

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**

11 Higher grain Zn concentration in ‘biofortified’ rice genotypes, bred for high grain Zn
12 concentration, should not be at the expense of reduced grain yield. This study examined the
13 grain yield and grain Zn concentration of Zn-biofortified genotypes in field experiments in the
14 Philippines. Zinc-biofortified genotypes (high grain Zn concentration in Zn-sufficient soil)
15 were compared with efficient genotypes (tolerant of soil Zn deficiency), inefficient genotypes
16 (sensitive to soil Zn deficiency) and check genotypes (popular local varieties) at four sites (Bay,
17 Bohol, Bukidnon and IRRI) with differing types and degrees of Zn deficiency, over five
18 cropping seasons (wet season 2012, 2014 and 2015 and dry season 2013 and 2015). A common
19 experimental design and plot size were used with treatments (genotypes and Zn fertilization)
20 arranged in a two-factorial randomized complete block design. The results showed that
21 biofortified genotypes achieved both the Philippine grain yield target (4.0 t ha⁻¹) and grain Zn
22 biofortification target (30 mg kg⁻¹) only when grown under Zn-sufficient conditions. In Zn-
23 deficient soils, most Zn-biofortified and deficiency-tolerant genotypes reached the Zn
24 concentration target but not the yield target, suggesting the need to correct the soil Zn-
25 deficiency to prevent yield penalty. Further, results from IRRI showed that only Zn-fertilized
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
28 more plant-available, grain Zn levels were all above the threshold, with or without Zn fertilizer.
29 This suggests that Zn fertilization may not be needed during the dry season in soils with
30 sufficient, potentially plant-available Zn.

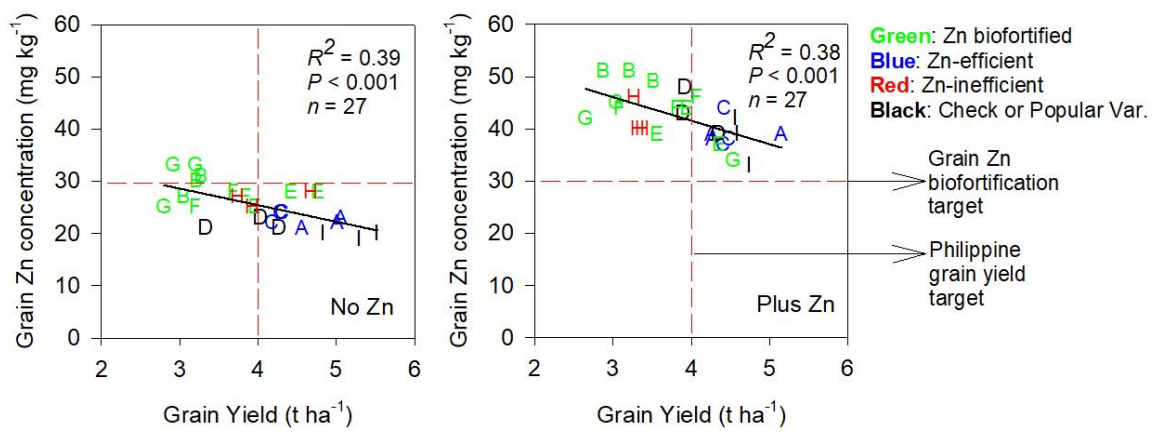
31 **Keywords:** Grain Zn biofortification, Zn fertilization, biofortified rice genotypes, Zn uptake,
32 improved grain yield performance

33 Highlights:

- 34 • Only Zn ‘biofortified’ genotypes achieved target grain-Zn concentrations
- 35 • No genotypes achieved target yields in Zn-deficient soils
- 36 • High grain Zn concentration was at the cost of grain yield
- 37 • 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

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

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

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

74 contrasting environments; (2) to investigate relationships between grain-Zn concentration and
75 grain yield, and dilution effects; and (3) to investigate Zn fertilization strategies for improving
76 rice growth and Zn enrichment in Zn-deficient conditions. We hypothesized that (1) grain
77 yields and Zn concentrations of contrasting genotypes vary with the degree of soil Zn
78 deficiency, (2) there is a trade-off between increased grain yield and increased grain Zn
79 concentration in Zn-biofortified genotypes, and (3) the effects of Zn fertilization on grain yield
80 and Zn concentration are different.

