertilization in excess of crop uptake could lead to problems, so judicious use should be advocated. For the short term it is relevant that on low-Zn soils Zn application may lead to higher grain yields. However, for wheat (Kalayci *et al.*, 1999) and rice (Gao *et al.*, 2006) under field conditions it was shown that in currently available varieties grain-Zn mass concentration is not easily increased by fertilization.

So developing rice varieties that would combine high yields with high grain-Zn mass concentrations in situations without high levels of available Zn is a desirable breeding objective. Hence, to evaluate Zn efficiency in breeding programmes, indices are needed that are based on both grain yield and grain-Zn mass concentration, and embedded in physiological insight into the Zn husbandry of the crop and applicable in field evaluation of advanced breeding material or in screening of existing varieties. In previous work (Jiang *et al.*, 2007, submitted a, b) we showed that the final mass of Zn in the rice grain is a function of (1) Zn availability in the soil, (2) the capacity of the roots to take up Zn, (3) the Zn demand of the growing crop, and (4) the partitioning of Zn within the crop. However, a large proportion of Zn is sequestered in the vegetative parts of the above-ground crop and in the panicle structure, so that relatively little of the Zn accumulates in the grains, in spite of the fact that stimulating Zn uptake after flowering increases Zn mass concentration in the grains. For rice we also showed that the physiological mechanisms of Zn husbandry in relation to grain-Zn mass concentration are cultivar-specific, indicating a potential of selection for increased Zn efficiency and increased Zn mass concentration in the grains, independent of grain yield.

Currently, two indices relating to 'Zn efficiency' in cereals are widely used. One is the grain yield efficiency index, first defined by Graham (1984) as the ratio of [yield of a genotype at low soil-Zn level/experimental mean yield at low soil Zn] to [yield of a genotype at high soil-Zn level/experimental mean yield at high soil Zn], to classify genotypes into efficient (grain yield efficiency index exceeding 1) and inefficient (grain yield efficiency index in the range of 0.0–0.5) groups. A genotype with a high grain yield efficiency index has the ability to produce a relatively high yield under Zn-limited soil conditions compared with its own yield under Zn-sufficient conditions and with yields of other genotypes tested. This agronomic definition is meaningful to a plant breeder selecting genetic material of cereals in the field.

The second index commonly used in cereals is the ratio of yield at low soil-Zn level to yield at high soil-Zn level (Graham et al., 1992; Cakmak *et al.*, 1994; Rengel & Graham, 1995). This index could reflect the genotype's ability to cope with Zn deficiency relative to its own yield under non-limiting conditions. This index is of interest to crop physiologists and soil scientists, as it may form the basis for further study of the mechanisms underlying Zn efficiency in cereals, including root system geometry, chemical modification of the root–soil interface, and internal Zn redistribution.

Within a given experiment, the ratio of [experimental mean yield at high Zn] to [experimental mean yield at low Zn] will be identical for all genotypes. Within one experiment, the two indices therefore differentiate between the genotypes in an identical way and only differ by a constant factor. However, for breeders the performance of genotypes under different environmental conditions (i.e., weather and/or soil) is of interest. In this paper we therefore shall only use the grain yield efficiency index (YEI).

Another criterion for evaluation of grain crops is the stress tolerance index (STI) (Fernandez, 1993), which is used to compare genotypic performance across years or in environments where stress is common. This STI is most commonly used for tolerance to drought or heat stress and is calculated as $[YP/XP] \times [YS/XS] \times [XS/XP]$, where YP and YS are the yields of a given genotype in non-stressed and stressed environments, respectively, and XP and XS the mean yields of all tested genotypes in non-stressed and stressed environments, respectively. Higher values of STI for a genotype indicate greater stress tolerance and higher yield potential. STI has been found effective in identifying genotypes that perform well under both stress and non-stress conditions (Porch, 2006). This index has the potential for supporting the identification of genotypes that perform relatively well under stress, but also take advantage of favourable conditions by yielding high in terms of production and/or quality.

The Zn efficiency indices described above are all related to the yield of the genotypes and not to quality criteria, such as Zn mass concentration. We hypothesize that the ranking of genotypes for grain yield will differ from the ranking for Zn mass concentration, both under low Zn and high Zn soil conditions, and that the currently available Zn efficiency indices will not be suitable to select for high Zn mass concentration. We propose new indices, combinations of existing ones, or combinations of existing and new ones that will perform better when screening for Zn mass concentration in combination with grain yield. Some of these new indices will be based on the STI as developed for yield response to drought stress in cereals (Fernandez, 1993).

