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Grain Zn concentrations and yield of Zn-biofortified versus deficiency-tolerant rice genotypes under contrasting growth conditions

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Abstract

Higher grain Zn concentration in 'biofortified' rice genotypes, bred for high grain Zn concentration, should not be at the expense of reduced grain yield. This study examined the grain yield and grain Zn concentration of Zn-biofortified genotypes in field experiments in the Philippines. Zinc-biofortified genotypes (high grain Zn concentration in Zn-sufficient soil) were compared with efficient genotypes (tolerant of soil Zn deficiency), inefficient genotypes (sensitive to soil Zn deficiency) and check genotypes (popular local varieties) at four sites (Bay, Bohol, Bukidnon and IRRI) with differing types and degrees of Zn deficiency, over five cropping seasons (wet season 2012, 2014 and 2015 and dry season 2013 and 2015). A common experimental design and plot size were used with treatments (genotypes and Zn fertilization) arranged in a two-factorial randomized complete block design. The results showed that biofortified genotypes achieved both the Philippine grain yield target (4.0 t ha^{-1}) and grain Zn biofortification target (30 mg kg^{-1}) only when grown under Zn-sufficient conditions. In Zn-deficient soils, most Zn-biofortified and deficiency-tolerant genotypes reached the Zn concentration target but not the yield target, suggesting the need to correct the soil Zn-deficiency to prevent yield penalty. Further, results from IRRI showed that only Zn-fertilized plants were able to achieve the Zn biofortification target during the wet season; whereas during the dry season, when the soil was less chemically-reduced and therefore the soil Zn probably more plant-available, grain Zn levels were all above the threshold, with or without Zn fertilizer. This suggests that Zn fertilization may not be needed during the dry season in soils with sufficient, potentially plant-available Zn.

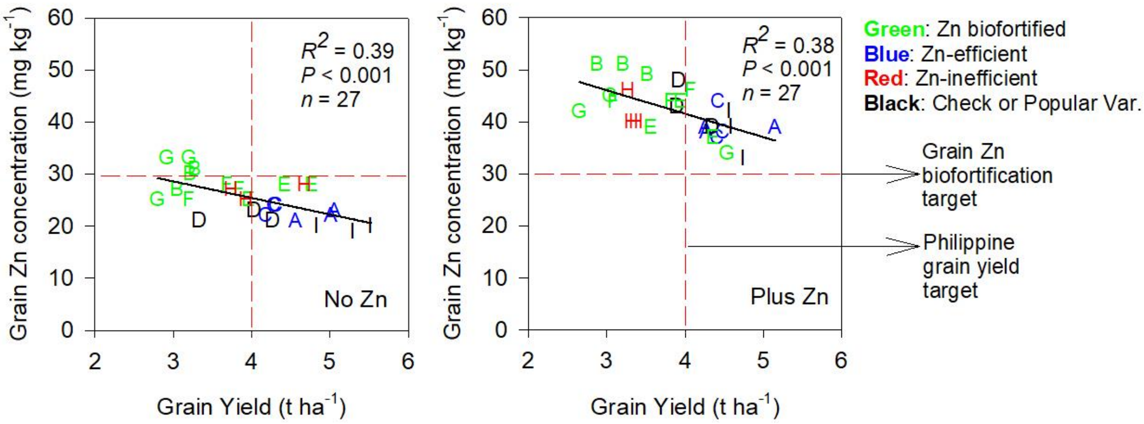
Keywords: Grain Zn biofortification, Zn fertilization, biofortified rice genotypes, Zn uptake, improved grain yield performance

Highlights:

- Only Zn ‘biofortified’ genotypes achieved target grain-Zn concentrations
- No genotypes achieved target yields in Zn-deficient soils
- High grain Zn concentration was at the cost of grain yield
- Zinc fertilizer increased grain-Zn in the wet season but not the dry season

Graphical abstract:

[We plan to put a simplified version of Fig. 5a here]



1. Introduction

Zinc (Zn) deficiency in human populations is a major global health problem (UNICEF/WHO/WB, 2013). Initiatives to address this include 'biofortification' of food crops with Zn, either by agronomic management or genetic improvement (Mayer et al., 2008; Hirschi, 2009; White and Broadley, 2011). Rice is one of the key crops being targeted for this (Bouis and Saltzman 2017). In general rice has a low content of micronutrients, particularly Zn, compared with other cereal grains, partly because of inherent genetic differences and partly because biogeochemical changes in submerged paddy soils result in Zn being immobilized and so made less-available for plant uptake (Kirk 2004; Izquierdo et al., 2016). However, there is wide genetic variation in grain Zn content in the rice germplasm, and this is being exploited in breeding programs aiming to produce Zn-biofortified varieties (Gregorio et al, 2002; HarvestPlus 2014). For example, at least nine Zn-biofortified varieties have been released for Boro season rice in Bangladesh (Bangladesh Rice Research Institute, 2016).

There has also been progress with agronomic enrichment of rice grain Zn concentrations by fertilizer and water management (Gao et al 2012; Johnson-Beebout et al 2016). Aerobic water management consistently shows a moderate increase in grain Zn concentration in rice compared with traditional continuously flooded (anaerobic) practice, but the effects of Zn fertilizer addition to the soil have been inconsistent (Cakmak 2008; HarvestPlus 2014; Tuyogon et al., 2016). A recent study suggested that Zn fertilization timing could be optimized in combination with water management to exploit increased Zn solubility with soil oxidation after moderate drying (Johnson-Beebout et al., 2016). Foliar Zn fertilization strategies have shown some promise to increase grain Zn concentration (Cakmak 2008; Mabesa et al., 2013), as has seedling dipping in Zn-containing slurry to overcome early-season Zn deficiency (Rehman et al., 2012).

Adoption of biofortification technologies by farmers will depend on grain yields in diverse and sometimes adverse conditions, including moderate-to-severe Zn-deficiency (Bouis et al., 2013). While there has been significant progress in understanding the physiological and environmental factors that determine Zn uptake, internal use efficiency and allocation to grain (Stomph et al., 2014; Mori et al., 2016; Rose et al., 2016; Jaksomsak et al., 2017; Affholder et al., 2017), there have been very few field studies addressing both grain yield and Zn concentration under limiting conditions (Wissuwa et al., 2008; Nanda and Wissuwa 2016; Beebout-Johnson et al 2016). The objectives of this study were: (1) to compare Zn-biofortified, Zn-efficient and Zn-inefficient rice genotypes for grain yield and Zn concentration in contrasting environments; (2) to investigate relationships between grain-Zn concentration and grain yield, and dilution effects; and (3) to investigate Zn fertilization strategies for improving rice growth and Zn enrichment in Zn-deficient conditions. We hypothesized that (1) grain yields and Zn concentrations of contrasting genotypes vary with the degree of soil Zn deficiency, (2) there is a trade-off between increased grain yield and increased grain Zn concentration in Zn-biofortified genotypes, and (3) the effects of Zn fertilization on grain yield and Zn concentration are different.

2. Materials and methods

Field experiments were made at four sites in the Philippines during the wet seasons (WS) of 2012, 2014 and 2015 and dry seasons (DS) of 2013 and 2015. The four sites were a Zn-sufficient site at the International Rice Research Institute (IRRI) in Los Baños, Laguna and three Zn-deficient sites at Bay, Laguna; Sagbayan, Bohol; and Musuan, Bukidnon (Table 1). Seventeen genotypes were compared: five 'efficient' genotypes tolerant of soil Zn deficiency (based on growth), four 'inefficient' genotypes sensitive to soil Zn deficiency, five 'biofortified' genotypes bred for high grain Zn content under non-limiting conditions, and three widely-grown checks (Table S1).

