

Yield and Zn content of biofortified rice genotypes in an Indonesian rice agro-ecosystem

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Abstract. *Susanto U, Barokah U, Hidayatullah A, Satoto, Swamy M. 2017. Yield and Zn content of biofortified rice genotypes in an Indonesian rice agro-ecosystem. Nusantara Bioscience 9: 288-294.* Approximately one-third of the world's population suffer from Zn deficiency causing significant socio-economic losses as a result of stunting and compromised immune system function. One strategy to overcome the problem is by developing rice cultivars with high grain Zn content (Zn Rice) to improve dietary intake. This study reports the yield and Zn content of 22 rice genotypes developed at the International Rice Research Institute (IRRI) and the Indonesian Centre for Rice Research (ICRR). The experiment was conducted in the Cirebon district of West Java province during the 2013 wet season (2013WS). Yield and grain Zn content (using an XRF machine) were measured. Five lines demonstrated higher yield (ranging from 7.0 to 8.9 t/ha) than the check variety Ciherang (5.2 t/ha), but had similar grain Zn content to the check variety Ciherang (23.4 ppm), ranging from 19.0 to 24.8 ppm. On the other hand, seven lines had higher grain Zn content (ranging from 30.0 to 34.2 ppm) compared to Ciherang, and five of these lines had comparable yield to Ciherang. The selected lines had acceptable agronomic traits, and are suitable for further testing and utilization, in addition to providing a foundation for future improvement in the dual goals of increasing the yield and nutritional value of rice.

Keywords: Indonesia, micronutrients, rice genotype, yield, Zn content

INTRODUCTION

The prevalence of micronutrient malnutrition, especially Zn, is recognized as a significant public health problem across much of the world (Mc Clean et al. 2009; Wessells and Brown, 2012), and this includes Indonesia. Zinc deficiency is more extensive in developing countries where more than 60 per cent of the population is at risk (www.harvestplus.org). Approximately one-third of the world's population are at risk of suffering Zn deficiency due to low dietary intake of Zn (Hotz and Brown 2004; Myers et al. 2015). Among them, 2 billion people are in Asia and 400 million are in sub-Saharan Africa (IRRI, 2006). The prevalence of Zn deficiency in Indonesia ranges from 10 to 90% according to varying parameters (such as demographic groups) used in the study of the prevalence level of Zn deficiency (Herman 2009). In particular, the prevalence of Zn deficiency in Indonesia for children under five years during 2006 was 31.6% (Herman 2007).

Zn is an important micronutrient for humans. Zn is a key component of more than 300 enzymes needed to repair wounds, maintain fertility, synthesize protein, and boost immunity among the many functions important to human health and productivity (Mares-Perlman et al. 1995). Zn is also important in vitamin A metabolism, and one effect of Zn deficiency is xerophthalmia (Morrison et al. 1978). Another effect of Zn deficiency is stunting during childhood (Herman 2007). The prevalence of stunting in Indonesia was around 45% in the 1990s and 36.2% in 2006 (Taufiqurrahman et al. 2009). It is also prevalent than in the

Philippines, which was reported as 39.1% during 2000 (Guno 2004).

Rice is consumed by almost a half the world's population. Nevertheless, it does not provide enough Zn to match human requirements, and there is a high prevalence of Zn deficiency in countries having rice as a staple food (Impa and Johnson-Beebot, 2012). Efforts have been directed at overcoming this Zn deficiency, such as supplementation, fortification, and biofortification. Zn fortification efforts have also been conducted in Indonesia (Herman et al. 2002). Nevertheless, the coverage of Zn fortification in the world is very low (Bhutta et al. 2013). A further complementary effort to overcome this nutrient deficiency is by biofortification (Bouis 2004, Bouis et al. 2011), i.e. developing plant varieties with increased micronutrient content, including Zn. This approach is sustainable and economically viable (Nakandalage et al. 2016). Improving Zn content in rice grains is believed to be one of the most feasible, sustainable, and economical approach to combat Zn deficiency in the world (Salunke et al. 2011; Atiqueur-Rehman et al. 2014).

Breeding efforts to conduct Zn biofortification for rice has been initiated at IRRI either by using conventional breeding approaches (Graham et al. 1999; Gregorio et al. 2000; Slamet-Leodin et al. 2015) or transgenic approaches (Trijatmiko et al. 2016), and efforts continue on both. Promising breeding materials have been developed and shared with collaborating countries, including Indonesia. Bangladesh and Philippines had just released Zn rice varieties for the certain countries.

Some promising lines had been shared with Indonesia and initially screened under irrigated lowland conditions. Promising lines have been selected for further testing. Nevertheless, the stability of yield and grain Zn content requires evaluation following each round of selection to ensure the dual goals of improved productivity and improved nutrition are met. This research aimed to test the yield and Zn content of 22 rice genotypes originating from IRRI and ICRR, Indonesia.

