Field Crops Research, Volume 234, 15 March 2019, Pages 26-32 DOI:10.1016/j.fcr.2019.01.011

# 1 Grain Zn concentrations and yield of Zn-biofortified versus deficiency-tolerant rice

- 2 genotypes under contrasting growth conditions
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# 10 Abstract

Higher grain Zn concentration in 'biofortified' rice genotypes, bred for high grain Zn 11 concentration, should not be at the expense of reduced grain yield. This study examined the 12 grain yield and grain Zn concentration of Zn-biofortified genotypes in field experiments in the 13 14 Philippines. Zinc-biofortified genotypes (high grain Zn concentration in Zn-sufficient soil) were compared with efficient genotypes (tolerant of soil Zn deficiency), inefficient genotypes 15 16 (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 17 cropping seasons (wet season 2012, 2014 and 2015 and dry season 2013 and 2015). A common 18 experimental design and plot size were used with treatments (genotypes and Zn fertilization) 19 20 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<sup>-1</sup>) and grain Zn 21 biofortification target (30 mg kg<sup>-1</sup>) only when grown under Zn-sufficient conditions. In Zn-22 deficient soils, most Zn-biofortified and deficiency-tolerant genotypes reached the Zn 23 concentration target but not the yield target, suggesting the need to correct the soil Zn-24 deficiency to prevent yield penalty. Further, results from IRRI showed that only Zn-fertilized 25 26 plants were able to achieve the Zn biofortification target during the wet season; whereas during 27 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. 28 This suggests that Zn fertilization may not be needed during the dry season in soils with 29 sufficient, potentially plant-available Zn. 30

31 Keywords: Grain Zn biofortification, Zn fertilization, biofortified rice genotypes, Zn uptake,

32 improved grain yield performance

# 33 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
- 38 Graphical abstract:
- 39 [We plan to put a simplified version of Fig. 5a here]



# 41 **1. Introduction**

Zinc (Zn) deficiency in human populations is a major global health problem 42 (UNICEF/WHO/WB, 2013). Initiatives to address this include 'biofortification' of food crops 43 with Zn, either by agronomic management or genetic improvement (Mayer et al., 2008; 44 Hirschi, 2009; White and Broadley, 2011). Rice is one of the key crops being targeted for this 45 (Bouis and Saltzman 2017). In general rice has a low content of micronutrients, particularly 46 47 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 48 49 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 50 51 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 52 Boro season rice in Bangladesh (Bangladesh Rice Research Institute, 2016). 53

There has also been progress with agronomic enrichment of rice grain Zn concentrations 54 by fertilizer and water management (Gao et al 2012; Johnson-Beebout et al 2016). Aerobic 55 water management consistently shows a moderate increase in grain Zn concentration in rice 56 compared with traditional continuously flooded (anaerobic) practice, but the effects of Zn 57 fertilizer addition to the soil have been inconsistent (Cakmak 2008; HarvestPlus 2014; 58 Tuyogon et al., 2016). A recent study suggested that Zn fertilization timing could be optimized 59 in combination with water management to exploit increased Zn solubility with soil oxidation 60 after moderate drying (Johnson-Beebout et al., 2016). Foliar Zn fertilization strategies have 61 shown some promise to increase grain Zn concentration (Cakmak 2008; Mabesa et al., 2013), 62 63 as has seedling dipping in Zn-containing slurry to overcome early-season Zn deficiency (Rehman et al., 2012). 64

Adoption of biofortification technologies by farmers will depend on grain yields in diverse 65 and sometimes adverse conditions, including moderate-to-severe Zn-deficiency (Bouis et al., 66 2013). While there has been significant progress in understanding the physiological and 67 environmental factors that determine Zn uptake, internal use efficiency and allocation to grain 68 (Stomph et al., 2014; Mori et al., 2016; Rose et al., 2016; Jaksomsak et al., 2017; Affholder et 69 al., 2017), there have been very few field studies addressing both grain yield and Zn 70 concentration under limiting conditions (Wissuwa et al., 2008; Nanda and Wissuwa 2016; 71 Beebout-Johnson et al 2016). The objectives of this study were: (1) to compare Zn-biofortified, 72 73 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.