2.1 Crop establishment

Land preparation included plowing and puddling followed by construction of levees to separate the Zn treatment and replications. To prevent contamination from previous experiments, plots with and without added Zn were kept separate at all sites over the multiple years of the experiment. A day before transplanting, a basal dose of fertilizer at a rate of 20 kg ha^{-1} each of N, P and K was broadcast and thoroughly mixed with the soil using a power weeder, followed by leveling at all sites. After breaking the seed dormancy at 50 °C for 3 days, the seeds were sown in a wet seed bed with NPK fertilizer recommendation of 80 g m^{-2} without added Zn. The seedlings were transplanted at 21 days after sowing at all the field sites except in Bay where 28-day old seedlings were used to avoid the damage caused by snails as brought upon by continuously deep flooded conditions. Molluscicide (Bayluscide) was sprayed at a rate of 1 L ha^{-1} one week before and after transplanting to minimize the snail damage on young seedlings. The water inside the field was maintained at saturation point until irrigation water was provided one week after transplanting, to further minimize snail damage. Continuous flooding was used throughout the experiment in all the sites, unless noted otherwise. Fertilizer NPK rates at each site were 100 kg N ha^{-1}, 20 kg P ha^{-1} and 20 kg K ha^{-1}. All of the P and K was added to the soil at basal stage together with 20% of N as complete (NPK) fertilizer with the remaining N broadcasted as urea in two split applications: 35% at 24 days after transplanting (DAT) and 45% at 42 DAT.

2.2 Experimental design and layout

The treatments in the different seasons were as follows. The genotypes used at each site and season are given in Table S1.

2.2.1 Wet season 2012

A two-factorial randomized complete block design (RCBD) was followed with two Zn treatments, either 14 (Bohol, Bukidnon and IRRI) or 8 (Bay) genotypes and 4 replications. The Zn treatments were either no added Zn or root dipping of rice seedlings in 4% ZnO for 15 min before transplanting. Plot size was 3 × 1.2 m^2 with six rows of 3-m length and 0.20-m spacing between rows and hills, with one seedling per hill.

2.2.2 Dry season 2013 and wet season 2014

A two-factorial RCBD was followed with two Zn treatments, either 9 (Bay and Bohol, DS 2013) or 10 (Bay, Bohol and IRRI, WS 2014) genotypes and 4 replications. The Zn treatments were either no Zn added or (a) for WS, root dipping of rice seedlings in 4% ZnO for 15 min before transplanting or (b) for DS, broadcast zinc sulfate heptahydrate to the soil with Zn foliar application during flowering stage at a split rate of 5 kg ha^{-1}. The size of each plot was 12 m^2 with 20 rows of 3-m length and 0.20-m spacing between rows and hills, with one seedling per hill.

2.2.3 Dry season 2015

A split-plot two-factor RCBD was followed with 4 fertilizer treatments, 4 genotypes and 3 replications. The Zn treatments were: (0) no added zinc; (1) soil basal + soil broadcast at 50% flowering; (2) soil basal + zinc foliar spray at 50% flowering; and (3) Zn foliar spray at mid-tillering (30 DAT) and flowering stages. Zinc sulphate heptahydrate fertilizer was used at a split rate of 5 kg ha^{-1}. The size of each plot was 12 m^2 with 20 rows of 3-m length, 0.20-m spacing between rows and hills and with continuous planting within main plots and bunds between main plots. The planting density was two seedlings per hill.

2.3 Soil analyses

Soil samples for initial soil characterization from 10 randomly selected subplots from each site were collected, combined and analysed at IRRI's Analytical Service Laboratory for particle size by the hydrometer method (Gee and Bauder, 1979), pH in KCl at 1:25 soil:extractant ratio (Reeuwijk, 2002), CEC by ammonium acetate pH 7 (Sumner and Miller, 1996), organic C by potassium dichromate (Walkley and Black, 1934), available Zn by DTPA extraction (Lindsay and Norvell, 1978) and available P by sodium bicarbonate for soil pH >7 (Olsen et al., 1954) or HCl and NH_4F for soil pH < 7 (Bray et. al., 1945). Wet soil samples were randomly collected again from each site at 14 DAT to estimate the available Zn during the experiment using a modified DTPA method (Beebout et al., 2009).

2.4 Plant analyses

Grain yield sampling was done according to the protocol described by Cassman et al. (1994). Plant samples from a 5-m^2 central area of each plot (125 hills) were taken for yield measurement. The total number of hills was recorded for each plot. The harvested plants were put in net bags, threshed and sun-dried in a glasshouse. After drying, grain was passed through a blower three times to remove any unfilled and partially filled spikelets. The cleaned grains were transferred to double-ply paper bags for weighing. Grain moisture content was measured using a grain moisture meter. Grain yield was calculated and adjusted to 14% and 3% moisture content. Thousand grain weight and actual harvest area were calculated as described by Cassman et al. (1994).

Prior to determination of Zn concentration, grain samples were dehulled to obtain brown rice. The brown rice samples were analysed at IRRI's Analytical Services Laboratory for Zn concentration by digestion in 1% nitric acid (HNO_3) and 2.8% perchloric acid ($HClO_4$) and analysis by ICP-OES (Optima 5300DV, Perkin Elmer, USA).

2.5 Statistical analyses

Grain yields and Zn concentrations were tested for normality using the Shapiro–Wilk normality test. Non-normally distributed data were transformed, but the original non-transformed values are presented in Results. A two-way analysis of variance (ANOVA) was carried out using the Statistix 8.0 software package (ref). Treatment mean differences were calculated using the Least Significant Difference at 5% level of significance. A Pearson correlation analysis was also performed between grain yield and grain Zn concentration.

3. Results

3.1 Grain yield performance of genotypes in contrasting soils and seasons

The 2012 WS results showed significant genotypic differences ($P < 0.0001$) in grain yield (Table 2). The grain yield ranged from 1.0 ± 0.2 to 3.7 ± 0.2 t ha^{-1} for Bay; 0.9 ± 0.1 to 4.6 t ha^{-1} for Bohol; 0.8 ± 0.2 to 3.7 ± 0.2 t ha^{-1} for Bukidnon and 1.7 ± 0.3 to 6.4 ± 0.3 t ha^{-1} for IRRI (Table S2). The highest grain yield was observed for IR55179 in Bay, Bohol and IRRI, and for IR68144 in Bukidnon (Table S2). In the 2013 DS, results still did not show significant effects of Zn fertilization on grain yield from two experimental sites; but did show significant differences of grain Zn at Bay. There were significant genotype effects on both grain yield and grain Zn in Bay. The highest grain yield was observed for genotype IR55179 in Bay (Table S3). In the WS of 2014, the significant effects of Zn fertilization ($P < 0.0001$) on grain yield were observed from two sites: Bohol and IRRI but not from Bay. On the other hand, the effects of genotypes on grain yield were significant ($P < 0.0001$) in all three sites: Bay, Bohol and IRRI (Table S4). The highest grain yield was observed in IR55179 for Bay, NSIC22, IR69144, IR55179 and BRRIdhan28 for Bohol and NSIC22, A69-1 for IRRI. In DS of 2015, results of experiments conducted at IRRI showed significant effects of Zn fertilization and genotype with BR7840 having the highest yield at 5.3 t ha^{-1} (Table S5) but significant interactions of these treatments were not observed (Table 2). Also, our results from 2012 to 2015 from all sites did not show significant interactions of treatments used such as Zn fertilization and genotypes (Table 2).