This study was, therefore, carried out to test the merits of current and new indices in screening genotypes for grain-Zn mass concentration and grain yield, separately and in combination. In our study, rice accessions were used that had been specifically bred for favourable performance under aerobic soil conditions (Bouman *et al.*, 2002; Yang *et al.*, 2005). Such soil conditions are potentially reducing soil-Zn availability, thus increasing the need to select for increased Zn efficiency and necessitating enhanced genotype performance (Gao *et al.*, 2006).

Materials and methods

The study comprised three data sets. A greenhouse experiment was set up to conceptualize the screening indices under relatively controlled conditions. A field experiment was carried out to validate these indices under agronomically relevant conditions. In the greenhouse experiment we did not observe statistically significant effects of Zn on grain yield or harvest index, though Zn uptake, Zn efficiency and Zn mass concentrations were strongly affected. In the field experiment, the grain yield was affected by Zn level, but the harvest index was not. We therefore identified a data set from literature to verify our results for conditions where Zn did affect grain yield and harvest index (Giordano & Mortvedt, 1974).

Both experiments consisted of a diverse set of genotypes. However, due to poor adaptation of some of the genotypes used in the greenhouse study to prevailing temperatures and photoperiods in the field, we were not able to carry out the field experiment with the same material. Some accessions caused considerable leverage in the regression analyses. The literature data set for verification also consisted of a diverse data set, but with cultivars not included in our experiments.

Greenhouse experiment

A pot experiment was carried out in a greenhouse at China Agricultural University, Beijing, China, from 24 May until 15 October 2003. The plants were grown in pots containing 7.5 kg soil (pH 6.8, DTPA-extractable Zn 0.3–0.4 mg kg^{-1}, i.e., well below the critical Zn concentration of 0.5 mg kg^{-1}; same soil as in the field experiment reported hereafter), either without amendment or amended with 10 mg Zn per kg soil, added as $ZnSO_4.7H_2O$. A basal dressing of 200 mg N per kg soil as $Ca(NO_3)_2$ and 100 mg P per kg soil as KH_2PO_4 was applied to all pots. All nutrients were mixed thoroughly with the soil before sowing. Sixteen aerobic rice (*Oryza sativa* L.) accessions were used. The seeds were obtained from the Aerobic Rice Research Center of China Agricultural University. Zn mass concentration in the hulled grain ranged from 9.7 to 15.4 mg kg^{-1}. The experiment was of a completely randomized factorial design

(16 accessions × 2 Zn levels) replicated three times. Ten seeds were sown in each pot, and the plant stand was thinned to four seedlings per pot soon after emergence. Pots were watered daily with de-ionized water to 80% of field capacity. Plants were grown under natural temperature and natural light during the summer season. On 1 September 2003, i.e., before flowering, all pots were transferred to a greenhouse where the temperature was maintained at 30 ± 1 °C during the day and 21 ± 1 °C during the night. Light intensity was about 85% of natural light intensity and 1000 μmol m^{-2} s^{-1} light was supplemented when it was cloudy. At physiological maturity (30 days after flowering), plants were harvested to determine dry weights and Zn mass concentrations.

Field experiment

The field experiment, comprising 14 aerobic rice accessions, was carried out in Mengcheng, Anhui province, China (33°55' N, 116°15' E) in 2004. Because photoperiod and temperature in the field were not suitable to many of the genotypes used in the greenhouse experiment, only four accessions tested in the greenhouse experiment were also tested in the field experiment. The soil at the experimental site was a Shajiang black soil (vertisol; Anon., 1998) with pH 6.8. DTPA-extractable Zn was 0.30–0.40 mg per kg soil, i.e., well below the widely accepted critical Zn concentration of 0.5 mg kg^{-1}. Seeds were obtained from the Aerobic Rice Research Center of China Agricultural University. Zn mass concentration in the hulled grain was 12.7–19.4 mg kg^{-1}. The experimental design was a split-plot, replicated three times. Main factor was Zn at two levels (+Zn, 22.5 kg ha^{-1} added as $ZnSO_4{\cdot}7H_2O$, and –Zn, no Zn added) and split factor was aerobic rice accessions – 14 in all. Plant spacing within the row was 0.15 m, and distance between rows 0.25 m. Composite fertilizer ($N–P_2O_5–K_2O$: 12–18–10) at a rate of 625 kg ha^{-1} and Zn fertilizer (only in the +Zn plots) were incorporated before planting and 50 kg N ha^{-1} was top-dressed as ammonium nitrate at tillering. Plants were grown under rainfed conditions, with supplemental irrigation once 1 day after sowing and once at flowering. Plant samples were collected at physiological maturity to determine dry weights and Zn mass concentrations, using standard procedures.