#### 81 **2. Materials and methods**

Field experiments were made at four sites in the Philippines during the wet seasons (WS) 82 of 2012, 2014 and 2015 and dry seasons (DS) of 2013 and 2015. The four sites were a Zn-83 84 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). 85 Seventeen genotypes were compared: five 'efficient' genotypes tolerant of soil Zn deficiency 86 (based on growth), four 'inefficient' genotypes sensitive to soil Zn deficiency, five 87 88 'biofortified' genotypes bred for high grain Zn content under non-limiting conditions, and three widely-grown checks (Table S1). 89

### 90 2.1 Crop establishment

Land preparation included plowing and puddling followed by construction of levees to 91 92 separate the Zn treatment and replications. To prevent contamination from previous 93 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 94 ha-1 each of N, P and K was broadcast and thoroughly mixed with the soil using a power 95 weeder, followed by leveling at all sites. After breaking the seed dormancy at 50 °C for 3 days, 96 the seeds were sown in a wet seed bed with NPK fertilizer recommendation of 80 g m<sup>-2</sup> without 97 added Zn. The seedlings were transplanted at 21 days after sowing at all the field sites except 98 99 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 100 101 of 1 L ha<sup>-1</sup> 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 102 was provided one week after transplanting, to further minimize snail damage. Continuous 103 flooding was used throughout the experiment in all the sites, unless noted otherwise. Fertilizer 104 NPK rates at each site were 100 kg N ha<sup>-1</sup>, 20 kg P ha<sup>-1</sup> and 20 kg K ha<sup>-1</sup>. All of the P and K 105 was added to the soil at basal stage together with 20% of N as complete (NPK) fertilizer with 106

the remaining N broadcasted as urea in two split applications: 35% at 24 days aftertransplanting (DAT) and 45% at 42 DAT.

# 109 2.2 Experimental design and layout

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

# 112 *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<sup>2</sup> with six rows of 3-m length and 0.20-m
spacing between rows and hills, with one seedling per hill.

# 118 *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<sup>-1</sup>. The size of each plot was 12 m<sup>2</sup> with 20 rows of 3-m length and 0.20-m spacing between rows and hills, with one seedling per hill.

#### 126 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<sup>-1</sup>. The size of each plot was 12 m<sup>2</sup> with 20 rows of 3-m length, 0.20m 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.

#### 134 2.3 Soil analyses

135 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

- 137 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,

- 139 1996), organic C by potassium dichromate (Walkley and Black, 1934), available Zn by
- 140 DTPA extraction (Lindsay and Norvell, 1978) and available P by sodium bicarbonate for soil
- 141 pH > 7 (Olsen et al., 1954) or HCl and NH<sub>4</sub>F 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
- 143 during the experiment using a modified DTPA method (Beebout et al., 2009).
- 144

# 145 *2.4 Plant analyses*

Grain yield sampling was done according to the protocol described by Cassman et al. 146 (1994). Plant samples from a  $5 \text{-m}^2$  central area of each plot (125 hills) were taken for yield 147 measurement. The total number of hills was recorded for each plot. The harvested plants were 148 put in net bags, threshed and sun-dried in a glasshouse. After drying, grain was passed 149 through a blower three times to remove any unfilled and partially filled spikelets. The cleaned 150 grains were transferred to double-ply paper bags for weighing. Grain moisture content was 151 measured using a grain moisture meter. Grain yield was calculated and adjusted to 14% and 152 3% moisture content. Thousand grain weight and actual harvest area were calculated as 153 154 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<sub>3</sub>) and 2.8% perchloric acid (HClO<sub>4</sub>) and analysis by ICP-OES (Optima 5300DV, Perkin Elmer, USA).

159 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 nontransformed 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.