In general, Zn-efficient and check genotypes had greater grain yield than Zn-biofortified genotypes. Grain yield results in WS 2012 showed that Zn-efficient genotypes performed better than check and Zn-biofortified genotypes in most sites (Fig. 1). Further trials conducted during DS 2013 Bay soils and WS 2014 Bay and IRRI soils, showed a comparable yield between Check and Zn-efficient genotypes (i.e. > Philippine yield threshold) but not with the Zn-biofortified genotypes which were consistently lower ($P < 0.05$) than Zn efficient genotypes (Fig. 2).

3.2 Grain Zn concentration of genotypes in contrasting soils and seasons

Generally, the highest grain Zn concentrations were achieved by Zn-biofortified genotypes. In WS 2012, Zn-biofortified genotypes consistently had the highest grain Zn concentrations though differences were only significant ($P < 0.05$) at IRRI and Bohol (Fig. 1). Similar results were also observed for WS 2014 IRRI and Bohol soils (Fig. 2). Specific results for grain Zn concentration also revealed significant influence by genotypes ($P < 0.0001$) but not by Zn fertilization during WS of 2012 for Bay, Bohol, Bukidnon and IRRI (Table 2). Grain Zn concentration ranged from 15.8 ± 0.7 to 21.8 ± 0.6 mg kg^{-1} for Bay; 18.1 ± 1.0 to 34.6 ± 1.3

mg kg^{-1} for Bohol; 23.2 ± 0.3 to 35.4 ± 0.8 mg kg^{-1} for Bukidnon; and 21.0 ± 0.8 to 35.1 ± 0.6 mg kg^{-1} for IRRI (Table S2). The highest grain Zn concentration observed was for IR68144 in Bay, and IR91143AC and IR68144 in Bohol, Bukidnon, and IRRI (Table S2). In the DS of 2013, grain Zn concentration differed ($P < 0.01$) between genotypes in Bay while the Zn fertilizer treatment did not show significant effects (Table 2). The highest grain Zn concentration was observed for genotypes IR69144 and IR8742 in Bay (Table S3). In the 2014 WS, grain Zn concentration differed ($P < 0.05$) both between genotypes and Zn treatments in Bohol and IRRI, while in Bay only Zn fertilization significantly influenced ($P < 0.05$) the grain Zn concentration (Table 2). In the DS 2015, grain Zn concentration differed ($P < 0.0001$) with both Zn treatment and genotypes significantly at IRRI. There were no significant interactions between genotypes and Zn treatments for grain Zn concentration (Table 2).

3.3 Relationship between grain yield and grain Zn concentration

In all seasons and sites there was generally an inversely relation between grain yield and grain Zn concentration. In the WS of 2012, grain yield was inversely related to grain Zn concentrations in Bohol ($R^2 = 0.48$, $P < 0.0001$) and IRRI ($R^2 = 0.26$, $P < 0.0001$). Grain Zn concentrations at Bay were all below the 30 mg Zn kg^{-1} threshold regardless of genotype, while the other sites showed some values that were above the threshold (Fig. 3). In DS 2013, similar pattern were observed at Bay (Fig. 4). Unlike in WS 2012, there were some data points that were above the grain Zn concentration threshold with yield above 3 t ha^{-1} (Fig. 4). Further, results in WS 2014 and DS 2015 at IRRI sites still showed negative correlations between grain yield and grain Zn concentration, with Zn treatment showing significant ($P < 0.0001$) effects on grain Zn concentrations in both seasons (Fig. 5; Table 2). For example, in the 2015 DS both no Zn and plus Zn treated rice plants had grain Zn concentrations above the 30 mg Zn kg^{-1} threshold, while in the 2014 wet season, the no Zn treatment had mostly grain Zn concentrations below the threshold and the plus Zn treated plants were all above the grain Zn biofortification target (Fig. 5).

4. Discussion

The grain yield results for the Zn-efficient, -inefficient and -biofortified genotypes were inconsistent between study sites and Zn treatments. The Zn-efficient genotypes did not always have greater grain yield. Various factors may contribute to this. First, Zn fertilizer quickly becomes unavailable to plants in flooded soils by forming insoluble complexes (Izquierdo et al., 2016), so the effectiveness of fertilizer applications in overcoming Zn deficiency varies greatly between soils. Second, the time to crop maturity increases under Zn deficiency

(Fairhurst et al., 2007), and this extra time may allow unfertilized plants to catch up with fertilized ones in terms of grain Zn concentration. However, in this study, harvest times in given genotypes did not vary with Zn fertilization (data not shown). Third, genotypic variation in nutrient use efficiency and yield performance are generally more important than Zn fertilizer management (Wissuwa et al., 2008; Impa et al., 2013; Nanda and Wissuwa, 2016). Our results during wet season 2012 (from four sites: Bay, Bohol, Bukidnon and IRRI), dry season 2013 (Bay), and wet season (Bay) support the latter explanation, where genotype differences explained variation in grain yield better than Zn fertilization ($P < 0.0001$ versus $P > 0.05$).

The results also revealed genotype differences in grain Zn concentrations were sensitive to soil conditions. In the wet season of 2012, none of the studied genotypes achieved the grain Zn biofortification target at the Bay site, which site is severely Zn deficient. However at Bohol, Bukidnon and IRRI, some genotypes (the biofortified genotypes IR68144 and IR91143AC) consistently exceeded the biofortification target. Surprisingly, Zn-inefficient K. Patong also achieved the biofortification target at Bohol and Bukidnon, while none of the Zn-efficient genotypes did so at any of the sites, except IR87842 at Bukidnon. The cases of K. Patong (high grain Zn concentration and low grain yield) and IR55179 (high grain yield and low grain Zn concentration) could be attributed to dilution effects, as shown by high grain total Zn uptake but low grain Zn concentration at IRRI, Bay and Bohol. Similar results have been obtained in other studies (Slafer et al., 1990; Ortiz-Monasterio et al., 1997; MacDonald et al., 2008). In the 2013 dry season and 2012 wet season at Bay, grain Zn concentrations were all below the threshold, except for the Zn-biofortified genotype IR68144. These results suggest that despite the effectiveness of some Zn-biofortified genotypes, the effects of soil type remained a limiting factor. The effect of soil Zn status was also apparent at IRRI in the 2014 wet season and 2015 dry season.

Although the Zn-biofortified genotypes achieved the highest grain Zn concentrations (P <0.0001), grain Zn concentration was strongly influenced by soil type and the degree of Zn deficiency. During the 2015 dry season at IRRI, although Zn fertilization had significant (P <0.0001) effects on grain Zn concentration, the levels of grain Zn without Zn fertilizer were still above the biofortification target. Conversely, during the 2014 wet season at IRRI, grain Zn concentration without Zn fertilizer were mostly below the biofortification target. These differences between wet and dry seasons indicate that Zn fertilization may not be needed during the dry season. Higher availability of soil Zn can be expected in the dry season the soil may be more oxidized and therefore soil Zn more soluble (Johnson-Beebout et al., 2016). Hence Zn fertilization management needs to be optimized based on cropping seasons.