MATERIALS AND METHODS

Study area

This study was focused on twenty-two selected rice lines consisted of genotypes introduced from IRRI along with Indonesian varieties. The experiment was conducted in Palimanan of Cirebon District, West Java Province, Indonesia during Wet Season of 2013. It is located in-6.705825, 108.435025 at around 15 m asl. The experiment was conducted under irrigated conditions.

Procedures

Field experiment

The trial was designed as a Randomized Complete Block Design with two replications. Seedlings (21 days after sowing) were transplanted at a spacing of 20cm x 20cm of in plots measuring 2m x 3m. Crop establishment and fertilizer applications followed local recommendations (equal to each 300 kg Urea, 50 kg SP-36 and 50 kg KCl for one ha, referred to Ministry of Agriculture Decree No. 40/Permentan/OT.140/04//2007), while pests and diseases were managed according to Integrated Crop Protection principles. The main traits to be measured were yield, which was converted into t/ha at 14% moisture content, and Zn content in rice grains. Some basic agronomic traits were also measured, i.e. heading date, plant height, tiller number, number of filled and unfilled grains/panicle, seed set, and 1000 grain weight (g).

Zn content measurement

The Zn content (ppm) of dehulled (brown rice) grains sample was measured using an XRF machine (Oxford Instrument X-Supreme) that had been validated by ICP method. The machine was located in the Plant Breeding Laboratory of ICRR (Indonesian Center for Rice Research) of IAARD (Indonesian Agency of Agricultural Research and Development) in Sukamandi, Subang District, West Java. Approximately 50g grain samples from each plot were de-hulled using Satake THU Testing Husker. Brown rice samples were then sorted to get only healthy and fully filled grain then used for Zn content measurement using the XRF machine.

Data analysis

Data analysis was executed using Excel and CropStat Ver 6.1. (IRRI 2007). Variance components were analysis based on Randomized Complete Block Design model (Table 1) and the analysis of heritability and genetic variability were calculated following (Pinaria et al. 1995; Yuwono et al. 2015).

Table 1. Source of variance and expected value of Randomized Complete Block Design

Source of variance	dF	Mean square	Expected value
Block	r-1	-	
Genotype	g-1	Mean Square of Genotype (MSG)	$\sigma_e^2 + r \sigma_g^2$
Error	(r-1)(g-1)	Mean Square of Error (MSE)	σ_e^2
Total	gt-1		

Note: σ_g^2 = Genetic Variance; σ_e^2 = Variance of environment.

Heritability was calculated by the formula:

$$\sigma_e^2 = \text{MSE}$$

$$\sigma_g^2 = (\text{MSG} - \text{MSE})/r$$

$$\sigma_p^2 = \sigma_g^2 + (\sigma_e^2/r)$$

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100\%$$

Genetic variability is defined as the tendency of individual genetic characteristics in a population to vary from one another (biology online, http://www.biology-online.org/bodict/index.php?title=Genetic_variability&action=edit). Genetic variability could be classified as wide or narrow, calculated by the comparison of genetic variability and standard deviation of genetic variability. Genetic variability was considered as wide if $\sigma_g^2 > 2 \sigma_{\sigma_g^2}$, and otherwise was narrow (Pinaria et al. 1995). Standard deviation of genetic variability was calculated as follows (Hallauer and Miranda 1995):

$$\sigma_{\sigma_g^2} = \sqrt{\left(\frac{2}{r^2} \left[\frac{\text{MSG}^2}{(dF_g) + 2} + \frac{\text{MSE}^2}{(dF_e) + 2} \right] \right)}$$

RESULTS AND DISCUSSION

Variations and genetic variability

The trial was conducted during wet season, and was fully irrigated during the complete plant growth cycle. There were no serious biotic stresses such as pests and diseases during the plant establishment. However, at the early vegetative stage irrigation water containing waste from spirit oil factory was accidentally used to irrigate the field, but did not significantly affect the plants.

Variance analysis showed that at P threshold of 0.05 there was significant variation among genotypes for all the observed traits, except for seed set (Table 2). At the minimum, two genotypes had significant differences for all traits except seed set. Further analysis allowed the partitioning of genetic variance, demonstrating variation among the genotypes, with some traits having larger genetic variances e.g., plant height, 1000 grain weight, and yield. On the other hand, heading date, tiller number/plant, panicle number / plant, filled grain/panicle, unfilled grain/

Table 2. Variance analysis and Heriability of agronomic traits of 22 Rice Genotypes, Cirebon, WS 2013/2014