81 **2. Materials and methods**

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

90 *2.1 Crop establishment*

91 Land preparation included plowing and puddling followed by construction of levees to
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
94 years of the experiment. A day before transplanting, a basal dose of fertilizer at a rate of 20 kg
95 ha⁻¹ each of N, P and K was broadcast and thoroughly mixed with the soil using a power
96 weeder, followed by leveling at all sites. After breaking the seed dormancy at 50 °C for 3 days,
97 the seeds were sown in a wet seed bed with NPK fertilizer recommendation of 80 g m⁻² without
98 added Zn. The seedlings were transplanted at 21 days after sowing at all the field sites except
99 in Bay where 28-day old seedlings were used to avoid the damage caused by snails as brought
100 upon by continuously deep flooded conditions. Molluscicide (Bayluscide) was sprayed at a rate
101 of 1 L ha⁻¹ one week before and after transplanting to minimize the snail damage on young
102 seedlings. The water inside the field was maintained at saturation point until irrigation water
103 was provided one week after transplanting, to further minimize snail damage. Continuous
104 flooding was used throughout the experiment in all the sites, unless noted otherwise. Fertilizer
105 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
106 was added to the soil at basal stage together with 20% of N as complete (NPK) fertilizer with

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

109 *2.2 Experimental design and layout*

110 The treatments in the different seasons were as follows. The genotypes used at each site
111 and season are given in [Table S1](#).

112 *2.2.1 Wet season 2012*

113 A two-factorial randomized complete block design (RCBD) was followed with two Zn
114 treatments, either 14 (Bohol, Bukidnon and IRRI) or 8 (Bay) genotypes and 4 replications.
115 The Zn treatments were either no added Zn or root dipping of rice seedlings in 4% ZnO for
116 15 min before transplanting. Plot size was $3 \times 1.2 \text{ m}^2$ with six rows of 3-m length and 0.20-m
117 spacing between rows and hills, with one seedling per hill.

118 *2.2.2 Dry season 2013 and wet season 2014*

119 A two-factorial RCBD was followed with two Zn treatments, either 9 (Bay and Bohol, DS
120 2013) or 10 (Bay, Bohol and IRRI, WS 2014) genotypes and 4 replications. The Zn
121 treatments were either no Zn added or (a) for WS, root dipping of rice seedlings in 4% ZnO
122 for 15 min before transplanting or (b) for DS, broadcast zinc sulfate heptahydrate to the soil
123 with Zn foliar application during flowering stage at a split rate of 5 kg ha^{-1} . The size of each
124 plot was 12 m^2 with 20 rows of 3-m length and 0.20-m spacing between rows and hills, with
125 one seedling per hill.

126 *2.2.3 Dry season 2015*

127 A split-plot two-factor RCBD was followed with 4 fertilizer treatments, 4 genotypes and
128 3 replications. The Zn treatments were: (0) no added zinc; (1) soil basal + soil broadcast at
129 50% flowering; (2) soil basal + zinc foliar spray at 50% flowering; and (3) Zn foliar spray at
130 mid-tillering (30 DAT) and flowering stages. Zinc sulphate heptahydrate fertilizer was used
131 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-
132 m spacing between rows and hills and with continuous planting within main plots and bunds
133 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
136 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
138 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 NH₄F for soil pH < 7 (Bray et. al., 1945). Wet soil
142 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*

146 Grain yield sampling was done according to the protocol described by Cassman et al.
147 (1994). Plant samples from a 5-m² central area of each plot (125 hills) were taken for yield
148 measurement. The total number of hills was recorded for each plot. The harvested plants were
149 put in net bags, threshed and sun-dried in a glasshouse. After drying, grain was passed
150 through a blower three times to remove any unfilled and partially filled spikelets. The cleaned
151 grains were transferred to double-ply paper bags for weighing. Grain moisture content was
152 measured using a grain moisture meter. Grain yield was calculated and adjusted to 14% and
153 3% moisture content. Thousand grain weight and actual harvest area were calculated as
154 described by Cassman et al. (1994).