The results revealed a significant inverse relationship between grain yield and grain Zn concentrations, irrespective of genotypic effects, which supports our hypothesis (2) that there is a trade-off between increased grain yield and increased grain Zn concentration in Zn-biofortified genotypes. Previous studies suggest that this relationship is due to yield dilution, whereby more grains are produced in distal spikelets and florets, which are known to have lower micronutrient concentrations (Slafer et al., 1990; Ortiz-Monasterio et al., 1997). Our results show that improving grain Zn concentration and at the same time achieving grain yield targets can be challenging. The Philippine yield target of 4 t ha^{-1} and the biofortification grain Zn target was only achieved by Zn-biofortified genotypes at IRRI. Although some Zn-efficient genotypes and Zn-biofortified genotypes were able to achieve the grain yield target in the dry season of 2013 at Bay, none achieved the 30 mg Zn kg^{-1} grain Zn biofortification target, suggesting that grain Zn biofortification performance of these genotypes is limited by soil conditions, consistent with our hypothesis (1). This agrees with previous studies (White and Zasoski, 1999; Cakmak, 2008; Graham et al., 2001; Tiong et al., 2015).

In the experiment on Zn fertilizer management at IRRI, we found significant grain yield differences between no Zn and the Zn fertilized treatments, but no significant difference among the Zn fertilized treatments. This could be because the Zn deficiency in the unfertilized soil was only moderate. However, we found higher grain Zn concentrations with foliar Zn fertilization compared with broadcast or basal fertilization. This supports our hypothesis (3), that the effects of Zn fertilization on grain yield and Zn concentration are different. Foliar Zn fertilization is evidently a superior means of improving grain Zn concentration. This may be partly due to the timing and frequency of applications. We made the foliar application during mid-tillering, when effects of Zn deficiency are most severe, and flowering, when Zn is needed for grain Zn loading (Boonchuay et al. 2013; Mabesa et al, 2013). This finding needs be evaluated in contrasting soils with varying degrees of Zn deficiency.

5. Conclusions

Our results confirmed a general superiority of ‘Zn-biofortified’ and ‘Zn-efficient’ genotypes over ‘Zn-inefficient’ genotypes in terms of grain yield and grain Zn concentration. However, these genotypes generally did not achieve both yield and biofortification targets in Zn-deficient soils, where high grain Zn concentrations tended to be at the cost of grain yield and vice versa. The advantage of Zn-biofortified and Zn-efficient genotypes was more apparent at sites with adequate levels of soil Zn. Further, there were differences between seasons, with Zn fertilization being less necessary during the dry season, probably due to better soil Zn availability under more-oxidized soil conditions. Overall, the findings demonstrate the strong influence of soil conditions on grain yield and grain Zn concentration of Zn-biofortified and Zn-efficient genotypes. Their superiority over Zn-inefficient genotypes was evident in soils with adequate soil Zn, where the Zn-biofortified genotypes performed better than Zn-deficiency tolerant genotypes in both yield and grain Zn concentration.

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Table 1 Properties of the soils at the four field sites in the Philippines.

	IRRI	Bay	Bohol	Bukidnon
Site grid reference	14° 8'46.93"N, 121°15'48.37"E	14°10'36.87"N, 121°17'24.99"E	9º55'0"N, 124º6'0"E	7°51'27.78"N, 125° 3'29.37"E
Classification (USDA, 1999)	Haplaquoll	Tropaquept	Aquic Argiudolls	Andisol
Clay (%)	36.0 ± 3.10	35.0 ± 0.63	23.0 ± 0.16	48.0 ± 0.50
Silt (%)	39.0 ± 1.20	47.0 ± 0.75	41.0 ± 0.49	29.0 ± 2.70
pH (KCl)	4.62 ± 0.10	6.30 ± 0.20	7.20 ± 0.02	4.80 ± 0.01
CEC (cmol$_c$ kg^{-1})	30.6 ± 0.70	42.8 ± 0.30	23.6 ± 0.33	13.5 ± 0.07
Organic carbon (%)	1.65 ± 0.10	4.65 ± 0.03	4.08 ± 0.21	1.98 ± 0.01
DTPA Zn (mg kg^{-1})	1.84 ± 0.13	0.32 ± 0.03	1.13 ± 0.10	0.43 ± 0.03
Available P (mg kg^{-1})	10.4 ± 1.71 (Bray)	25.0 ± 0.45 (Olsen)	9.80 ± 0.12 (Olsen)	9.65 ± 0.14 (Bray)

Table 2 Analysis of variance for grain yield and grain Zn concentration of rice plants as influenced by genotypes and Zn fertilization grown in contrasting soils and different seasons from 2012 to 2015.

Season and site	Treatment	df	Grain yield *P values*	Grain Zn concentration *P values*
WS 2012				
Bohol	Genotype	11	0.0001***	0.0001***
	ZF	1	0.2321ns	0.6036ns
	Genotype*ZF	11	0.5622ns	0.3196ns
Bay	Genotype	7	0.0001***	0.01690*
	ZF	1	0.3940ns	0.8466ns
	Genotype*ZF	7	0.5528ns	0.9984ns
Bukidnon	Genotype	11	0.0001***	0.0001***
	ZF	1	0.1695ns	0.2053ns
	Genotype*ZF	11	0.8137ns	0.8137ns
IRRI	Genotype	11	0.0001***	0.0001***
	ZF	1	0.2208ns	0.6053ns
	Genotype*ZF	11	0.5970ns	0.9904ns
DS 2013				
Bay	Genotype	8	0.0001***	0.0019***
	ZF	1	0.9089ns	0.0718ns
	Genotype*ZF	8	0.6970ns	0.8275ns
WS 2014				
Bay	Genotype	9	0.0001***	0.9354ns
	ZF	1	0.8621ns	0.0111*
	Genotype*ZF	9	0.1550ns	0.9058ns
Bohol	Genotype	9	0.0136*	0.0001***
	ZF	1	0.0008***	0.0001***
	Genotype*ZF	9	0.8206ns	0.0562ns
IRRI	Genotype	9	0.0001***	0.0001***
	ZF	1	0.0001***	0.0273*
	Genotype*ZF	9	0.2448ns	0.0915ns
DS 2015				
IRRI	Genotype	3	0.0001***	0.0001***
	ZF	3	0.0162*	0.0082*
	Genotype*ZF	9	0.7522ns	0.3126ns

ns, non-significant at 5% level.
* Significant at 5% level.
** Significant at 1% level.
***Significant at 0.1% level
ZF = Zn fertilization; *WS*= wet season; DS= dry season.

Table 3 Analysis of variance for the grain Zn uptake of rice genotypes in contrasting soils during WS 2012.

Site	Treatments	df	Grain Zn uptake	Rachis Zn uptake	Stem Zn uptake	Leaf Zn uptake
WS 2012						
Bohol	Genotype	11	0.0001***	ND	ND	ND
	ZF	1	0.4086ns			
	Genotype*ZF	11	0.3464ns			
Bay	Genotype	7	0.0001***	0.0000***	0.0379*	0.0117*
	ZF	1	0.8706ns	0.3115ns	0.5133ns	0.4118ns
	Genotype*ZF	7	0.7268ns	0.0093**	0.3353ns	0.7547ns
Bukidnon	Genotype	11	0.0001***	ND	ND	ND
	ZF	1	0.1391ns			
	Genotype*ZF	11	0.4336ns			
IRRI	Genotype	11	0.0001***	ND	ND	ND
	ZF	1	0.4560ns			
	Genotype*ZF	11	0.7288ns			

ND= no data
ns, non-significant at 5% level.
* Significant at 5% level.
** Significant at 1% level.
***Significant at 0.1% level

Table S1 Rice genotypes contrasting in tolerance of soil Zn deficiency (‘efficient’ or ‘inefficient’) and in grain Zn concentration under Zn-sufficiency (‘biofortified’) grown at different sites.