Measurement of Zn mass concentrations

Plant samples from both experiments were transported to the laboratory and partitioned into shoot (without panicle), panicle and grain. Samples were rinsed three times with double-de-ionized water, and then oven-dried at 75 °C for 48 h. Each component was weighed; the grain was hulled. Dried plant samples were ground in a stainless steel mill and passed through a 0.25-mm sieve before analysis. Sub-samples of 0.5 g of the dried and ground samples were digested in a bi-acid mixture (HNO_3:$HClO_4$ = 4:1). Zn was determined by atomic absorption spectroscopy (SPECTRAA-55, Karian Australia, Mulgrave, Australia).

Definition of Zn efficiency indices

The following indices for Zn efficiency were calculated:
Grain yield efficiency index (YEI) (Graham, 1984):

$$\mathrm{YEI} = (Y_L/\tilde{Y}_L)/(Y_H/\tilde{Y}_H),$$

Zn mass concentration efficiency index (ZnMCEI):

$$\mathrm{ZnMCEI} = (\mathrm{ZnMC}_L/\widetilde{\mathrm{Zn}}\mathrm{MC}_L)/(\mathrm{ZnMC}_H/\widetilde{\mathrm{Zn}}\mathrm{MC}_H),$$

Grain-Zn mass concentration and yield efficiency index (ZnMCYEI):

$$\mathrm{ZnMCYEI} = (\mathrm{YEI})\ (\mathrm{ZnMCEI}),$$

Low-Zn tolerance index for grain yield (TIY) (based on Fernandez, 1993):

$$\mathrm{TIY} = (Y_L/\tilde{Y}_L)\ (Y_H/\tilde{Y}_H)\ (\tilde{Y}_L/\tilde{Y}_H) = (Y_L)\ (Y_H)/(\tilde{Y}_H)^2,$$

Low-Zn tolerance index for grain-Zn mass concentration (TIZnMC):

$$\mathrm{TIZnMC} = (\mathrm{ZnMC}_L/\widetilde{\mathrm{Zn}}\mathrm{MC}_L)\ (\mathrm{ZnMC}_H/\widetilde{\mathrm{Zn}}\mathrm{MC}_H)\ (\widetilde{\mathrm{Zn}}\mathrm{MC}_L/\widetilde{\mathrm{Zn}}\mathrm{MC}_H)$$
$$= (\mathrm{ZnMC}_L)\ (\mathrm{ZnMC}_H)/(\widetilde{\mathrm{Zn}}\mathrm{MC}_H)^2,$$

Low-Zn tolerance index for grain-Zn mass concentration and grain yield (TIZnMCY):

$$\mathrm{TIZnMCY} = (\mathrm{TIY})\ (\mathrm{TIZnMC}),$$

where Y_H is the genotypic yield at high Zn; Y_L the genotypic yield at low Zn; $\tilde{Y}_H$ the mean yield over all genotypes at high Zn; $\tilde{Y}_L$ the mean yield at low Zn. ZnMC_H is the genotypic grain-Zn mass concentration at high Zn; ZnMC_L the genotypic grain-Zn mass concentration at low Zn; $\widetilde{\mathrm{Zn}}\mathrm{MC}_H$ the mean grain-Zn mass concentration over all genotypes in the high-Zn environment; $\widetilde{\mathrm{Zn}}\mathrm{MC}_L$ the mean grain-Zn mass concentration in the low-Zn environment.

To help the reader understand the different acronyms being used the following summary might be useful: Y stands for yield, MC is mass concentration, EI is efficiency index, TI is tolerance index and Zn stands for zinc. All indices are by definition dimensionless.

Data analysis

Regression analysis and analysis of variance (ANOVA) were performed with SAS (Anon., 2001).

In addition to data from our own experiments, the data set from Giordano & Mortvedt (1974) was used to analyse the correlation between all defined indices.

Results

Grain yield and grain-Zn mass concentration

The accessions significantly differed in grain yield and harvest index (Table 1). In the greenhouse experiment, Zn treatment did not significantly affect grain yield or harvest index, but did affect grain yield in the field experiment (Table 1). Tables 2 and 3 show the effect of Zn and genotype on the grain-Zn yield (i.e., the mass of Zn per plant present in the grain at the end of the growing period), shoot-Zn content (i.e., the mass of Zn per plant in the above-ground plant dry matter), Zn use efficiency (i.e., the shoot dry matter production per unit of Zn uptake) and Zn harvest index (i.e., grain-Zn yield divided by shoot-Zn content). In the greenhouse experiment (Table 2), additional Zn supply increased grain-Zn yield and shoot-Zn content for all genotypes, but reduced Zn use efficiency and had a variable effect on Zn harvest index. Genotypes showed large variation in all characteristics listed in Table 2. In the field experiment (Table 3), additional Zn supply increased grain-Zn yield, shoot-Zn content and Zn harvest index for most genotypes, but not in all. Zn use efficiency was not affected by Zn application, and was consistently higher than in the greenhouse experiment.

Table 1. Statistical significance [1] of F values derived from Analysis of Variance of two experiments studying the effects of aerobic rice accessions, Zn level and their interaction for various variables, and the coefficients of variation of these variables.