155 Prior to determination of Zn concentration, grain samples were dehulled to obtain brown
156 rice. The brown rice samples were analysed at IRRI's Analytical Services Laboratory for Zn
157 concentration by digestion in 1% nitric acid (HNO₃) and 2.8% perchloric acid (HClO₄) and
158 analysis by ICP-OES (Optima 5300DV, Perkin Elmer, USA).

159 *2.5 Statistical analyses*

160 Grain yields and Zn concentrations were tested for normality using the Shapiro–Wilk
161 normality test. Non-normally distributed data were transformed, but the original non-
162 transformed values are presented in Results. A two-way analysis of variance (ANOVA) was
163 carried out using the Statistix 8.0 software package (ref). Treatment mean differences were
164 calculated using the Least Significant Difference at 5% level of significance. A Pearson
165 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*

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

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

193 3.2 Grain Zn concentration of genotypes in contrasting soils and seasons

194 Generally, the highest grain Zn concentrations were achieved by Zn-biofortified genotypes.
195 In WS 2012, Zn-biofortified genotypes consistently had the highest grain Zn concentrations
196 though differences were only significant ($P < 0.05$) at IRRI and Bohol (Fig. 1). Similar results
197 were also observed for WS 2014 IRRI and Bohol soils (Fig. 2). Specific results for grain Zn
198 concentration also revealed significant influence by genotypes ($P < 0.0001$) but not by Zn
199 fertilization during WS of 2012 for Bay, Bohol, Bukidnon and IRRI (Table 2). Grain Zn
200 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

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

212 3.3 Relationship between grain yield and grain Zn concentration

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

227 4. Discussion

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

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

242 The results also revealed genotype differences in grain Zn concentrations were sensitive to
243 soil conditions. In the wet season of 2012, none of the studied genotypes achieved the grain Zn
244 biofortification target at the Bay site, which site is severely Zn deficient. However at Bohol,
245 Bukidnon and IRRI, some genotypes (the biofortified genotypes IR68144 and IR91143AC)
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
248 genotypes did so at any of the sites, except IR87842 at Bukidnon. The cases of K. Patong (high
249 grain Zn concentration and low grain yield) and IR55179 (high grain yield and low grain Zn
250 concentration) could be attributed to dilution effects, as shown by high grain total Zn uptake
251 but low grain Zn concentration at IRRI, Bay and Bohol. Similar results have been obtained in
252 other studies (Slafer et al., 1990; Ortiz-Monasterio et al., 1997; MacDonald et al., 2008). In the
253 2013 dry season and 2012 wet season at Bay, grain Zn concentrations were all below the
254 threshold, except for the Zn-biofortified genotype IR68144. These results suggest that despite
255 the effectiveness of some Zn-biofortified genotypes, the effects of soil type remained a limiting
256 factor. The effect of soil Zn status was also apparent at IRRI in the 2014 wet season and 2015
257 dry season.

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

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

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

293 **5. Conclusions**

294 Our results confirmed a general superiority of ‘Zn-biofortified’ and ‘Zn-efficient’
295 genotypes over ‘Zn-inefficient’ genotypes in terms of grain yield and grain Zn concentration.
296 However, these genotypes generally did not achieve both yield and biofortification targets in
297 Zn-deficient soils, where high grain Zn concentrations tended to be at the cost of grain yield
298 and vice versa. The advantage of Zn-biofortified and Zn-efficient genotypes was more apparent
299 at sites with adequate levels of soil Zn. Further, there were differences between seasons, with
300 Zn fertilization being less necessary during the dry season, probably due to better soil Zn

301 availability under more-oxidized soil conditions. Overall, the findings demonstrate the strong
302 influence of soil conditions on grain yield and grain Zn concentration of Zn-biofortified and
303 Zn-efficient genotypes. Their superiority over Zn-inefficient genotypes was evident in soils
304 with adequate soil Zn, where the Zn-biofortified genotypes performed better than Zn-
305 deficiency tolerant genotypes in both yield and grain Zn concentration.

