Fe and Zn content of various genetic background of released rice varieties in Indonesia

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Abstract. Fe and Zinc deficiency is one of the most prevalence micro nutrient disorder in Indonesia. Increasing of Fe and Zn content in rice grain is expected to be useful to reduce the micronutrient deficiency. This research was aimed to identify rice varieties containing high Fe and Zn content and initially study the correlation among some related traits. The experiment was conducted in October – December 2016 by testing 176 released varieties and 4 elite rice lines. The samples were prepared as brown rice. Fe and Zn contents were measured using XRF Oxford machine which is validated by ICP method. The results showed that Fe content of the tested genotypes is normally distributed ranged from 9.70 ppm to 17.40 ppm with the mean of 13.03 ppm and the Zn content is not normally distributed ranged from 15.80 ppm to 31.10 ppm with the mean of 22.41 ppm. Majority of the released rice varieties in Indonesia had lower Fe and Zn content compared to the most popular variety, Ciherang (12.20 ppm and 24.70 ppm). Kalimutu had the highest both for Fe and Zn content (17.40 ppm and 31.10 ppm). Fe and Zn content are positively correlated and breeding effort for both traits is prospective.

1. Introduction

Micro nutrient deficiency or hidden hunger has affecting around two billion people in developing country [1]. Fe and Zinc deficiency is one of the most prevalence micro nutrient disorder in the world include Indonesia. Deficiency of both micro nutrients caused big losses to the country due to decrease productivity, livelihood quality of the people, etc. Zn deficient itself caused 0.04-0.16% loss of GDB annually [2]. It is predicted that one-third of the world's population are at risk of Zn deficiency [3]. The prevalence of Zn deficiency in Indonesia ranged from 10 to 90 % according to demographical groups [4].

Genetically increasing of Fe and Zn content in rice grain, which called as Fe and Zn biofortification [5], is expected to be useful to reduce the Fe and Zn deficiency in the world. Evaluation of micro nutrient content of rice accession had been started at the early this milenia. Nearly 7000 samples in IRRI and around 500 samples in Indonesia had been evaluated indicating diverse variation on Fe and Zinc content [6-7]. Genetic study, molecular analysis, and also transgenic approach [8-11] had been reported to explore possible effort to increase Fe and Zn content in rice.

Searching of high Fe and Zn content of available genotypes in Indonesia is required to find genetic donors for the further breeding and scientific efforts. Identification of old and new released variety
with high Fe and Zn content would hopefully provide short cut strategy to combat Fe and Zn deficiency by utilizing high Fe and Zn content available released varieties. This research aimed to identify variety having high Fe and Zn content and initially study the correlation among some traits in rice.

2. Methodology
The experiment was conducted in October – December 2016 in Plant Breeding Laboratory of Indonesian Center for Rice Research Institute (ICRR) in Sukamandi, Subang, West Java, Indonesia. The materials to be tested were 176 released varieties and 4 elite rice lines. The grains samples were provided by ICRR germ plasm collection. The samples were prepared as brown rice. Fe and Zn content were measured using XRF Oxford machine which is validated by ICP method. About 50 g grains from each accession were unhusked by Satake THU Testing Husker. Brown rice samples were then sorted to get only good health and fully filled grain for Zn content measurement. Descriptive statistical analysis was conducted using Microsoft Excel 2010 and Minitab ver. 14.0 software.

3. Results and Discussion
The results showed that Fe content of the tested 180 genotypes is normally distributed ranged from 9.70 to 17.40 ppm with the mean of 13.03 ppm for Fe (Figure 1) but not normally distributed for the Zn which ranged from 15.80 to 31.10 ppm with the mean of 22.41 ppm for Zn (Figure 1). Cihetang, the most popular variety, had Fe and Zn content of 12.20 and 24.70 ppm, respectively. The three highest Zn content varieties are Kalimutu (31.10 ppm), Situ Patenggang (29.60 ppm), and Inpago 9 (28.50 ppm), while three highest Fe content variety are Kalimutu (17.40 ppm), Lambur (17.10 ppm), and Inpago 8 (16.10 ppm) (Table 1). Kalimutu had the highest value either for Fe or Zn content. Nevertheless, around 64% of the genotypes had Zn content lower than the population mean, indicating fewer genotypes having relatively high Zn content. Thus, most of the released varieties needed to be improved in term of Zn content.