Character	Grand mean	MS Gen	MS E	P Gen	Var. Gen	Var. Phe.	St. Dev. Gen.	Gen. Var.	H ² (%)	CV (%)
Heading date (DAS)	87.50	51.33	16.47	0.01	11.62	56.8239	8.11	Narrow	20.45	4.60
Plant height (cm)	115.40	643.83	33.43	0.00	203.47	654.974	95.11	Wide	31.06	5.00
Tiller number/plant	23.82	66.23	27.55	0.03	12.89	75.4121	10.91	Narrow	17.10	22.00
Panicle number/plant	19.77	62.57	24.53	0.03	12.68	70.7482	10.19	Narrow	17.93	24.60
Filled grain/panicle	80.10	652.58	298.10	0.05	118.16	751.943	109.70	Narrow	15.71	21.80
Unfilled grain/panicle	52.59	483.18	167.04	0.01	105.38	538.862	77.12	Narrow	19.56	24.80
Seed set (%)	60.45	191.78	95.49	0.07	32.10	223.614	32.93	Narrow	14.35	16.20
1000 grains weight (g)	24.77	21.06	1.21	0.00	6.62	21.4653	32.35	Wide	30.82	4.50
Yield (t/ha)	5.14	7.41	0.74	0.00	2.22	7.65509	3.11	Wide	29.02	17.30
Zn content (ppm)	26.56	51.21	7.97	0.00	14.41	53.8654	1.10	Narrow	26.76	22.60

Note: MS Gen = Mean Square of Genotype; MS E = Mean Square of Error; P Gen = Probability of Genotype; H² = Broad Sense Heritability; CV = Coefficient of Variation; DAS = Days After Sowing; Seed Set = proportion of filled grains over total grain per panicle

panicle, and Zn content had smaller genetic variance components. The greater the genetic variability the greater the opportunity to improve the trait through selection among genotypes included in the study. Lower genetic variability for a trait indicates uniformity across the studied genotypes, thus little chance to select outstanding lines among the genotypes (Ruchjaningsih 2006).

Heritability

Heritability represents the proportion of variation due to genetic effects compared to the total variation in the expression of a trait (Sleper and Poehlman 2006). Broad sense heritability in the trial ranged from 15 to 31%. Among the traits, plant height, 1000 grain weight, yield, and Zn content had the highest levels of heritability ranging from 26.8% to 31.1% (Table 2). It indicated that for the performance of genotypes for these traits, genetic factors were responsible for around one-third of the variation noted. Thus, selection for those traits is warranted, despite the fact that genetic variation is a minor portion of the total variation noted.

On the basis of testing these genotypes, selection among them to develop high yielding with high Zn content variety is feasible. Further, management options (agronomic management) to maximize yield and Zn content may also be beneficial. Slaton et al. (2001) reported that either Zn seed treatment or soil Zn fertilizer increase Zn content in rice plant tissue compared to control. It is a different case with Iron, which crop Fe fertilization is not very effective due to Fe soil insolubility (Sperotto et al. 2012). On the other hand, organic farming system decreases Fe and Zn content (Sakagami et al. 2016). While it was suggested that plant height, heading time, or grain shape are not of primary importance in controlling economic variation (include Zn) in rice grain (Pinson et al. 2015). Selection for specific growth duration best suited to the production environment and specific plant stature is also possible, given the variation demonstrated.

The genotypes tested in this study demonstrated that all the traits demonstrated a range of genetic variability, with the greatest genetic variation for plant height, 1000 grain

weight, and yield (Table 2). This highlights the opportunities for selection of lines which match the yield of locally adapted types, such as Ciherang, and which match for agronomic characteristics such as plant height and grain weight.

Yield and Zn content

Based on yield performance, IR64 (8.87 t/ha), BR28 (8.14 t/ha), IR91152AC-81 (8.03 t/ha), NSICRc222 (7.29 t/ha), NSICRc238 (7.04 t/ha) had higher yield compared to Ciherang (popular variety; 5.16 t/ha) (Figure 1). The variety IR64 in this research originated from IRRI, and had the highest yield in this trial, reflecting its adaptability and productivity. IR64 is commonly referred to as a mega variety in the tropical rice growing areas of the world. The genotypes which had relatively high yield were mostly recently developed varieties, not local varieties. Many of these modern varieties have IR64 as one of the parents in the pedigree, or have the parents of IR64 in the pedigree. The plant architecture and physiological characteristics of the modern varieties and their derivatives had been optimized for high yield, including elements such as large panicle size and appropriate tillering capacity. Those traits are important in increasing yield capacity of the plant (Peng et al. 2008).

Nevertheless, in this study, those high yielding genotypes had relatively low Zn content (19.00-24.80 ppm). For further efforts, these genotypes should be used as recurrent or recipient parents to combine high grain Zn content in combination with high yield. Harvest Plus is an international program which developed initiatives to address human micronutrient malnutrition through improving the micro nutrient concentration of staple foods. This program has targeted Zn level of brown and polished rice up to 30 ppm and 28 ppm respectively (Johnson-Beebout et al. 2009, Trijatmiko et al. 2016).