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445 **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	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 ± 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)

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.

Season and site	Treatment	df	Grain yield <i>P values</i>	Grain Zn concentration <i>P values</i>
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

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.

455

456

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			

457

ND= no data

458

ns, non-significant at 5% level.

459

* Significant at 5% level.

460

** Significant at 1% level.

461

***Significant at 0.1% level

462 **Table S1** Rice genotypes contrasting in tolerance of soil Zn deficiency ('efficient' or
 463 'inefficient') and in grain Zn concentration under Zn-sufficiency ('biofortified') grown at
 464 different sites.
 465

Genotype	Referred in text as	Color code in Figure 5	Years/seasons			
			WS 2012	DS 2013	WS 2014	DS 2015
<u>Efficient</u>						
A69-1	A69-1	Blue	✓	✓	✓	
IR55179	IR55179	Blue	✓	✓	✓	✓
RIL46	RIL46	Blue	✓			
IR87839-4-1-1-1-2-	IR87839	Blue	✓			
BAY B						
IR87842-5-1-3-1-B	IR87842	Blue	✓	✓		
<u>Inefficient</u>						
Kinandang Patong	KPatong	Red	✓	✓	✓	
IR26	IR26	Red	✓			
IR74	IR74	Red	✓			
<u>Biofortified</u>						
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			✓	✓
<u>Checks</u>						
IR64	IR64	Black	✓			✓
NSIC222	NSIC222	Black		✓	✓	

466

467 **Table S2** Mean and standard error of grain yield and grain Zn concentration of rice plants as
 468 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 (<i>n</i> =8)			Bukidnon (<i>n</i> =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 $P < 0.05$.

471 **Table S3** Mean and standard error of grain yield and grain Zn concentration of rice plants as
 472 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)
<u>Bay (n=8)</u>			
	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
	<u>Zn fertilization¹</u>		
	Z0	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 ¹ 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⁻¹.

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
 479 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

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

483 **Table S5** Mean and standard error of grain yield and grain Zn concentration of rice plants as
 484 influenced by genotypes and Zn fertilization grown in IRRI soils during the dry season of
 485 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

486
 487 Means in columns (per treatment) followed by the same letter are not significantly different from one
 488 another at $P < 0.05$.

489 Z0= No Zn added

490 Z1= Soil basal + Soil Soil 50% flowering

491 Z2= Soil basal + foliar 50% flowering

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

493 **Figure legends**

494 **Fig. 1.** Grain Zn concentration and yield of Zn-efficient, Zn-biofortified and check genotypes
495 at the four field site in WS 2012. Data are means \pm standard error; common letters in a panel
496 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](#)).