Experiment	Variable	Accessions	Zn level	Accessions × Zn level	CV (%)
Greenhouse	Grain yield	**	ns	ns	8.3
	Harvest index	**	ns	ns	8.5
	Grain ZnMC [2]	**	**	**	11.7
	Grain-Zn yield	**	**	**	15.3
	Shoot-Zn content	**	**	**	13.6
	Zn use efficiency	**	**	**	13.0
	Zn harvest index	**	**	**	17.0
Field	Grain yield	**	*	ns	8.6
	Harvest index	**	ns	**	6.2
	Grain ZnMC	**	**	**	13.7
	Grain-Zn yield	**	**	*	20.0
	Shoot-Zn content	**	**	ns	15.1
	Zn use efficiency	**	*	ns	13.5
	Zn harvest index	**	**	**	11.1

[1] * = $P < 0.05$; ** = $P < 0.01$; ns = not statistically significant.

[2] Grain ZnMC = grain-Zn mass concentration.

Table 2. Grain-Zn yield, shoot-Zn content, Zn use efficiency and Zn harvest index for the accessions studied in the greenhouse experiment. For the grouping of the accessions see Table 4.

Accession	Grain-Zn yield		Shoot-Zn content		Zn use efficiency		Zn harvest index	
	–Zn	+Zn	–Zn	+Zn	–Zn	+Zn	–Zn	+Zn
	(μg Zn per plant)				(g shoot DM/ μg Zn)			
90B10-1	102	729	781	4810	0.022	0.003	0.13	0.15
91B8-30-3	105	598	1010	5480	0.018	0.003	0.10	0.11
Handao277	184	959	816	6090	0.020	0.003	0.23	0.16
91BTe3	96	552	972	6750	0.017	0.002	0.10	0.08
89B271Mozhuxi	117	627	932	8970	0.018	0.002	0.13	0.07
Handao9	100	537	762	5790	0.018	0.003	0.13	0.09
Handao72	114	580	780	5270	0.020	0.003	0.15	0.11
89D108-11-1	127	617	1100	7050	0.013	0.002	0.12	0.09
TB Mozhuxi	119	529	989	6070	0.017	0.003	0.12	0.09
K150	140	590	892	5720	0.015	0.002	0.16	0.10
89B271-17Hun	148	520	946	5300	0.019	0.003	0.16	0.10
Handao99-19	114	401	571	4950	0.018	0.002	0.20	0.08
Hongkelaoshuya	106	552	1320	5550	0.012	0.003	0.08	0.10
Handao502	133	567	1000	6430	0.017	0.003	0.13	0.09
Baxiludao	118	499	808	5210	0.020	0.003	0.15	0.10
Handao297	143	557	1170	3990	0.014	0.004	0.12	0.14
Mean	123	588	928	5840	0.017	0.003	0.14	0.10
SED [1]	40		335		0.001		0.01	

[1] SED = standard error of the difference between means (+Zn versus –Zn) in the same row.

Under low-Zn conditions, grain yield varied between 2.5 and 4.5 g plant^{-1} in the greenhouse and between 213 and 457 g m^{-2} in the field experiment (Tables 4 and 5). Zn application significantly increased Zn mass concentration in the grains (Tables 1, 4 and 5), and there were strong interactions between Zn level and accession (Table 1). Grain ZnMC responded differently to Zn fertilization amongst accessions. Four accessions in the greenhouse experiment and five accessions in the field experiment were found to show a markedly strong response to Zn fertilization, with an increase in grain ZnMC exceeding 3 times the standard error of the difference between means (SED). Other accessions were less responsive to Zn fertilization (Tables 4 and 5). Accessions tested in the greenhouse and in the field experiment strongly varied in yield, grain-Zn mass concentration and partitioning of dry matter and Zn. Under low soil-Zn conditions without Zn supply, grain-Zn mass concentration varied from 27.3 (Handao9) to 50.5 (89D108-11-1) mg kg^{-1} in the greenhouse experiment and from 12.0 (Baxiludao) to 26.3 (Hongkelaoshuya) mg kg^{-1} in the field experiment. With additional Zn, grain-Zn mass concentration varied from 28.9 (Handao99-19) to 57.5 mg kg^{-1} (89D108-11-1) in the greenhouse experiment and from 16.3 (Haogelao-5) to 29.6

Table 3. Grain-Zn yield, shoot-Zn content, Zn use efficiency and Zn harvest index for the accessions studied in the field experiment. For the grouping of the accessions see Table 4.