Based on grain Zn content, seven lines had higher Zn content compared to the current most popular variety in Indonesia, i.e. Ciherang (23.35 ppm). Among the seven, five lines had comparable yield to Ciherang, i.e. BR7840-54-2-5-1 (33.08 ppm; 5.75 t/ha), IR68144-2B-2-2-3-1-166

(34.22 ppm; 5.08 t/ha), IR84020-84-2-3-2 (29.9 ppm; 5.70 t/ha), Vanjakohonandiana (31.73 ppm; 4.39 t/ha), IR10M195 (IR84842-35-3-1-1-2-2) (35.68 ppm; 3.95 t/ha). While these lines had relatively high grain Zn content, this was combined with modest yield performance compared to the best conventional check variety (Table 3). However, two lines, i.e. IR10M196 (IR84842-131-1-2-1-1-3) (34.48 ppm; 2.74 t/ha) and BR7840-54-1-1-2-5 (29.98 ppm; 3.29 t/ha) had relatively low yield. It is possible that these lines could hopefully be utilized as donors for high Zn content in the breeding program, proving that the relatively higher grain Zn content was not driven by the lower yield.

Correlation between yield and grain Zn content in this trial was significantly negative (-0.46) with *t* value of -2.286 and $t_{5\%}=1.73$. This contrasts with the results reported by Sala and Geetha (2015) in which grain Zn content and yield were positively correlated. They used F_4 lines developed from two cross combinations, *viz.*, ADT37 × IR68144-3B-2-2-3 and TRY(R)2 × Mapillaisamba. They noted the importance of choosing appropriate parental genotypes to dissect the relationship between Zn content and grain yield, and concluded that the combination of high yield and high grain Zn was possible.

Agronomic characteristics of selected genotypes

Agronomic characteristics among the lines were varied however the genotypes mostly had similar characteristics to modern varieties in Indonesia such as Ciherang. The genotypes had growth duration of around 115 days, plant height of around 100 cm, and erect leaves (ICRR 2015). Heading dates ranged from 76 days (Pokkali) to 96 days (IR83286-22-1-2-1-1), and mostly the high Zn materials had a longer time to flower compared to Ciherang (85 days). Plant height ranged from 76.70 cm (IR68144-2B-2-2-3-1-166) to 158.80 cm (Pokkali), while Ciherang was 113.20 cm. Tiller number ranged from 14 (IR10M195 (IR84842-35-3-1-1-2-2)) to 41 (IR68144-2B-2-2-3-1-166) and Ciherang had an average of 19 tillers. Most of the high Zn materials had more tillers than Ciherang. Filled grains/panicle varied between 43 (IR83286-22-1-2-1-1) to 124 (IR69428-6-1-1-3-3), while Ciherang had 81 filled grains per panicle. Seed set ranged from 35.27 % (IR83286-22-1-2-1-1) to 74.62 % (IR91152AC-819), while Ciherang had 64.46 %. Weight of 1000 grains ranged from 15.80 g (IR68144-2B-2-2-3-1-166) to 30.90 g (Pokkali), while Ciherang had 26.30 g (Figure 2). Selection of the

best genotypes among the tested materials in each traits and combine the best traits might be useful to get the ideal plants which have a combination of high yielding traits, such as more tiller number, more filled grain per panicle, higher seed set, and bigger grain size consequencing heavier of 1000 grain weight. Grain number, panicle seed setting rate, panicle number and grain weight are the most important components of rice grain yield (Li et al. 2013).

Discussion

The target level of breeding for high Zn content is 24-28 ppm Zn content in polished rice grains which is essential to attain 30% of the estimated average requirement (EAR) for humans (Bouis et al. 2011). Nevertheless, studies have reported that for current varieties, the polished rice grains supply only one fifth of daily Zn requirements (Prom-uthai et al. 2010, Sharma et al. 2013).

The highest Zn content identified in this study was 35.68 ppm achieved by IR10M195 (IR84842-35-3-1-1-2-2). It was measured on brown rice, in which the bran and the germ remain intact, and naturally, carry higher Zn and other nutrients. The Fe and Zn are mostly located in aleurone layer, but the percentage of Zn in polished rice (endosperm only) varies from around 75-84 % of the total Zn content including the aleurone layer. The case for Fe is only around 19-30% (Johnson et al. 2011). Assuming 75% of the Zn is located in endosperm, IR10M195 (IR84842-35-3-1-1-2-2) is predicted to have Zn content in polished grain of around 26.76 ppm which is within the range of the targeted levels. Nevertheless, the line had a relatively low yield (3.95 t/ha).