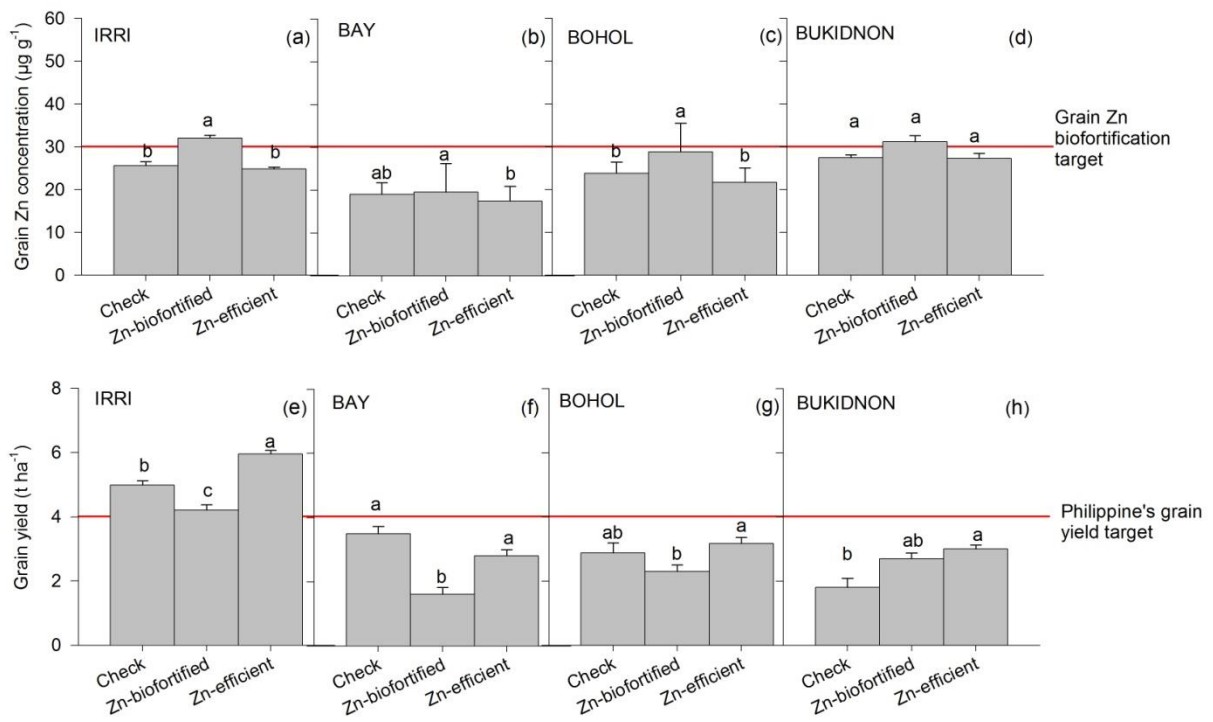
499 **Fig. 2.** Grain Zn concentration and yield of Zn-efficient, Zn-biofortified and check genotypes
500 at the indicated sites in DS 2013 and WS 2014. Data are means \pm standard error; common
501 letters in a panel indicate means not significantly different at $P < 0.05$.

502 **Fig. 3.** The relationship between grain yield and grain Zn concentration at the four sites in WS
503 2012.

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

505 **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 =
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).