#### 166 **3. Results**

167 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 168 (Table 2). The grain yield ranged from  $1.0 \pm 0.2$  to  $3.7 \pm 0.2$  t ha<sup>-1</sup> for Bay;  $0.9 \pm 0.1$  to 4.6 t 169 ha<sup>-1</sup> for Bohol;  $0.8 \pm 0.2$  to  $3.7 \pm 0.2$  t ha<sup>-1</sup> for Bukidnon and  $1.7 \pm 0.3$  to  $6.4 \pm 0.3$  t ha<sup>-1</sup> for 170 IRRI (Table S2). The highest grain yield was observed for IR55179 in Bay, Bohol and IRRI, 171 and for IR68144 in Bukidnon (Table S2). In the 2013 DS, results still did not show significant 172 effects of Zn fertilization on grain yield from two experimental sites; but did show significant 173 differences of grain Zn at Bay. There were significant genotype effects on both grain yield and 174 grain Zn in Bay. The highest grain yield was observed for genotype IR55179 in Bay (Table 175 S3). In the WS of 2014, the significant effects of Zn fertilization (P < 0.0001) on grain yield 176 were observed from two sites: Bohol and IRRI but not from Bay. On the other hand, the effects 177 of genotypes on grain yield were significant (P < 0.0001) in all three sites: Bay, Bohol and 178 IRRI (Table S4). The highest grain yield was observed in IR55179 for Bay, NSIC22, IR69144, 179 IR55179 and BRRIdhan28 for Bohol and NSIC22, A69-1 for IRRI. In DS of 2015, results of 180 experiments conducted at IRRI showed significant effects of Zn fertilization and genotype with 181 BR7840 having the highest yield at 5.3 t ha<sup>-1</sup> (Table S5) but significant interactions of these 182 treatments were not observed (Table 2). Also, our results from 2012 to 2015 from all sites did 183 not show significant interactions of treatments used such as Zn fertilization and genotypes 184 185 (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 Znbiofortified genotypes which were consistently lower (P < 0.05) than Zn efficient genotypes (Fig. 2).

193 *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  $15.8 \pm 0.7$  to  $21.8 \pm 0.6$  mg kg<sup>-1</sup> for Bay;  $18.1 \pm 1.0$  to  $34.6 \pm 1.3$ 

mg kg<sup>-1</sup> for Bohol;  $23.2 \pm 0.3$  to  $35.4 \pm 0.8$  mg kg<sup>-1</sup> for Bukidnon; and  $21.0 \pm 0.8$  to  $35.1 \pm 0.6$ 201 mg kg<sup>-1</sup> for IRRI (Table S2). The highest grain Zn concentration observed was for IR68144 in 202 Bay, and IR91143AC and IR68144 in Bohol, Bukidnon, and IRRI (Table S2). In the DS of 203 2013, grain Zn concentration differed (P < 0.01) between genotypes in Bay while the Zn 204 fertilizer treatment did not show significant effects (Table 2). The highest grain Zn 205 concentration was observed for genotypes IR69144 and IR8742 in Bay (Table S3). In the 2014 206 WS, grain Zn concentration differed (P < 0.05) both between genotypes and Zn treatments in 207 Bohol and IRRI, while in Bay only Zn fertilization significantly influenced (P < 0.05) the grain 208 Zn concentration (Table 2). In the DS 2015, grain Zn concentration differed (P < 0.0001) with 209 both Zn treatment and genotypes significantly at IRRI. There were no significant interactions 210 between genotypes and Zn treatments for grain Zn concentration (Table 2). 211

## 212 *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 213 grain Zn concentration. In the WS of 2012, grain yield was inversely related to grain Zn 214 concentrations in Bohol ( $R^2 = 0.48$ , P < 0.0001) and IRRI ( $R^2 = 0.26$ , P < 0.0001). Grain Zn 215 concentrations at Bay were all below the 30 mg Zn kg<sup>-1</sup> threshold regardless of genotype, while 216 the other sites showed some values that were above the threshold (Fig. 3). In DS 2013, similar 217 pattern were observed at Bay (Fig. 4). Unlike in WS 2012, there were some data points that 218 were above the grain Zn concentration threshold with yield above 3 t  $ha^{-1}$  (Fig. 4). Further, 219 results in WS 2014 and DS 2015 at IRRI sites still showed negative correlations between grain 220 yield and grain Zn concentration, with Zn treatment showing significant (P < 0.0001) effects 221 on grain Zn concentrations in both seasons (Fig. 5; Table 2). For example, in the 2015 DS both 222 223 no Zn and plus Zn treated rice plants had grain Zn concentrations above the 30 mg Zn kg<sup>-1</sup> threshold, while in the 2014 wet season, the no Zn treatment had mostly grain Zn 224 concentrations below the threshold and the plus Zn treated plants were all above the grain Zn 225 biofortification target (Fig. 5). 226