Genotype	Referred in text as	Color code in Figure 5	Years/seasons			
			WS 2012	DS 2013	WS 2014	DS 2015
Efficient						
A69-1	A69-1	Blue	✓	✓	✓	
IR55179	IR55179	Blue	✓	✓	✓	✓
RIL46	RIL46	Blue	✓			
IR87839-4-1-1-1-2-BAY B	IR87839	Blue	✓			
IR87842-5-1-3-1-B	IR87842	Blue	✓	✓		
Inefficient						
Kinandang Patong	KPatong	Red	✓	✓	✓	
IR26	IR26	Red	✓			
IR74	IR74	Red	✓			
Biofortified						
IR69428-6-1-1-3-3	IR69428	Green	✓	✓	✓	
IR68144-2B-2-2-3-1-166	IR68144	Green	✓	✓	✓	
IR85800-41-3-2-1-2	IR85800	Green	✓	✓		
BR7840-54-3-1	BR7840	Green			✓	✓
Checks						
IR64	IR64	Black	✓			✓
NSIC222	NSIC222	Black		✓	✓	

Table S2 Mean and standard error of grain yield and grain Zn concentration of rice plants as influenced by genotypes grown in contrasting soils during the wet season of 2012.

Genotype	Grain yield ($t\ ha^{-1}$)	Grain Zn concentration ($mg\ kg^{-1}$)	Genotype	Grain yield ($t\ ha^{-1}$)	Grain Zn concentration ($mg\ kg^{-1}$)
	Bay (*n*=8)			Bukidnon (*n* =8)	
A69-1	3.45±0.24a	15.8±0.66c	A69-1	2.42±0.19cde	23.2±0.25f
IR87839	1.62±0.6cd	16.8±1.18bc	IR87839	1.96±0.16ef	27.1±0.69de
IR87842	2.50±0.40b	16.7±1.03bc	IR87842	3.37±0.10ab	31.6±1.16b
IR26	3.45±0.23a	19.1±1.32ab	IR26	2.32±0.16de	26.4±0.90e
IR55179	3.68±0.19a	19.1±1.07ab	IR55179	2.73±0.74cd	23.7±0.25f
IR64	3.47±0.21a	19.1±0.91ab	IR64	2.10±0.29ef	27.0±0.56de
IR68144	1.03±0.20d	21.8±0.56a	IR68144	3.67±0.19a	33.8±0.69a
IR91143AC	2.19±0.22bc	17.5±0.56bc	IR69428	2.06±0.22ef	27.5±0.32de
			IR74	2.95±0.21bc	28.8±0.61cd
			IR85800	1.59±0.12fg	30.6±1.22bc
			IR91143AC	1.33±0.15gh	35.4±0.80a
			K Patong	0.83±0.13h	31.0±0.80b
	Bohol (*n*=8)			IRRI (*n* =8)	
A69-1	3.20±0.51bcd	21.0±0.70de	A69-1	6.10±0.26a	24.0±0.89def
IR87839	3.37±0.30bcd	18.1±0.97e	IR87839	5.87±0.22a	21.0±0.76g
IR87842	2.70±0.34cde	21.5±0.66cd	IR87842	5.80±0.19a	26.0±0.82cd
IR26	3.67±0.17b	21.6±0.53cd	IR26	6.28±0.23a	22.5±0.53fg
IR55179	4.59±0.27a	22.5±0.90cd	IR55179	6.38±0.25a	25.7±0.31cd
IR64	2.88±0.35bcd	23.8±0.95cd	IR64	4.99±0.13b	25.6±0.96cde
IR68144	1.94±0.35ef	33.7±2.46ab	IR68144	2.93±0.18d	35.1±0.61a
IR69428	2.57±0.29de	24.5±0.62c	IR69428	4.93±0.18bc	31.1±0.58b
IR74	3.34±0.24bcd	22.8±0.69cd	IR74	5.10±0.20b	26.5±0.73efg
IR85800	3.52±0.26bc	22.5±0.37cd	IR85800	4.64±0.24bc	26.5±0.73c
IR91143AC	1.12±0.13f	34.6±1.33a	IR91143AC	4.34±0.26c	34.0±1.30a
K Patong	1.44±0.22f	31.0±1.66b	K Patong	4.58±0.19bc	27.5±1.21c

Means in columns (per site) followed by the same letter are not significantly different from one another at $P < 0.05$.

Table S3 Mean and standard error of grain yield and grain Zn concentration of rice plants as influenced by genotypes and Zn fertilization grown in Bay during the dry season of 2013.

Dry season 2013	Genotype	Grain yield (t ha^{-1})	Grain Zn concentration (mg kg^{-1})
Bay (*n*=8)			
	A69-1	5.75±0.25ab	19.8±1.32c
	IR55179	6.30±0.20a	20.2±0.79c
	IR68144	4.62±0.12c	28.8±1.42a
	IR69428	5.35±0.17b	23.0±1.03bc
	IR85800	3.89±0.24d	25.0±1.48b
	IR87842	3.85±0.18d	28.6±1.54a
	IR91143AC	3.73±0.20d	24.1±2.01b
	K Patong	3.81±0.24d	22.5±1.51bc
	NSIC222	5.94±0.26ab	15.7±0.57d
	Zn fertilization[1]		
	Z0	4.80±0.22a	21.8±0.87a
	Z1	4.81±0.19a	24.3±1.02a

Means in columns (treatment) followed by the same letter are not significantly different from one another at $P < 0.05$.

[1] Zn fertilizer (zinc sulfate heptahydrate) applied broadcast to the soil with Zn foliar application during flowering stage at a split rate of 5 kg ha^{-1}.

Table S4 Mean and standard error of grain yield and grain Zn concentration of rice plants as influenced by genotypes and Zn fertilization grown in contrasting soils: Bay, Bohol and IRRI during the wet season of 2014.

Wet Season 2014	Genotype	Grain Yield (t/ha)	Grain Zn concentration (mg kg^{-1})
Bay (*n*=8)			
	A69-1	5.12±0.33b	31.0±3.94a
	BR7840	4.30±0.22cd	30.3±6.88a
	BRRIdhan28	4.82±0.29bc	28.6±5.95a
	IR55179	6.15±0.29a	29.0±6.29a
	IR68144	5.09±0.20b	30.3±4.50a
	IR69428	4.05±0.19d	30.0±6.94a
	IR91143AC	3.66±0.24d	22.5±1.94a
	K Patong	4.01±0.22d	24.9±1.65a
	NSIC222	5.32±0.33b	33.6±4.21a
	Zn fertilization[1]		
	Z0	4.70±0.19a	24.4±1.59b
	Z1	4.72±0.15a	33.3±2.40a
Bohol (*n*=8)	Genotype		
	A69-1	2.46±0.25ab	25.4±1.88cd
	BR7840	2.04±0.25bc	31.7±2.94a
	BRRIdhan28	2.39±0.25ab	24.8±2.41cd
	IR55179	2.41±0.25ab	25.5±2.22cd
	IR68144	2.57±0.27ab	31.7±1.85a
	IR69428	2.23±0.20b	25.3±1.05cd
	IR91143AC	1.45±0.45c	28.8±2.43ab
	K Patong	1.95±0.26bc	26.8±2.43bc
	NSIC222	3.04±0.284a	23.0±1.03d
	Zn fertilization		
	Z0	1.95±0.15b	22.7±0.53b
	Z1	2.58±0.11a	30.5±0.83a
IRRI (*n*=8)	Genotype		
	A69-1	4.72±0.17ab	30.3±3.74de
	BR7840	3.18±0.09f	39.8±4.74a
	BRRIdhan28	3.90±0.26cde	28.6±4.04e
	IR55179	4.35±0.04bc	31.5±3.79cde
	IR68144	4.16±0.18cd	33.5±3.08bcd
	IR69428	3.62±0.16ef	35.5±3.08b
	IR91143AC	3.19±0.28f	35.6±4.06bc
	K Patong	3.72±0.22de	35.3±2.93bc
	NSIC222	4.92±0.16a	34.3±3.57bc
	Zn fertilization		
	Z0	4.10±0.15a	24.7±0.72b
	Z1	3.84±0.13b	41.3±0.96a