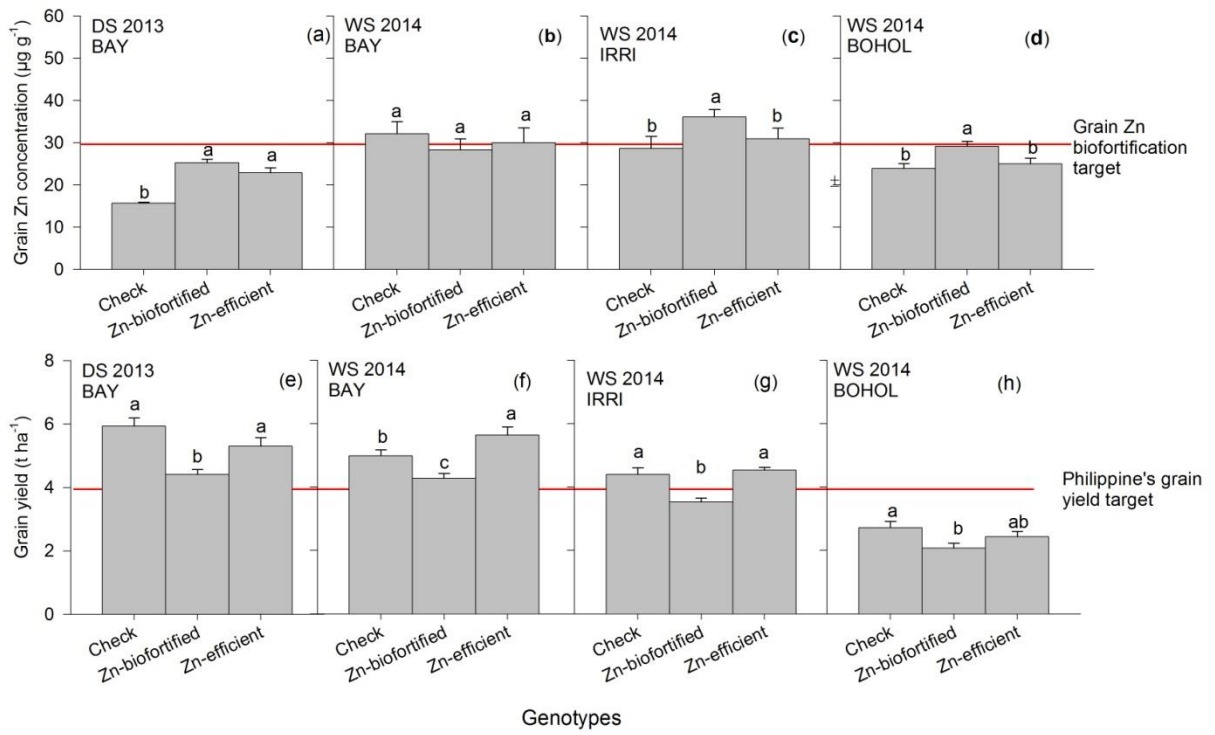
510 **Fig. 6.** Grain Zn uptake of Zn-biofortified (ZnB), Zn-efficient (ZnT), and Zn-inefficient (ZnS)
511 in Bay, Bohol, Bukidnon and IRRI soils in WS 2012. Data are means \pm standard error; common
512 letters in a panel indicate means not significantly different at $P < 0.05$.