# 227 **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 234 fertilized ones in terms of grain Zn concentration. However, in this study, harvest times in 235 given genotypes did not vary with Zn fertilization (data not shown). Third, genotypic variation 236 237 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 238 during wet season 2012 (from four sites: Bay, Bohol, Bukidnon and IRRI), dry season 2013 239 (Bay), and wet season (Bay) support the latter explanation, where genotype differences 240 explained variation in grain yield better than Zn fertilization (P < 0.0001 versus P > 0.05). 241

242 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 243 biofortification target at the Bay site, which site is severely Zn deficient. However at Bohol, 244 Bukidnon and IRRI, some genotypes (the biofortified genotypes IR68144 and IR91143AC) 245 246 consistently exceeded the biofortification target. Surprisingly, Zn-inefficient K. Patong also 247 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 248 grain Zn concentration and low grain yield) and IR55179 (high grain yield and low grain Zn 249 concentration) could be attributed to dilution effects, as shown by high grain total Zn uptake 250 but low grain Zn concentration at IRRI, Bay and Bohol. Similar results have been obtained in 251 other studies (Slafer et al., 1990; Ortiz-Monasterio et al., 1997; MacDonald et al., 2008). In the 252 2013 dry season and 2012 wet season at Bay, grain Zn concentrations were all below the 253 threshold, except for the Zn-biofortified genotype IR68144. These results suggest that despite 254 the effectiveness of some Zn-biofortified genotypes, the effects of soil type remained a limiting 255 factor. The effect of soil Zn status was also apparent at IRRI in the 2014 wet season and 2015 256 257 dry season.

Although the Zn-biofortified genotypes achieved the highest grain Zn concentrations (P 258 <0.0001), grain Zn concentration was strongly influenced by soil type and the degree of Zn 259 deficiency. During the 2015 dry season at IRRI, although Zn fertilization had significant (P 260 <0.0001) effects on grain Zn concentration, the levels of grain Zn without Zn fertilizer were 261 still above the biofortification target. Conversely, during the 2014 wet season at IRRI, grain Zn 262 concentration without Zn fertilizer were mostly below the biofortification target. These 263 differences between wet and dry seasons indicate that Zn fertilization may not be needed during 264 the dry season. Higher availability of soil Zn can be expected in the dry season the soil may be 265 more oxidized and therefore soil Zn more soluble (Johnson-Beebout et al., 2016). Hence Zn 266 fertilization management needs to be optimized based on cropping seasons. 267

The results revealed a significant inverse relationship between grain yield and grain Zn 268 concentrations, irrespective of genotypic effects, which supports our hypothesis (2) that there 269 is a trade-off between increased grain yield and increased grain Zn concentration in Zn-270 271 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 272 lower micronutrient concentrations (Slafer et al., 1990; Ortiz-Monasterio et al., 1997). Our 273 results show that improving grain Zn concentration and at the same time achieving grain yield 274 targets can be challenging. The Philippine yield target of 4 t ha<sup>-1</sup> and the biofortification grain 275 Zn target was only achieved by Zn-biofortified genotypes at IRRI. Although some Zn-efficient 276 genotypes and Zn-biofortified genotypes were able to achieve the grain yield target in the dry 277 season of 2013 at Bay, none achieved the 30 mg Zn kg<sup>-1</sup> grain Zn biofortification target, 278 suggesting that grain Zn biofortification performance of these genotypes is limited by soil 279 conditions, consistent with our hypothesis (1). This agrees with previous studies (White and 280 281 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 282 differences between no Zn and the Zn fertilized treatments, but no significant difference among 283 the Zn fertilized treatments. This could be because the Zn deficiency in the unfertilized soil 284 was only moderate. However, we found higher grain Zn concentrations with foliar Zn 285 fertilization compared with broadcast or basal fertilization. This supports our hypothesis (3), 286 that the effects of Zn fertilization on grain yield and Zn concentration are different. Foliar Zn 287 fertilization is evidently a superior means of improving grain Zn concentration. This may be 288 partly due to the timing and frequency of applications. We made the foliar application during 289 mid-tillering, when effects of Zn deficiency are most severe, and flowering, when Zn is needed 290 291 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. 292