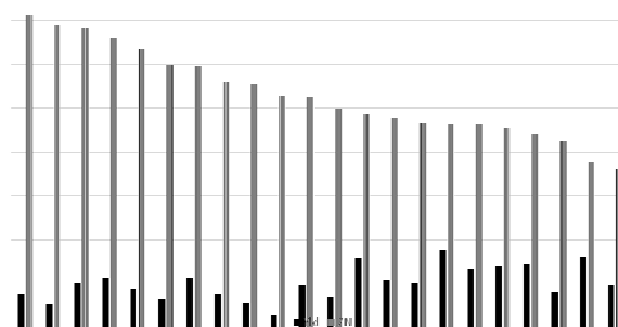


Figure 1. Yield (solid) and Zn Content (vertical lines) of 22 rice genotypes grown at Cirebon, WS 2013/2014

Table 3. Agronomic traits of Five selected lines based on Zn content and yield, Cirebon, WS 2013/2014

Genotype	Heading date (DAS)	Plant height (cm)	Tiller number	Panicle number	Filled grains /panicle	Unfilled grain/ panicle	Seed Set (%)	1000 grain weight (g)	Yield (t/ha)	Zn (ppm)
BR7840-54-2-5-1	89	104.50	26	16	86	57	61.60	21.80	5.75	33.08
IR84020-84-2-3-2	89	103.10	31	28	68	37	65.04	21.45	5.69	29.90
IR10M195 (IR84842-35-3-1-1-2-2)	93	107.30	14	12	92	56	62.43	26.59	3.95	35.68
IR68144-2B-2-2-3-1-166	85	76.70	41	32	66	24	73.73	15.80	5.08	34.22
Vanjakohonandian	83	148.90	29	15	46	80	36.38	28.95	4.39	31.73
Ciherang	85	113.20	19	17	81	44	64.46	26.30	5.16	23.35

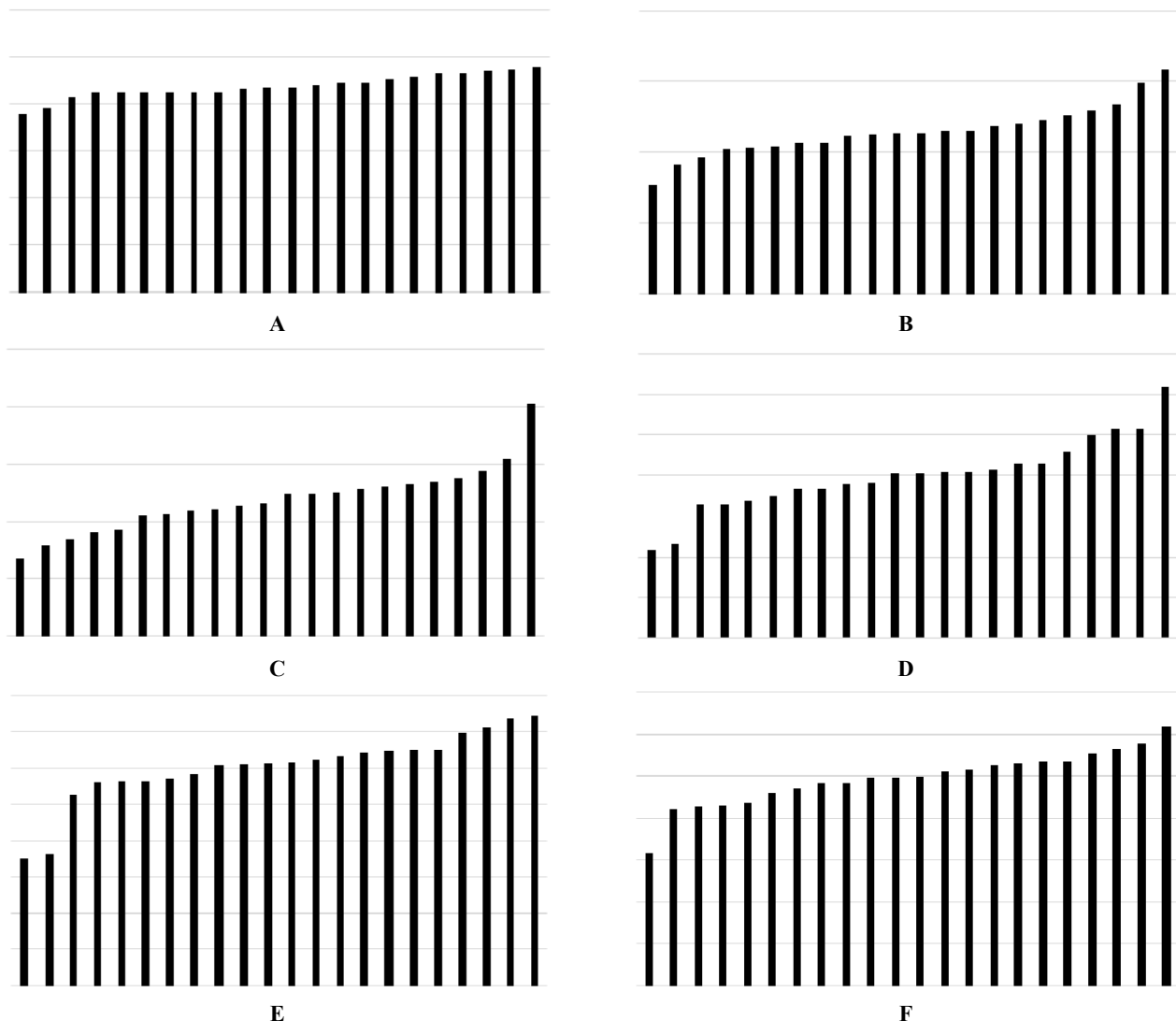


Figure 2. Agronomic traits of 22 Rice Genotypes, Cirebon, WS 2013/2014. A. Heading date (days After Sowing), B. Plant Height (cm), C. Tiller Number, D. Filled Grain/Panicle, E. Seed Set (%), F. 1000 Grain Weight (g)