Means in columns (per site) followed by the same letter are not significantly different from one another at $P < 0.05$. [1] Zn fertilizer applied via root dipping of rice seedlings in 4% ZnO for 15 min before transplanting.

Table S5 Mean and standard error of grain yield and grain Zn concentration of rice plants as influenced by genotypes and Zn fertilization grown in IRRI soils during the dry season of 2015.

Dry season 2015	Genotype	Grain yield (t/ha)	Grain Zn concentration (mg kg^{-1})
IRRI	Genotype		
	BR7840	5.32±0.12a	42.3±0.63b
	IR55179	3.29±0.16d	38.0±2.54bc
	IR64	3.95±0.09c	33.0±2.96c
	IR91143AC	4.51±0.12b	50.0±2.20a
	Zn fertilization		
	Z0	3.95±0.23b	36.0±1.80c
	Z1	4.55±0.26a	38.7±2.32bc
	Z2	4.45±0.26a	43.0±2.76ab
	Z3	4.25±0.23a	46.0±3.54a

Means in columns (per treatment) followed by the same letter are not significantly different from one another at $P < 0.05$.

Z0= No Zn added

Z1= Soil basal + Soil Soil 50% flowering

Z2= Soil basal + foliar 50% flowering

Z3= Foliar mid-tillering (30DAT) + Foliar 50% flowering

Figure legends

Fig. 1. Grain Zn concentration and yield of Zn-efficient, Zn-biofortified and check genotypes at the four field site in WS 2012. Data are means ± standard error; common letters in a panel indicate means not significantly different at $P < 0.05$. The indicated biofortification target grain Zn concentration is set by HarvestPlus (2014); the indicated grain yield target is set by the Philippines Dept of Agriculture (Department of Agriculture, 2012).

Fig. 2. Grain Zn concentration and yield of Zn-efficient, Zn-biofortified and check genotypes at the indicated sites in DS 2013 and WS 2014. Data are means ± standard error; common letters in a panel indicate means not significantly different at $P < 0.05$.

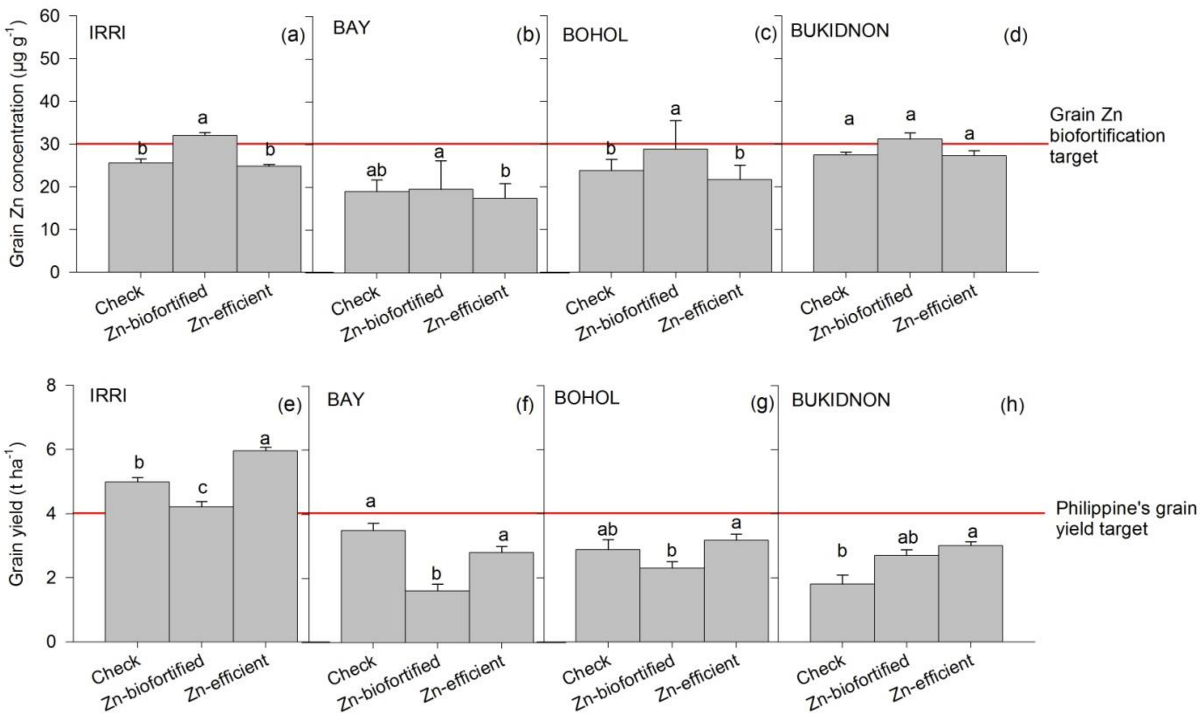
Fig. 3. The relationship between grain yield and grain Zn concentration at the four sites in WS 2012.

Fig. 4. The relationship between grain yield and grain Zn concentration at Bay in DS 2013.

Fig. 5. The relationship between grain yield and grain Zn concentration at IRRI in (a) WS 2014 and (b) DS 2015. In (a): A = A69-1, B = BR7840 , C = IR55179, D = IR64, E = IR68144, F = IR69428, G = IR91143AC, H = KPatong and I = NSIC222. In (b): A = BR7840, B = IR55179, C = IR64, D = IR91142AC, and numbers 1-3 indicate Zn fertilization regime (Section 2.2.3). Data points are means of three (3).

Fig. 6. Grain Zn uptake of Zn-biofortfied (ZnB), Zn-efficient (ZnT), and Zn-inefficient (ZnS) in Bay, Bohol, Bukidnon and IRRI soils in WS 2012. Data are means ± standard error; common letters in a panel indicate means not significantly different at $P < 0.05$.