Accession	Grain-Zn yield		Shoot-Zn content		Zn use efficiency		Zn harvest index	
	–Zn	+Zn	–Zn	+Zn	–Zn	+Zn	–Zn	+Zn
	------- (µg Zn per m²) --------				(g shoot DM/ µg Zn)			
Qinai-3Hun	4140	7450	14500	18600	0.06	0.05	0.29	0.40
Henghan1	3620	5710	16400	15900	0.05	0.05	0.22	0.36
Handao7	4650	9750	16400	22900	0.07	0.05	0.28	0.42
Handao65	4820	7420	14900	16300	0.07	0.06	0.32	0.46
Liaohan109	5990	8900	13500	19300	0.07	0.06	0.44	0.46
Yunnanhandao	5410	8320	20400	22300	0.07	0.07	0.27	0.37
90B290	11100	12000	20800	24000	0.07	0.06	0.53	0.49
91B8-14	5070	5900	15200	17300	0.08	0.07	0.33	0.34
91BTe9-7	7040	6720	17100	18400	0.07	0.07	0.41	0.37
Haogelao-5	6560	5920	18500	19300	0.07	0.07	0.36	0.31
Handao297	4850	8660	15000	17600	0.08	0.07	0.32	0.49
Handao502	6860	10200	23000	23800	0.07	0.06	0.30	0.43
Baxiludao	5400	7390	19700	20700	0.08	0.08	0.27	0.36
Hongkelaoshuya	7910	9440	28800	27300	0.05	0.06	0.28	0.35
Mean	5960	8120	18200	20300	0.07	0.06	0.33	0.40
SED [1]	1009		2075		0.006		0.029	

[1] SED = standard error of the difference between means (+Zn versus –Zn) in the same row.

(Qinai-3Hun) $mg\ kg^{-1}$ in the field experiment (Tables 4 and 5). Among the four accessions used in the greenhouse as well as in the field experiment, grain-Zn mass concentration was lowest in Baxiludao and highest in Hongkelaoshuya.

Low-Zn tolerance indices

Accessions differed in the indices YEI, ZnMCEI, ZnMCYEI, TIY, TIZnMC, and TIZnMCY (Tables 4 and 5). Handao277 (in the greenhouse experiment) and 90B290 (in the field experiment) were outliers combining very high values for both TIY and TIZnMC, indicating good performance (low-Zn tolerance) under low-Zn conditions, with high grain yield and high grain-Zn mass concentration potentials at high Zn supply in comparison to all other accessions tested. Handao99-19 as well as Henghan1 showed low Zn efficiency, with low grain yield and low grain-Zn mass concentration under low-Zn conditions. The ranking of the accessions differed depending on the index for which they were ranked, although YEI and TIY were both based on grain yield, whereas ZnMCEI and TIZnMC were both based on grain-Zn mass concentration (Tables 4 and 5). There was no statistically significant correlation between YEI and TIY, or between ZnMCEI and TIZnMC (Table 6).

Table 4. Grain yield, grain-Zn mass concentration, and Zn efficiency indices of the 16 accessions in the 2003 greenhouse experiment. The accessions are grouped into a first set of 4 accessions with an increase in grain ZnMC exceeding 3 times the standard error of the difference between means (SED), a second set of 8 accessions with a difference less than 3 times the SED, and a third group of 4 accessions that were also included in the field experiment (Table 5) and with differences less than 3 times the SED.

Accession	Grain yield		Grain ZnMC [1]		Zn efficiency index [2]							
	−Zn	+Zn	−Zn	+Zn	YEI	ZnMCEI	ZnMCYEI	TIY	Rank	TIZnMC	Rank	TIZnMCY
	- - (g plant^{-1}) - -		- - (mg kg^{-1}) - -									
90B10-1	3.2	3.3	31.7	55.6	0.99	0.67	0.67	0.84	13	1.00	4	0.85
91B8-30-3	3.7	3.5	28.6	42.8	1.07	0.78	0.84	1.03	8	0.70	12	0.72
Handao277	4.5	4.5	41.2	53.7	1.02	0.89	0.91	1.60	1	1.26	2	2.01
91BTe3	3.4	3.5	27.9	39.5	1.00	0.82	0.83	0.96	10	0.63	14	0.60
89B271Mozhuxi	4.0	3.9	29.5	40.0	1.03	0.86	0.89	1.25	2	0.67	13	0.83
Handao9	3.7	3.9	27.3	35.0	0.97	0.91	0.88	1.14	3	0.54	16	0.62
Handao72	3.6	3.7	32.1	39.7	1.00	0.94	0.93	1.05	6	0.72	11	0.76
89D108-11-1	2.5	2.7	50.5	57.5	0.96	1.02	0.98	0.55	16	1.65	1	0.90
TBMozhuxi	3.0	3.0	40.3	44.4	1.01	1.06	1.06	0.71	14	1.02	3	0.72
K150	3.5	3.7	40.5	40.2	0.97	1.17	1.13	1.02	9	0.93	6	0.94
89B271-17Hun	3.8	3.5	39.2	37.0	1.09	1.24	1.36	1.06	5	0.82	8	0.88
Handao99-19	3.4	3.5	34.0	28.9	0.99	1.37	1.36	0.94	11	0.56	15	0.52
Hongkelaoshuya	2.8	3.2	38.1	43.7	0.89	1.02	0.91	0.70	15	0.95	5	0.67
Handao-502	3.7	3.6	36.5	40.0	1.05	1.07	1.11	1.05	7	0.83	7	0.87
Baxiludao	3.2	3.4	37.4	37.1	0.96	1.17	1.12	0.86	12	0.79	10	0.67
Handao-297	3.6	3.9	39.5	36.0	0.95	1.28	1.22	1.12	4	0.81	9	0.91
SED [3]	0.21		3.07									
Mean	3.45 $=\tilde{Y}_L$	3.53 $=\tilde{Y}_H$	35.9 $=\tilde{Z}nMC_L$	42.0 $=\tilde{Z}nMC_H$								