514

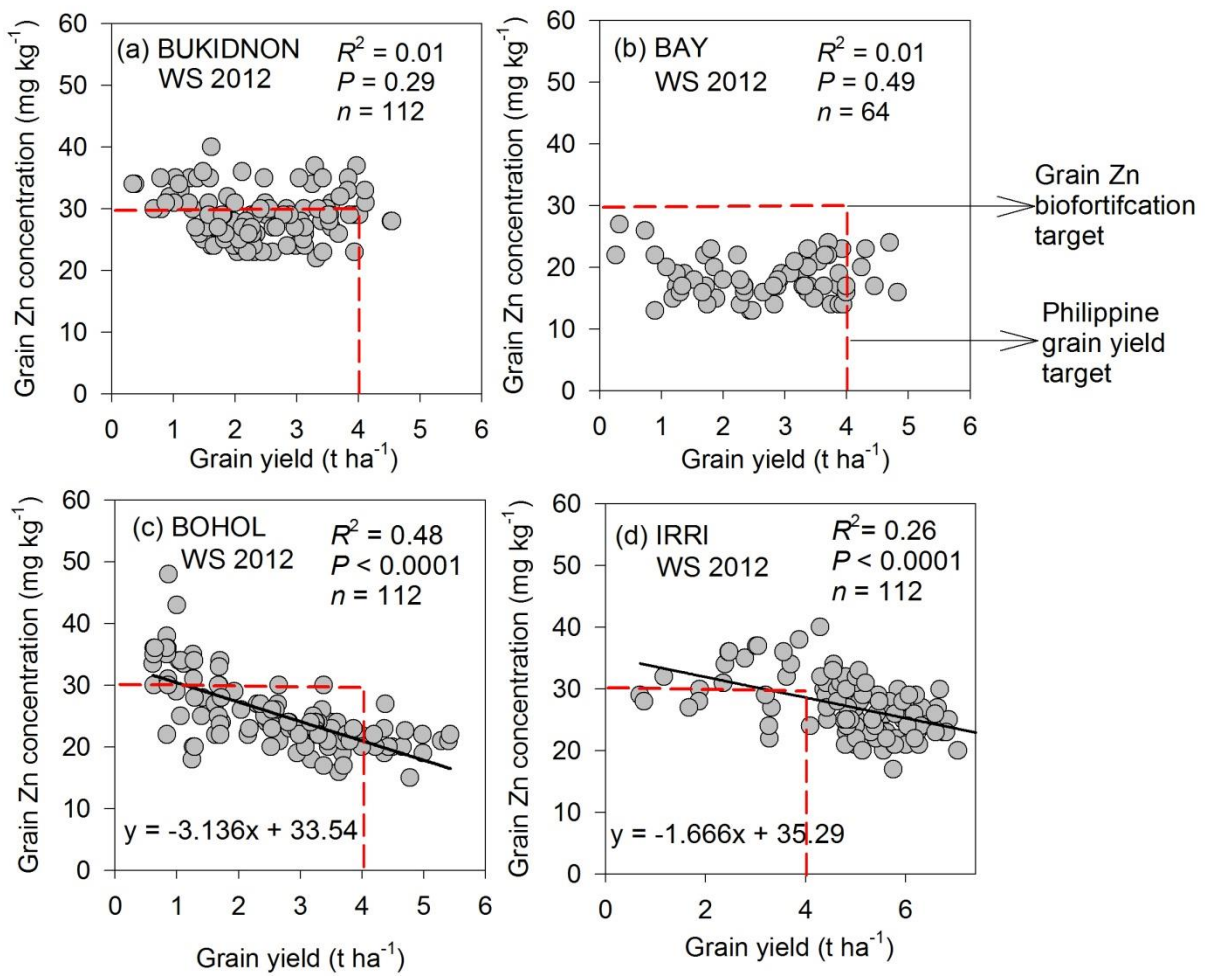
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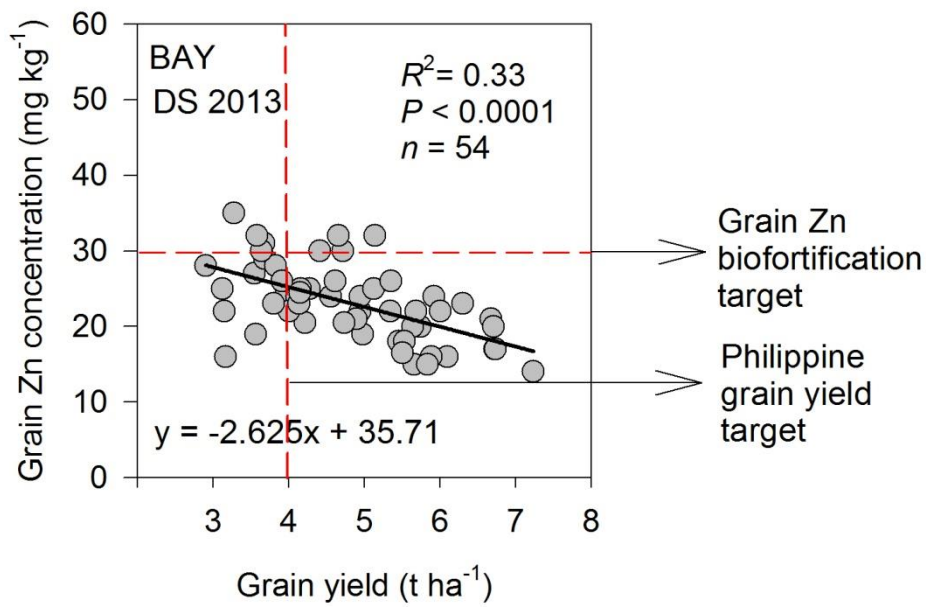
516 Fig. 2