Previous study showed that Cihetang had Fe content of 11.93 ppm and Zn content of 23.04 ppm in a trial in Central Java. It was identified a high Zinc content line IR 97477-115-1-CRB-0-SKI-1-SKI-0-2 (29.61 ppm Zn) without any penalty on yield [12]. The line had been release in 2019 as a new variety termed as Inpah IR Nutri Zinc [13]. On the other hand, a set of rice lines having Zn content ranged from around 16 ppm to 26 ppm while Cihetang having 23.45 ppm [14]. Testing on ultra-early maturing rice lines had also found variation of Zn content in rice grains, ranging from 20.03 ppm (Inpah 13) to 27.3 ppm (UG1) [15]. It is also reported that a set of lines having Zinc content ranged from around 20 ppm to 29 ppm while Cihetang in the trial having 22 ppm Zn content [16]. There was a variation on the Fe content across environment, due to genetic and environment interaction [17]. Always including check variety as standard such as the most popular variety in each location trial will give more robust result.

The Harvest Plus breeding programs for biofortified rice targeted 13 ppm Fe content and 28 Zn content in polished rice to reach 30% of the Estimated Average Requirement (EAR) [18]. Measurement in this study and some reported ones in Indonesia was based on brown rice condition. Polishing is predicted to reduce Zn content for about 20%, remaining the Zn in the endosperm for around 80% [19]. It means that to achieve 28 ppm Zn in polished rice required to have roughly approximated at around 35 ppm Zn content in brown rice. Previous study at a trial in West Java had also identified IR68144-2B-2-2-3-1-166 having 34.22 ppm of Zn with comparable yield with Cihetang [20]. Development of properly high Zinc as well as high yield and resistant varieties remained challenging. Fe content is mostly accumulated in aleuron layer which the polished rice tended to have very low Fe content [21]. Therefore, special effort was needed to accumulate more Fe in the endosperm. Transgenic approach might be more powerful to solve such problem [11].
Ten genotypes having the highest Zn content showed that mostly the higher Zn content genotypes are upland rice variety from various year of release. On the other hand, ten genotypes having the lowest Zn content showed that mostly the lower Zn content genotypes are swampy rice variety of various year of release. Higher Zn content genotypes had broader genetic background, indicated by the history of the parental, which some of them have introduces genetic background from various countries. IR 64, former most popular variety in Indonesia, unfortunately has Zn content at medium level. Ciherang, the recent most popular variety which has dominantly IR 64 genetic background, also has Zn content at medium level (Table 1). It indicated that utilization of high Zn content genetic varieties supposed to give advantage in the resulted breeding materials, in term of increasing of Zn content of the future released variety for various targeted agroecosystem. Furthermore, searching of rice genotypes having higher level of Zinc content is still required to attain the target of 28 ppm Zn content in polished rice [18] or roughly approximated at least 35 ppm Zn content in brown rice.