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[1] Grain ZnMC =grain-Zn mass concentration.

[2] YEI = grain yield efficiency index; ZnMCEI = grain-Zn mass concentration efficiency index; ZnMCYEI = grain-Zn mass concentration and yield efficiency index; TIY = low-Zn tolerance index for grain yield; TIZnMC = low-Zn tolerance index for grain-Zn mass concentration; TIZnMCY = low-Zn tolerance index for grain-Zn mass concentration and grain yield.

[3] SED = standard error of the difference between two means (+Zn versus −Zn) in the same row.

Table 5. Grain yield, grain-Zn mass concentration, and Zn efficiency indices of the 14 accessions in the 2004 field experiment. The accessions are grouped into a first set of 5 accessions with an increase in grain ZnMC exceeding 3 times the standard error of the difference between means (SED), a second set of 5 accessions with a difference less than 3 times the SED, and a third group of 4 accessions that were also included in the greenhouse experiment (Table 4) and of which the first 2 with differences less than 3 times the SED.

Accession	Grain yield		Grain ZnMC [1]		Zn efficiency index [2]							
	–Zn	+Zn	–Zn	+Zn	YEI	ZnMCEI	ZnMCYEI	TIY	Rank	TIZnMC	Rank	TIZnMCY
	- - ($g\ plant^{-1}$) - -		- - ($mg\ kg^{-1}$) - -									
Qinai-3Hun	244	252	17.1	29.6	1.02	0.75	76	0.45	13	1.01	4	0.45
Henghan1	213	221	16.9	25.9	1.01	0.85	87	0.35	14	0.87	5	0.30
Handao7	353	439	13.1	21.9	0.85	0.78	66	1.14	6	0.57	11	0.65
Handao65	290	300	16.5	24.8	1.02	0.87	89	0.64	11	0.81	6	0.52
Liaohan109	424	428	14.1	20.9	1.04	0.88	92	1.33	4	0.59	9	0.78
YunnanHandao	457	503	12.0	16.5	0.96	0.95	90	1.69	1	0.39	14	0.65
90B290	437	440	25.3	27.0	1.04	1.22	128	1.41	2	1.36	2	1.93
91B8-14	281	301	18.0	19.5	0.98	1.21	119	0.62	12	0.70	7	0.43
91BTe9-7	372	385	18.9	17.4	1.02	1.42	145	1.05	7	0.65	8	0.69
Haogelao-5	361	364	18.3	16.3	1.04	1.46	152	0.96	9	0.59	9	0.57
Handao297	385	416	12.5	20.7	0.97	0.79	77	1.18	5	0.52	12	0.61
Handao502	351	376	19.5	27.0	0.98	0.94	93	0.97	8	1.05	3	1.02
Baxiludao	444	410	12.0	18.0	1.14	0.87	101	1.33	3	0.43	13	0.58
Hongkelaoshuya	300	318	26.3	28.5	0.99	1.21	120	0.76	10	1.49	1	1.05
SED [3]	22.9		1.9									
Mean	351 $=\tilde{Y}_L$	368 $=\tilde{Y}_H$	17.2 $=\widetilde{ZnMC}_L$	22.4 $=\widetilde{ZnMC}_H$								

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[1] Grain ZnMC = grain-Zn mass concentration.

[2] YEI = grain yield efficiency index; ZnMCEI = grain-Zn mass concentration efficiency index; ZnMCYEI = grain-Zn mass concentration and yield efficiency index; TIY = low-Zn tolerance index for grain yield; TIZnMC = low-Zn tolerance index for grain-Zn mass concentration; TIZnMCY = low-Zn tolerance index for grain-Zn mass concentration and grain yield.

[3] SED = standard error of the difference between two means (+Zn versus –Zn) in the same row.