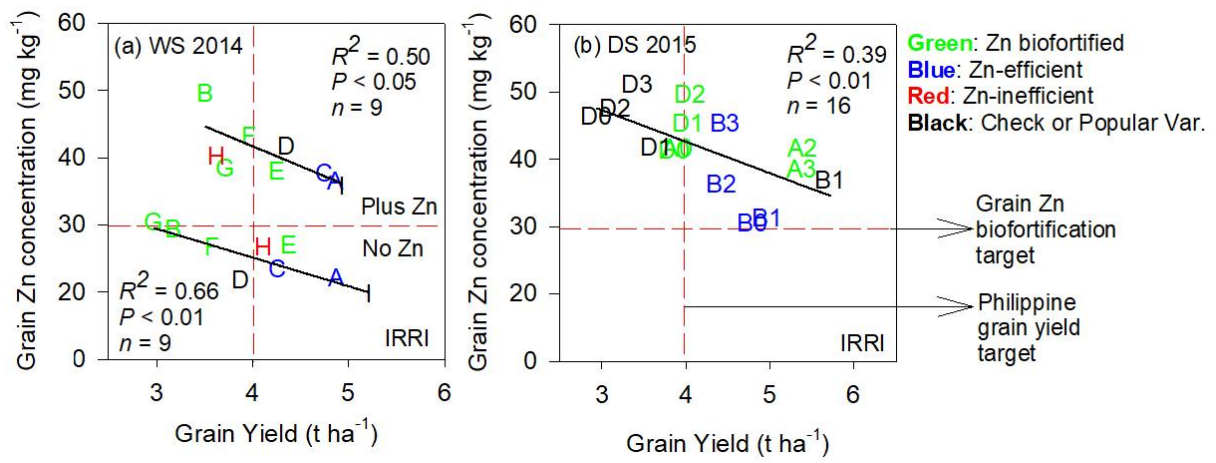


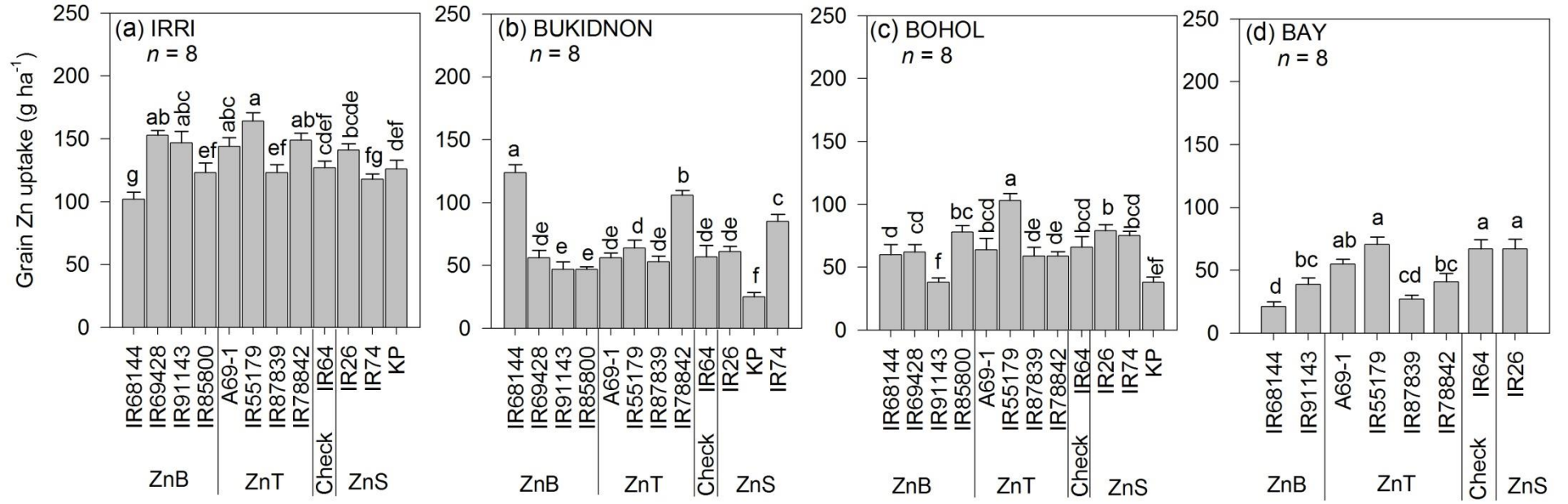
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518









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

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