**Table 1.** Comparison of Fe and Zn content from several accessions both 10 highest and lowest Zn content

| Accession Number | Variety       | Release Year | Fe (ppm) | Zn (ppm) | Ecosystem | Parental or Pedigree | Genetic Background |
|------------------|---------------|--------------|----------|----------|-----------|----------------------|--------------------|
| Genotypes having Highest Zn Content |               |              |          |          |           |                      |                    |
| 7319             | Kalimutu      | 1994         | 17.4     | 31.1     | Upland    | -                    | Introduced         |
| 1484             | Situ          | 2003         | 14       | 29.6     | Upland    | Kartuna /TB47H-MR-10 | Local              |
|                  | Patenggang    |              |          |          |           |                      |                    |
|                  | Inpago 9      | 2012         | 15.5     | 28.5     | Upland    | UPLRI/IRAT15         | Introduced         |
| 9337             | Digul         | 1996         | 14.6     | 27.8     | Lowland   | IR19661//IR 64//IR19661 | Introduced         |
|                  | Celebes       | 2000         | 11.4     | 27.7     | Lowland   | IR31892-100-3-3-3-3 | Introduced         |
|                  |               |              | 16       | 27.3     | Lowland   | -                    | -                  |
| 9336             | Inpago 8      | 2011         | 16.1     | 26.9     | Upland    | Cirata/TB177         | Local              |
| 4547             | Wayrarem      | 1994         | 16.1     | 26.8     | Upland    | IR83/Careon/B981k    | Introduced         |
| 7318             | Inpago 6      | 2010         | 11.4     | 26.7     | Upland    | IRAM2165/NC1281      | Introduced         |
| 4929             | Danau Tempe   | 1991         | 13.4     | 26.4     | Upland    | IR83/Careon/B981k    | Introduced         |
| Zn Content of most popular variety |               |              |          |          |           |                      |                    |
| 4842             | Ciherang      | 2000         | 12.2     | 24.7     | Lowland   | IR18249-53//IR19661-131-3-1//IR19661-131///IR64//IR 64 | Introduced         |
| 1461             | IR 64         | 1986         | 13.6     | 23.9     | Lowland   | IR5657/IR2061        | Introduced         |
This study showed that Fe and Zn content are positively correlated, while Fe content had negative correlation with growth duration of the plant (Table 2). It is in accordance with a reported that Fe and Zn content are moderately correlated at 0.5 for brown rice and 0.3 for polished rice [21]. It indicated that indirect selection of Fe content through Zn content might be possible. Selecting high Zn content might also provide high Fe content.

**Table 2.** Prediction of correlation value among traits of 180 rice genotypes.

|    | 1    | 2    | 3    | 4    |
|----|------|------|------|------|
| 2  | 0.276* |      |      |      |
| 3  | 0.025  | -0.052 |      |      |
| 4  | 0.099  | 0.023 | -0.01 |      |
| 5  | -0.232* | -0.154 | -0.03 | -0.025 |

Remark: 1. Fe content (ppm); 2. Zn content (ppm); 3. 1000 grain weight (g); 4. Amylose content (%); 5. Plant Growth Duration (days)

High Zinc rice variety had been released in some countries, include Indonesia and Bangladesh. It had been released a variety namely BRRI Dhan 84 which has Zn content of 27.6 ppm in polished grains. It is a cross of BRRIDhan29/IR68144//BRRIDhan28///BR11 [22]. Indonesia had release high Zn rice variety at 2019 namely Inpari IR Nutri Zinc with potential Zn content of 34.5 ppm in brown rice grains. It is made from the cross of IR91153-AC 82/IR05F102//IR 68144-2B-2-3-1-166///IRR1145 [13]. Both varieties shared the same parent, i.e. IR68144. It migh indicate that IR68144 was one of the source of high Zn content character in rice plant. Searching of genotypes having higher Zn content than this variety is important to further increase the Zn content of next released variety.

4. Conclusions

Based on brown rice sample measurement, Fe content among 180 genotypes ranged from 9.70 ppm to 17.40 ppm with the mean of 13.03 ppm, while Zn content ranged from 15.80 ppm to 31.10 ppm with the mean of 22.41 ppm. The recent most popular variety, Ciherang, has Fe (12.20 ppm) and Zn (24.70 ppm) content at medium level. None of the tested varieties having enough Zn level to provide 30% of...
the Estimated Average Requirement. Searching of rice genotypes having higher Fe and Zn content is still needed for more powerful breeding efforts. Fe and Zn content are moderately correlated, so that selection for high Zn content has chance to get high in Fe simultaneously. Overall, breeding for high Fe and Zn content is prospective and challenging.

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