Table 6. Linear correlation coefficients between grain yield or grain-Zn mass concentration and Zn efficiency indices for the field and greenhouse experiments and for additional data from the literature (Giordano & Mortvedt, 1974). (Note: autocorrelation was found in all cases.)

Grain yield/ Grain-Zn mass concentration/ Indices	Data set[1]	Zn efficiency indices[2]						
		YEI	ZnMCEI	ZnMCYEI	TIY	TIZnMC	TIZnMCY	TIZnMCY[3]
LYIELD[4]	F	0.18	0.12	0.16	0.98**	−0.36	0.46	
	G	0.57*[5]	−0.12	0.03	0.98**	−0.35	0.56*	0.09
	D	0.77*	0.22	0.64	0.95**	−0.28	0.88**	
HYIELD[4]	F	−0.15	0.04	0.01	0.98**	−0.39	0.43	
	G	0.26	−0.08	−0.01	0.98**	−0.35	0.57*	0.02
	D	−0.07	−0.29	−0.17	0.79*	−0.50	0.62	
LGZnMC[6]	F	0.12	0.59*	0.57*	−0.28	0.91**	0.64*	
	G	−0.24	0.47	0.41	−0.31	0.84**	0.39	0.50
	D	0.29	0.57	0.49	−0.28	0.91**	0.21	
HGZnMC[6]	F	−0.08	−0.32	−0.32	−0.52	0.84**	0.33	
	G	−0.07	−0.57*	−0.59*	−0.16	0.84**	0.53*	0.45
	D	−0.26	−0.35	−0.29	−0.49	0.85**	−0.15	
YEI	F&G				ns[7]			
ZnMCEI	F&G					ns		
ZnMCYEI	F&G						ns	

[1] F = data from field experiment; G = data from greenhouse experiment; D = data from literature.

[2] YEI = grain yield efficiency index; ZnMCEI = grain-Zn mass concentration efficiency index; ZnMCYEI = grain-Zn mass concentration and yield efficiency index; TIY = low-Zn tolerance index for grain yield; TIZnMC = low-Zn tolerance index for grain-Zn mass concentration; TIZnMCY = low-Zn tolerance index for grain-Zn mass concentration and grain yield.

[3] Linear correlation coefficient when the outlier accession Handa0277 was excluded.

[4] LYIELD = grain yield at low Zn level; HYIELD = grain yield at high Zn level.

[5] Levels of statistical significance: * = $P < 0.05$; ** = $P < 0.01$.

[6] LGZnMC = grain-Zn mass concentration at low Zn level; HGZnMC = grain-Zn mass concentration at high Zn level.

[7] ns = not statistically significant.

Correlations between Zn-efficiency indices and grain yield or grain-Zn mass concentration

In both experiments, TIZnMC was strongly correlated with grain-Zn mass concentration, and TIY was correlated with grain yield under both low- and high-Zn conditions. TIZnMCY was correlated with grain yield, but only in the greenhouse experiment, and was not consistently correlated with grain Zn mass concentration (Table 6). The other indices, including YEI and ZnMCEI, were not consistently correlated with either grain yield or grain-Zn mass concentration. Thus, TIY was effective in identifying accessions with high and stable grain yield potential, whereas TIZnMC was effective in identifying accessions with high grain Zn mass concentration. The correlations of the combination of TIY–TIZnMC and TIY–TIZnMCY with grain yield and grain-Zn mass concentration were always weaker than those of the individual component indices (Table 6).

Test of indices with additional data

As in the greenhouse experiment no effect of Zn on grain yield and harvest index was observed, and in the field experiment no effect on harvest index, we used a data set from literature for which statistically significant effects on these parameters had been observed (Giordano & Mortvedt, 1974). For this data set we found correlations between all indices and grain yield and grain-Zn mass concentration that were similar to those found for our own data sets. This suggests that the two low-Zn tolerance indices also perform well under conditions in which Zn availability has more pronounced effects on crop performance.

Discussion

Genotypic variation in grain-Zn mass concentration

Genotypic variation in grain-Zn mass concentration in rice has been reported by Giordano & Mortvedt (1974), Yang *et al.* (1998), Fageria (2001), Gregorio (2002) and Gao *et al.* (2005). We too observed strong variation in grain-Zn mass concentration (grain ZnMC) among the accessions tested in both experiments, and Zn supplementation resulted in significantly higher grain ZnMC (Tables 1, 4 and 5). This genotypic variation was associated with variation in Zn use efficiency and Zn harvest index. The statistically significant interaction observed between Zn application and accession implies a statistically significant genotype × environment interaction. Grain ZnMC was correlated with the ZnMC of the panicle structure. However, grain ZnMC was not correlated with Zn harvest index or Zn mass concentration in the shoot, except in the situation without Zn supplementation in the greenhouse experiment (data not shown). This suggests that the differences in grain-Zn mass concentration among genotypes were due to a difference in loading ability of Zn from the panicle to the grains, and were not directly determined by Zn harvest index or shoot Zn content. This is consistent with the results of Grusak *et al.* (1999), indicating that the ability to maintain xylem influx into the panicle during seed formation and the ability to load the grain from that xylem are essential for realizing a high grain-Zn mass concentration. For a thorough understanding of grain-Zn mass concentration also the carbohydrate and protein accumulation in the different parts of the kernel, and their relation to Zn accumulation, should be taken into account.