Zn content in rice grains is a quantitative trait which is either directly or indirectly affected by some other traits simultaneously. It had medium heritability, reflecting that environmental effect is presented in expressing Zn content. Regarding this one, crop genetic and management practices improvement should be conducted simultaneously considering the environmental change. The interaction of environmental and genetic factors on Zn homeostasis should be taken into account. Different processing technologies, promoters, and inhibitors of Zn bioavailability in rice grains is also important (Nakandalage et al. 2016). Achieving the target of 30 ppm Zn content in brown rice requires strategic use of Zn fertilizers as many as rice fields have low available Zn (Johnson-Beebout et al. 2009). Fertilizer N and P applications during grain filling also promote Zn uptake and remobilization (Khan et al. 2015). Soil redox potential

also affects heavy metals (including Fe, Zn, and Cd) availability for uptake by the plants. Flooding practice increases Fe availability but decreases Zn and Cd availability (Slamet-Leodin et al. 2015).

Zn accumulation incorporates many processes, such as uptake, remobilization, transport in the plant, and environmental interaction. It is important to study genetic properties of each of these component traits. Increasing the efficiency of each of these processes affecting Zn uptake from roots to its accumulation in endosperm should allow the maximum accumulation of Zn in the grain. Zn-efficient and non-efficient varieties should be tested under Zn sufficient and deficient conditions at various growth stages to define the genetic capacity of Zn uptake (Nakandalage et al. 2016). On the other hand, transgenic approaches by modifying genes controlling those traits may result in greater grain Zn content compared to using conventional

breeding, however, this introduces further complexity in gaining regulatory approval for such varieties to be grown.

Global climate change increases CO₂ concentration in the air. Some studies indicated that increasing CO₂ affects plant growth, yield, and quality of cereals, including rice. Without any other limitations, increasing the CO₂ level increased photosynthesis and thus yield. Nevertheless, for grain quality, increased CO₂ level reduces all micro nutrient content, including Zn content in rice (Nakandalage et al. 2016; Myers et al. 2014). Thus, as CO₂ rises, greater efforts may be needed to retain Zn levels at sufficient levels to supply Zn requirement of rice consumers in the future.

In this study, five lines, i.e. IR64 (8.87 t/ha), BR28 (8.14 t/ha), IR91152AC-81 (8.03 t/ha), NSICRc222 (7.29 t/ha), NSICRc238 (7.04t/ha) had higher yield compared to Ciherang (popular variety; 5.16 t/ha). Nevertheless, The lines had relatively low Zn content (19.00-24.80 ppm). On the other hand, seven lines had higher Zn content compared to Ciherang (23.35 ppm), and among them five lines had comparable yield to Ciherang, i.e. BR7840-54-2-5-1 (5.75 t/ha; 33.08 ppm), IR84020-84-2-3-2 (5.70 t/ha; 29.9 ppm), IR68144-2B-2-2-3-1-166 (5.08 t/ha; 34.22 ppm), Vanjakohonandiana (4.39 t/ha; 31.73 ppm), IR10M195 (IR84842-35-3-1-1-2-2) (3.95 t/ha; 35.68 ppm). These lines had comparable agronomic characteristics with current popular varieties and thus have good prospects for further testing and utilization. This finding is a good start in developing varieties with high grain Zn in Indonesia. Some efforts should be conducted simultaneously and continuously, i.e. searching for new and better donors for high Zn content in rice grain, genetic and physiological studies of Zn uptake, transport, and accumulation, crossing and selection of breeding materials, and further evaluations required prior to variety release. Resistance to brown plant hopper (BPH) biotype 1 and bacterial leaf blight (BLB) strain III are required for releasing lowland rice varieties in Indonesia. Additionally, resistance to other BPH biotypes and BLB strains, and other diseases such as blast and tungro are also important for varietal durability in farmer's fields. Growth duration, grain shape, and physicochemical characteristic would also determine stake holders acceptance of the variety.

Further effort to compile yield and Zn content is needed. Genetic materials in this study provided genotypes having high Zn content and high yield. Hybridization among them may allow selection of lines having the combination of high Zn and high yield in one genotype. Previous studies suggested that wild and primitive rice have large and useful genetic variation in grain Zn concentration. Nevertheless, it has not been utilized maximally to improve grain Zn concentration in its bioavailability (Nakandalage et al. 2016). On the other hand, molecular and transgenic approaches have been used to generate high grain Zn content rice varieties. The transgene used to increase Fe and Zn content is from the Os-NAS gene family and resulted in Fe content up to 15 ppm and Zn content of 45.7 ppm in polished rice grains (Trijatmiko et al. 2016).