Fig. 1



Grain Zn concentration (µg g-1)
IRRI
(a)
BAY
(b)
BOHOL
(c)
BUKIDNON
(d)
Grain Zn biofortification target
Check
Zn-biofortified
Zn-efficient
Grain yield (t ha-1)
IRRI
(e)
BAY
(f)
BOHOL
(g)
BUKIDNON
(h)
Philippine's grain yield target

Fig. 2

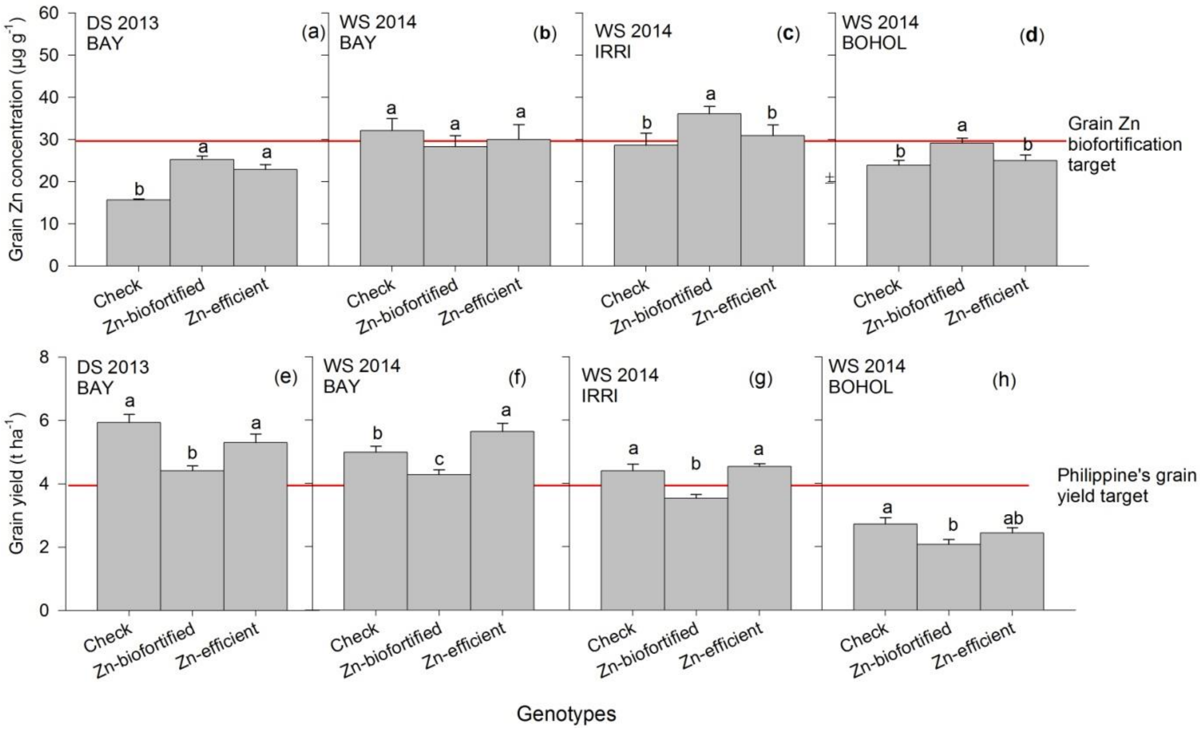
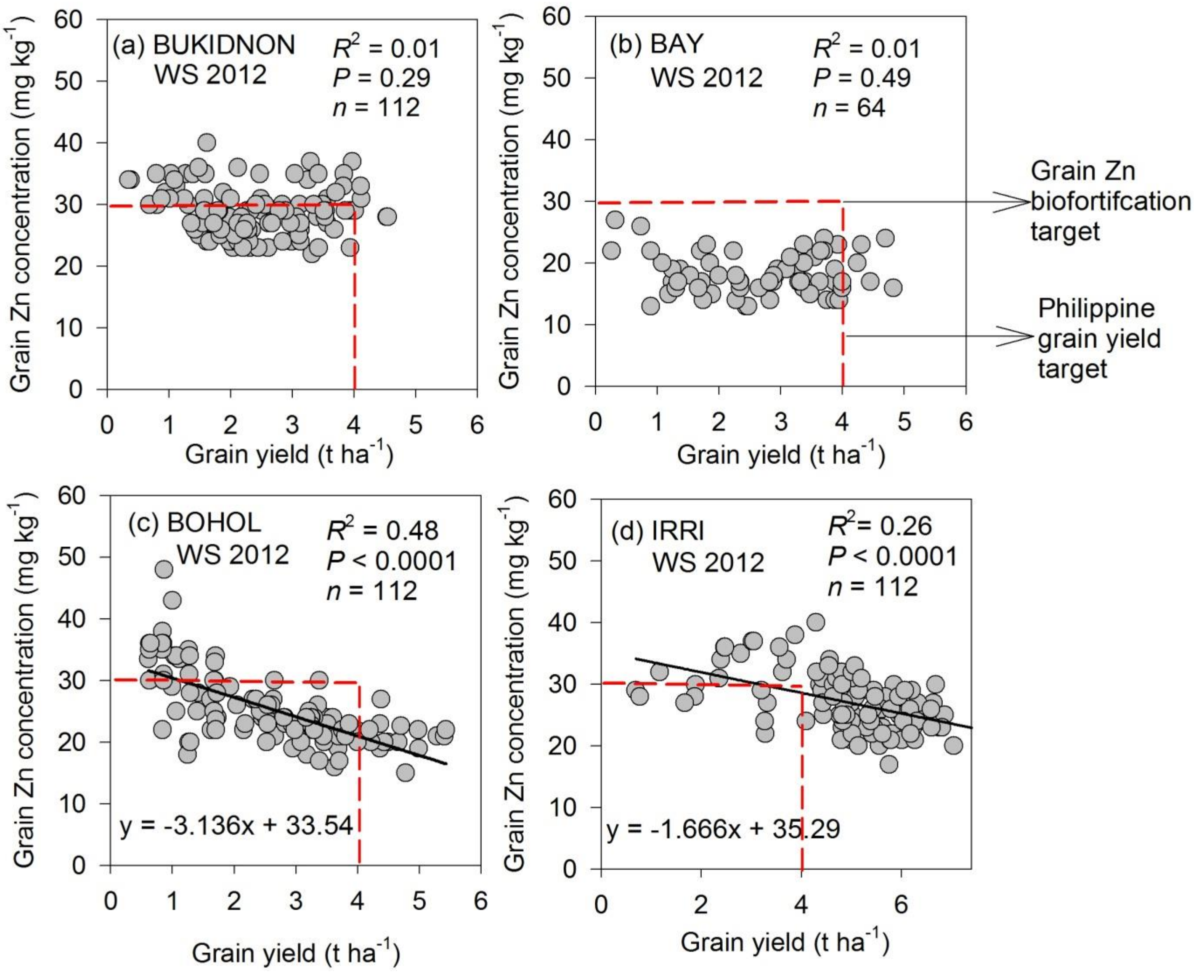
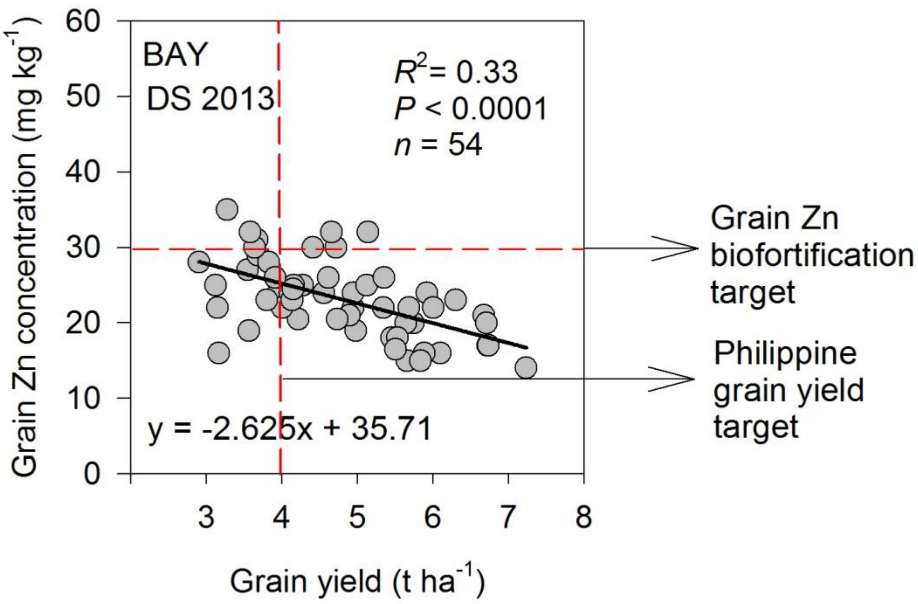