Zn efficiency or low-Zn tolerance indices in screening

Genotypes characterized by high grain yield efficiency indices (YEI) have the ability to produce relatively high yields under Zn-limited soil conditions in comparison to their yield under Zn-sufficient soil conditions and in comparison to yields of other accessions (Graham, 1984). However, in this study we found no consistent correlation between grain yield efficiency index and grain yield (Table 6).

Genotypic variation in Zn efficiency has been studied in various crops, including common bean (Ambler & Brown, 1969; Hacisalihoglu *et al.*, 2004), wheat (Graham & Rengel, 1993; Cakmak *et al.*, 1997; Kalayci *et al.*, 1999; Hacisalihoglu *et al.*, 2001; 2003) and rice (Fageria, 2001; Gao *et al.*, 2005). Insights into the mechanisms underlying high Zn efficiency are increasing, i.e., more information is becoming available on how the plant is able to maintain reasonable growth rates and yields under conditions of low Zn availability in the growth medium. Potential mechanisms include relatively efficient Zn uptake and translocation, and effective and efficient biochemical utilization of Zn. However, many questions with respect to these mechanisms still remain (Rengel & Graham 1995, Cakmak, 2000; Hacisalihoglu *et al.*, 2001; 2003; Hacisalihoglu & Kochian, 2003). It is essential to note, though, that a mechanism such as efficient biochemical utilization of low leaf-Zn levels necessary for production has no inherent contribution to high grain-Zn loading capability and may in fact be fully unrelated.

Similarly to the grain yield efficiency index, the grain-Zn mass concentration efficiency index (ZnMCEI) is not correlated with grain-Zn mass concentration, neither under low nor under sufficient Zn conditions. So ZnMCEI only reflects the accession's ability to produce a relatively high grain-Zn mass concentration under Zn-limited soil conditions, in comparison to its grain-Zn mass concentration under Zn-sufficient soil conditions, and not its ability to use high Zn-availability conditions to attain a high grain-Zn mass concentration.

The two new Zn-indices derived from drought stress research, i.e., the low-Zn tolerance index for grain yield (TIY) and the low-Zn tolerance index for grain-Zn mass concentration (TIZnMC), attain higher values for genotypes characterized by greater low-Zn tolerance in terms of grain yield or grain-Zn mass concentration, respectively, and higher yield or grain-Zn mass concentration potential, under low and sufficient soil-Zn conditions, respectively. Moreover, in both experiments, TIY and TIZnMC were highly correlated with grain yield and grain-Zn mass concentration, respectively (Table 6).

However, TIZnMCY, the combination of TIY and TIZnMC, did not correlate with grain yield or grain-Zn mass concentration (when the outlier accession Handao277 was excluded) (Table 6). So TIY and TIZnMC are effective in identifying genotypes that perform well in terms of yield or grain-Zn mass concentration, respectively, under both Zn-limited and Zn-sufficient conditions, but an effective indicator for a combination of the two characteristics could not be identified.

In the greenhouse experiment the ranking based on TIY of the four accessions included in both experiments (Handao297, Handao502, Hongkelaoshuya, Baxiludao) differed from that in the field experiment (Table 6). In studies reported in literature, different rankings of a set of genotypes have been observed on the same site but in different experimental years (Kalayci *et al.*, 1999), which could be the result of differences in plant-available Zn, and, therefore, in Zn-stress intensity between experiments. However, the overlap in terms of accessions between our experiments was too limited for any further analysis.

Conclusions

Differences in grain-Zn mass concentration among genotypes were due to a difference in loading ability of Zn from the panicle to the grains, and were not directly determined by Zn harvest index or shoot-Zn content. Therefore, there is no clear relation between grain-Zn mass concentration and Zn efficiency for grain production. For rice breeding programmes, the indices TIY and TIZnMC appear promising for screening genotypes in which high tolerances to low Zn based on grain yield and grain-Zn mass concentration are combined. The same indices also appear promising for exploring higher Zn availability through higher yield and grain-Zn mass concentration potentials under non-stressed conditions. As the two indices gave different rankings and the correlation between the compound index TIZnMCY and yield or grain-ZnMC was much weaker than for the individual indices, it seems important for breeding programmes to separate the analyses of both traits.

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