# 293 **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 Zndeficiency tolerant genotypes in both yield and grain Zn concentration.

# 306 Acknowledgements

We thank Ellen Genil, Jerone Onoya, Isagani De Castro, Jeff Gaco and Francis Rubianes
for assistance during the conduct of the experiments, sample preparation and chemical
extractions. This research was funded by grants from (a) the UK's Biotechnology and
Biological Sciences Research Council (Grant Ref. BB/J011584/1) under the Sustainable Crop

Production Research for International Development (SCPRID) programme, a joint multi-

national initiative of BBSRC, the UK Government's Department for International

313 Development (DFID) and the Bill & Melinda Gates Foundation; and (b) HarvestPlus. The

314 contents of this manuscript are solely the responsibility of the authors.

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- intercropping with gramineous species: a review. Agron. Sustain. Dev. 29, 63–71.

	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	9°55'0''N, 124°6'0''E	7°51'27.78"N, 125° 3'29.37"E
Classification (USDA, 1999)	Haplaquoll	Tropaquept	Aquic Argiudolls	Andisol
Clay (%)	$36.0 \pm 3.10$	$35.0\pm0.63$	$23.0\pm0.16$	$48.0\ \pm 0.50$
Silt (%)	39.0 ± 1.20	$47.0\pm0.75$	$41.0\pm0.49$	$29.0\pm2.70$
pH (KCl)	$4.62\pm0.10$	$6.30\pm0.20$	$7.20\pm0.02$	$4.80\pm0.01$
CEC (cmol <sub>c</sub> kg <sup>-1</sup> )	$30.6\pm0.70$	$42.8\pm0.30$	$23.6\pm0.33$	$13.5\pm0.07$
Organic carbon (%)	$1.65 \pm 0.10$	$4.65\pm0.03$	$4.08\pm0.21$	$1.98\pm0.01$
DTPA Zn (mg kg <sup>-1</sup> )	1.84 ± 0.13	$0.32 \pm 0.03$	$1.13 \pm 0.10$	$0.43 \pm 0.03$
Available P (mg kg <sup>-1</sup> )	10.4 ± 1.71 (Bray)	25.0 ± 0.45 (Olsen)	$9.80 \pm 0.12$ (Olsen)	9.65 ± 0.14 (Bray)

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

- 447 **Table 2** Analysis of variance for grain yield and grain Zn concentration of rice plants as
- 448 influenced by genotypes and Zn fertilization grown in contrasting soils and different seasons

449

from 2012 to 2015.