Grain Zn concentration (μg g-1)
DS 2013
BAY
(a)
WS 2014
BAY
(b)
WS 2014
IRRI
(c)
WS 2014
BOHOL
(d)
Grain Zn
biofortification
target
Check
Zn-biofortified
Zn-efficient
Grain yield (t ha-1)
DS 2013
BAY
(e)
WS 2014
BAY
(f)
WS 2014
IRRI
(g)
WS 2014
BOHOL
(h)
Philippine's grain
yield target
Genotypes

Fig. 3



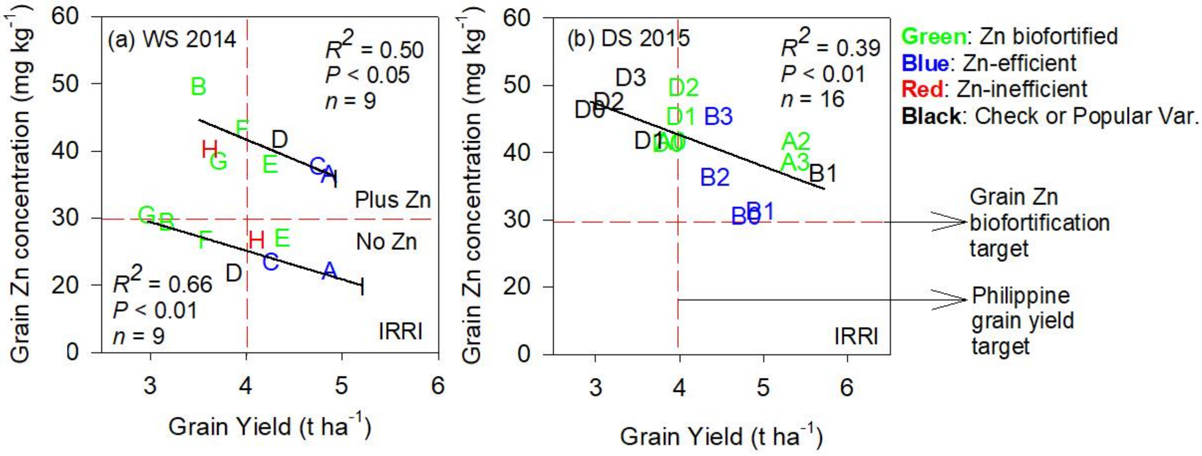
(a) BUKIDNON
WS 2012
R2 = 0.01
P = 0.29
n = 112
(b) BAY
WS 2012
R2 = 0.01
P = 0.49
n = 64
Grain Zn
biofortifcation
target
Philippine
grain yield
target
(c) BOHOL
WS 2012
R2 = 0.48
P < 0.0001
n = 112
y = -3.136x + 33.54
(d) IRRI
WS 2012
R2= 0.26
P < 0.0001
n = 112
y = -1.666x + 35.29
Grain Zn concentration (mg kg-1)
Grain yield (t ha-1)

Fig. 4



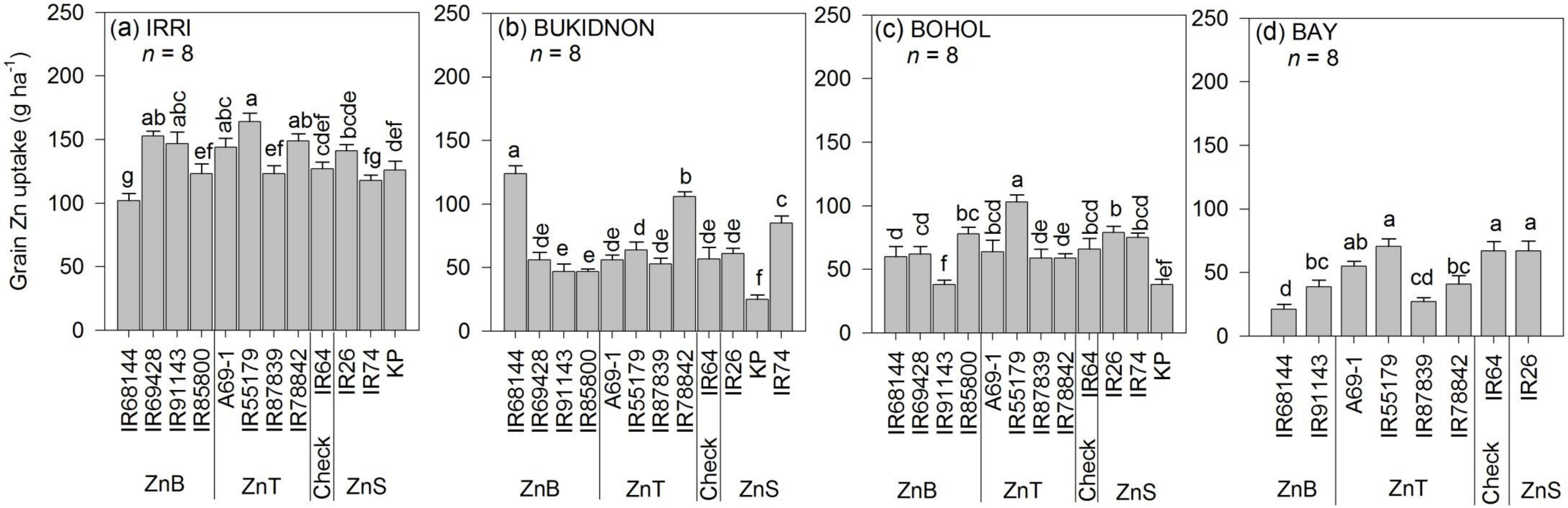
BAY
DS 2013
R^2= 0.33
P < 0.0001
n = 54
y = -2.625x + 35.71
Grain Zn biofortification target
Philippine grain yield target
Grain Zn concentration (mg kg^{-1})
Grain yield (t ha^{-1})
0
10
20
30
40
50
60
3
4
5
6
7
8

Fig. 5



(a) WS 2014
R2 = 0.50
P < 0.05
n = 9
Plus Zn
No Zn
R2 = 0.66
P < 0.01
n = 9
IRRI
(b) DS 2015
R2 = 0.39
P < 0.01
n = 16
IRRI
Grain Zn concentration (mg kg-1)
Grain Yield (t ha-1)
Green: Zn biofortified
Blue: Zn-efficient
Red: Zn-inefficient
Black: Check or Popular Var.
Grain Zn biofortification target
Philippine grain yield target

Fig. 6



(a) IRRI
n = 8
(b) BUKIDNON
n = 8
(c) BOHOL
n = 8
(d) BAY
n = 8
Grain Zn uptake (g ha-1)
250
200
150
100
50
0
IR68144
IR69428
IR91143
IR85800
A69-1
IR55179
IR87839
IR78842
IR64
IR26
IR74
KP
ZnB
ZnT
Check
ZnS