Utilization of high Zn content rice varieties in targeted areas with known Zn deficiency among the population will

improve Zn intake among those at risk from micronutrient malnutrition. This is contingent on developing varieties with high Zn content in the rice grain while maintaining or increasing yield. Once the variety is disseminated and adopted, this becomes a sustainable method of improving nutrition among the population of rice consumers, provided that seed purity (and thus nutritional benefit) is maintained, and farmers elect to use the variety.

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REFERENCES

- Atique-ur-Rehman FM, Nawaz A, and Ahmad R. 2014. Influence of boron nutrition on the rice productivity, kernel quality and biofortification in different production systems. *Field Crops Res.* 169, 123-131. DOI: 10.1016/j.fcr.2014.09.010
- ICRR [Indonesian Center for Rice Research]. 2015. Description of Rice Variety. Indonesian Center for Rice Research, Subang. [Indonesian]
- Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, Webb P. 2013. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost?. *The Lancet* 382 (9890): 452-477.
- Bouis H. 2004. The potential of biofortified rice for reducing micronutrient malnutrition. In: *Proceedings of the 1st International Conference on 'Rice for the future'*. 31 August-2 September, Bangkok, Thailand.
- Bouis HE, Hotz C, McClafferty B, Meenakshi JV, Pfeiffer WH. 2011. Biofortification: a new tool to reduce micronutrient malnutrition. *Food Nutr Bull* 32: S31-S40.
- Graham R, Senadhira D, Beebe S, Iglesias C, Monasterio I. 1999. Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. *Fields Crops Res* 60: 57-80.
- Gregorio GB, Senadhira D, Htut T, Graham RD. 2000. Breeding for trace mineral density in rice. *Food Nutr Bull* 21 (4): 382-386.
- Guno MJV. 2004. Nutrition status of mother and children in the Philippines. In: *Hardinsyah, Puruhita A (eds.). Proceeding of Food and Nutrition Innovation for Optimization of Children Growth and Development*. Mei 10-11, 2004, Jakarta-Indonesia, American Soybean Association, NY. [Indonesian]
- Hallauer AR, Miranda JB. 1995. *Quantitative genetics in maize breeding*. 2nd. Iowa State University Press, Ames. United States of America.
- Herman S. 2009. Review on the problem of Zinc deficiency, program prevention and its prospect. *Media Penelit. dan Pengembang. Kesehatan*. 29 (Suppl. 2): S47-S83.
- Herman S. 2007. Study of micro nutrient in Indonesia (special emphasis on Vitamin A deficiency, Anemia, and Zinc). [Research Report]. Puslitbang Gizi, Bogor. [Indonesian]
- Herman S, Griffin IJ, Suwanti S, Ernawati F, PermaesihDewi, Pambudi D, Steven AA. 2002. Cofortification of iron-fortified flour with zinc sulfate, but not zinc oxide, decreases iron absorption in Indonesian children. *Am J Clin Nutr* 76: 813-817.
- Hotz C, Brown KH. 2004. Assessment of the risk of Zn deficiency in populations and options for its control. *Food Nutr Bull* 25: S91-S204.
- Impa SM, Johnson-Beebout SE. 2012. Mitigating zinc deficiency and achieving high grain Zn in rice through integration of soil chemistry and plant physiology research. *Plant Soil* 361: 3-41.
- IRRI. 2006. *Bringing Hope, Improving Lives: Strategic Plan 2007-2015*. International Rice Research Institute, Los Banos, The Philippines.
- IRRI. 2007. *Crop Stat Ver 6.1. Tutorial Manual*. International Rice Research Institute, Los Banos, The Philippines.
- Johnson AAT, Kyriacou B, Callahan DL, et al. 2011. Constitutive overexpression of the osnas gene family reveals single gene strategies