Treatment	df	Grain yield	Grain Zn concentration
		P values	P values
	11	0.0001***	0 0001***
Genotype	1	0.0001***	0.0001***
	1	0.2321ns	0.6036ns
Genotype*ZF	11	0.5622ns	0.3196ns
Genotype	7	0.0001***	0.01690*
ZF	1	0.3940ns	0.8466ns
Genotype*ZF	7	0.5528ns	0.9984ns
Genotype	11	0.0001***	0.0001***
ZF	1	0.1695ns	0.2053ns
Genotype*ZF	11	0.8137ns	0.8137ns
Genotype	11	0.0001***	0.0001***
ZF	1	0.2208ns	0.6053ns
Genotype*ZF	11	0.5970ns	0.9904ns
Genotype	8	0.0001***	0.0019***
ZF	1	0.9089ns	0.0718ns
Genotype*ZF	8	0.6970ns	0.8275ns
Genotype	9	0.0001***	0.9354ns
ZF	1	0.8621ns	0.0111*
Genotype*ZF	9	0.1550ns	0.9058ns
Genotype	9	0.0136*	0.0001***
ZF	1	0.0008***	0.0001***
Genotype*ZF	9	0.8206ns	0.0562ns
Genotype	9	0.0001***	0.0001***
ZF	1	0.0001***	0.0273*
Genotype*ZF	9	0.2448ns	0.0915ns
Genotype	3	0.0001***	0.0001***
ZF	3	0.0162*	0.0082*
Genotype*ZF	9	0.7522ns	0.3126ns
	Treatment Genotype ZF Genotype*ZF Genotype*ZF Genotype*ZF Genotype*ZF Genotype*ZF Genotype*ZF Genotype*ZF Genotype*ZF Genotype*ZF Genotype*ZF Genotype*ZF Genotype*ZF Genotype*ZF Genotype*ZF	TreatmentdfGenotype11ZF1Genotype*ZF11Genotype7ZF1Genotype*ZF7Genotype*ZF11ZF1Genotype*ZF11ZF1Genotype*ZF11ZF1Genotype*ZF11Genotype*ZF11Genotype*ZF11Genotype*ZF1Genotype*ZF9ZF1Genotype*ZF9Genotype*ZF9ZF1Genotype*ZF9Genotype*ZF9ZF1Genotype*ZF9ZF1Genotype*ZF9ZF1Genotype*ZF9ZF1Genotype*ZF9ZF3ZF3Genotype*ZF9	Treatment         df         Grain yield P values           Genotype         11 $0.0001^{***}$ ZF         1 $0.2321ns$ Genotype*ZF         11 $0.5622ns$ Genotype         7 $0.0001^{***}$ ZF         1 $0.3940ns$ Genotype*ZF         7 $0.5528ns$ Genotype         11 $0.0001^{***}$ ZF         1 $0.3940ns$ Genotype*ZF         7 $0.5528ns$ Genotype         11 $0.0001^{***}$ ZF         1 $0.8137ns$ Genotype         11 $0.0001^{***}$ ZF         1 $0.5970ns$ Genotype*ZF         11 $0.5970ns$ Genotype*ZF         1 $0.9089ns$ Genotype*ZF         8 $0.6970ns$ Genotype*ZF         9 $0.136^*$ ZF         1 $0.8621ns$ Genotype*ZF         9 $0.0001^{***}$ Genotype*ZF         9 $0.0001^{***}$ Genotype*ZF         9 $0.244$

450 *ns*, non-significant at 5% level.

451 \* Significant at 5% level.

452 \*\* Significant at 1% level.

453 \*\*\*Significant at 0.1% level

454 ZF = Zn fertilization; WS = wet season; DS = dry season.

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

456	Table 3 Analysis of	variance for the grain	Zn uptake of rice	genotypes in con	ntrasting soils duri	ng WS 2012.
100		fullance for the grain	Lin aptaile of fiee	Seller, pes III eo	industring soms dan	

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			
		U	WS	DS	WS	DS
			2012	2013	2014	2015
<u>Efficient</u>						
A69-1	A69-1	Blue	$\checkmark$	$\checkmark$	$\checkmark$	
IR55179	IR55179	Blue	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
RIL46	RIL46	Blue	$\checkmark$			
IR87839-4-1-1-1-2-	IR87839	Blue	$\checkmark$			
BAY B						
IR87842-5-1-3-1-B	IR87842	Blue	$\checkmark$	$\checkmark$		
Inefficient						
Kinandang Patong	KPatong	Red	$\checkmark$	$\checkmark$	$\checkmark$	
IR26	IR26	Red	$\checkmark$			
IR74	IR74	Red	$\checkmark$			
<b>Biofortified</b>						
IR69428-6-1-1-3-3	IR69428	Green	$\checkmark$	$\checkmark$	$\checkmark$	
IR68144-2B-2-2-3-	IR68144	Green	$\checkmark$	$\checkmark$	$\checkmark$	
1-166						
IR85800-41-3-2-1-2	IR85800	Green	$\checkmark$	$\checkmark$		
BR7840-54-3-1	BR7840	Green			$\checkmark$	$\checkmark$
<u>Checks</u>						
IR64	IR64	Black	$\checkmark$			$\checkmark$
NSIC222	NSIC222	Black		$\checkmark$	$\checkmark$	