- for effective iron-and zinc-biofortification of rice endosperm. *PLoS ONE* 6 (9): e24476. DOI: 10.1371/journal.pone.0024476
- Johnson-Beebout SE, Lauren J, Duxbury JM. 2009. Immobilization of Zinc fertilizer in flooded soils monitored by adapted DTPA soil test. *Commun. Soil Sci Plant Anal* 40: 1842-1861.
- Khan WUD, Faheem M, Khan MY, Hussain S, Maqsood MA, Aziz T. 2015. Zinc requirement for optimum grain yield and zinc biofortification depends on phosphorus application to wheat cultivars. *Roman Agric Res* 32: 1-9.
- Li S, Li W, Huang B, et al. 2013. Natural variation in PTB1 regulates rice seed setting rate by controlling pollen tube growth. *Nature Commun* 4: 2793-2807. DOI: 10.1038/ncomms3793
- Mares-Perlman JA, Subar AF, Block G, Greger JL, Luby MH. 1995. Zinc intake and sources in the US adult population: 1976-1980. *J Am Coll Nutr* 14: 349-357.
- Mc Clean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. 2009. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. *Public Health Nutr* 12: 444-454.
- Morrison SA, Russell RM, Carney EA, Oaks EV. 1978. Zinc deficiency: a cause of abnormal dark adaptation in cirrhotics. *Am J Clin Nutr* 31: 276-281.
- Myers SS, Wessells KR, Kloog I, Zanobetti A, Schwartz J. 2015. Effect of increased concentrations of atmospheric carbon dioxide on the global threat of zinc deficiency: a modelling study. *Lancet Global Health* 3: e639-e645. DOI: 10.1016/s2214-109x(15)00093-95.
- Nakandalage N, Nicolas M, Norton RM, Hirotsu N, Milham PJ, Seneweera S. 2016. Improving rice zinc biofortification success rates through genetic and crop management approaches in a changing environment. *Front Plant Sci*. DOI: 10.3389/fpls.2016.00764.
- Peng S, Khus GS, Virk P, Tang Q, Zou Y. 2008. Progress in ideotype breeding to increase rice yield potential. *Field Crops Res* 108: 32-38.
- Pinaria A, Baihaki A, Setiamiharja R, Daradjat AA. 1995. Genetic variability and heritability of biomass characters of 53 soy bean genotypes. *Zuriat* 6 (2): 88-92. [Indonesian]
- Pinson SRM, Tarpley L, Yan W, et al. 2015. Worldwide Genetic Diversity for Mineral Element Concentrations in Rice Grain. *Crop Sci*. 55: 294-311. DOI: 10.2135/cropsci2013.10.0656
- Prom-u-thai C, Rerkasem B, Cakmak I, Huang L. 2010. Zinc fortification of whole rice grain through parboiling process. *Food Chem*. 120: 858-863. DOI: 10.1016/j.foodchem.2009.11.027.
- Ruchjaningsih. 2006. Mulcing effect to phenotypic performance and genotypic parameters of 13 potato genotypes in medium elevation field areas of Jatinangor. *Jurnal Hortikultura*. 16 (4): 290-298. [Indonesian]
- Sakagami N, Shiotsu F, Agustiani N, Komatsuzaki M, Nitta Y. 2016. Characteristics of elemental compositions and organic component of Indonesian rice: Examples of several products in Indonesia including organic rice. *Trop Agric Dev* 60 (2): 65-70.
- Sala M, Geetha S. 2015. Correlation and path analysis for iron and zinc content in segregating population of rice. *Rice Genom Genet* 6 (1): 1-12.
- Salunke R, Neelam K, Rawat N, Tiwari VK, Dhaliwal HS, Roy P. 2011. Bioavailability of iron from wheat aegilops derivatives selected for high grain iron and protein contents. *J Agric Food Chem* 59: 7465-7473.
- Sharma A, Patni B, Shankhdhar D, Shankhdhar SC. 2013. Zinc-an indispensable micronutrient. *Physiol Mol Biol Plants* 19: 11-20. DOI: 10.1007/s12298-012-0139-131
- Slamet-Leodin IH, Johnson-Beebout SE, Impa S, Tsakirpaloglou N. 2015. Enriching rice with Zn and Fe while minimizing Cd risk. *Front Plant Sci* 6 (121): 1-9. DOI:10.3389/fpls.2015.00121
- Sleper DA, Poehlman JM. 2006. *Breeding Field Crops*. 5th ed. Wiley-Blackwell Publ., New York.
- Slaton NA, Charles E. Wilson Jr., Ntamatungiro S, Norman, RJ, Boothe, DL. 2001. Evaluation of zinc seed treatments for rice. *Agron J* 93: 152-157.
- Sperotto RA, Ricachenevsky FK, Waldow VA, Fett JP. 2012. Iron biofortification in rice: It's a long way to the top. *Plant Science* 190: 24-39.
- Taufiqurrahman, Hadi H, Julia A, Herman S. 2009. Vitamin A and Zinc deficiency as risk factors for stunting in infants in West Nusa Tenggara. *Media Penelit. dan Pengembang. Kesehatan*. 29 (Suppl. 2): S84-S94. [Indonesian]
- Trijatmiko KR, Dueñas C, Tsakirpaloglou N, Torrizo L, Arines FM, Adeva C, Balindong J, Oliva N, Sapasap MV, Borrero J, Rey J, Francisco P, Nelson A, Nakanishi H, Lombi E, Tako E, Glahn RP, Stangoulis J, Chadha-Mohanty P, Johnson AAT, Tohme J, Barry G, Slamet-Loedin, IH. 2016. Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. *Sci Rep* 6 (19792): 1-13. DOI: 10.1038/srep/19792.
- Wessells KR, Brown KH. 2012. Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. *PLoS ONE* 7: e50568. DOI: 10.1371/journal.pone.0050568
- Yuwono PD, Murti RH, Basunanda P. 2015. Morphological genetic variations of twenty sweet corn inbred lines s7 generations. *Ilmu Pertanian* 18 (3): 127-134.