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	Grain Zn	Genotype	Grain yield	Grain Zn
	$(t ha^{-1})$	concentration		$(t ha^{-1})$	concentration
		$(mg kg^{-1})$			$(mg kg^{-1})$
	Bay ( <i>n</i> =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 ( <i>n</i> =8)			IRRI ( <i>n</i> =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

469 Means in columns (per site) followed by the same letter are not significantly different from

470 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	Grain Zn concentration
		$(t ha^{-1})$	$(mg kg^{-1})$
Bay ( <i>n</i> =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 <sup>1</sup>		
	ZO	4.80±0.22a	21.8±0.87a
	Z1	4.81±0.19a	24.3±1.02a

473 Means in columns (treatment) followed by the same letter are not significantly different from

474 one another at P < 0.05.

475 <sup>1</sup> Zn fertilizer (zinc sulfate heptahydrate) applied broadcast to the soil with Zn foliar

476 application during flowering stage at a split rate of 5 kg ha<sup>-1</sup>.

477 **Table S4** Mean and standard error of grain yield and grain Zn concentration of rice plants as

478 influenced by genotypes and Zn fertilization grown in contrasting soils: Bay, Bohol and IRRI

Wet Season 2014	Genotype	Grain Yield	Grain Zn concentration
- / 0		(t/ha)	$(\operatorname{mg} \operatorname{kg}^{-1})$
Bay ( <i>n</i> =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 <sup>1</sup>		
	ZO	4.70±0.19a	24.4±1.59b
	Z1	4.72±0.15a	33.3±2.40a
Bohol ( <i>n</i> =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		
	ZO	1.95±0.15b	22.7±0.53b
	Z1	2.58±0.11a	30.5±0.83a
IRRI ( <i>n</i> =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

479 during the wet season of 2014.

480 Means in columns (per site) followed by the same letter are not significantly different from

481 one another at P < 0.05.<sup>1</sup> Zn fertilizer applied via root dipping of rice seedlings in 4% ZnO 482 for 15 min before transplanting.

#### Table S5 Mean and standard error of grain yield and grain Zn concentration of rice plants as 483

influenced by genotypes and Zn fertilization grown in IRRI soils during the dry season of 484 2015.

485

Dry season 2015	Genotype	Grain yield	Grain Zn concentration
		(t/ha)	$(mg kg^{-1})$
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

486

487 Means in columns (per treatment) followed by the same letter are not significantly different from one

another at P < 0.05. 488

Z0= No Zn added 489

Z1= Soil basal + Soil Soil 50% flowering 490

491 Z2= Soil basal + foliar 50% flowering

Z3= Foliar mid-tillering (30DAT) + Foliar 50% flowering 492

#### 493 **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  $\pm$  standard error; common letters in a panel indicate means not significantly different at *P* < 0.05. The indicated biofortification target grain
- 497 Zn concentration is set by HarvestPlus (2014); the indicated grain yield target is set by the
- 498 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  $\pm$  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 WS2012.
- **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
- 506 and (b) DS 2015. In (a): A = A69-1, B = BR7840 , C = IR55179, D = IR64, E = IR68144, F = IR681444, F = IR681444, F = IR681444, F =
- 507 IR69428, G = IR91143AC, H = KPatong and I = NSIC222. In (b): A = BR7840, B = IR55179,
- 508 C = IR64, D = IR91142AC, and numbers 1-3 indicate Zn fertilization regime (Section 2.2.3).
- 509 Data points are means of three (3).
- 510 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  $\pm$  standard error; common
- 512 letters in a panel indicate means not significantly different at P < 0.05.





(d)

а

Zn-efficient

a

а

Grain Zn \_biofortification target



Fig. 1 513



#### Fig. 2





525 Fig. 5





527 Fig. 6



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# Grain Zn concentrations and yield of Zn-biofortified versus Zn-efficient rice genotypes under contrasting growth conditions

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