High-zinc rice as a breakthrough for high nutritional rice breeding program

U Barokah1,5, U Susanto2, M Swamy3, D W Djoar4 and Parjanto4

1Master student Agronomy Program Graduate School of Sebelas Maret University, Jl. Ir. Sutami 36 A, Kentingan, Surakarta, Central Java 57126 Indonesia
2Indonesian Center of Rice Research, Sukamandi, Jl. Raya, Patok Besi, Subang No.9, Rancajaya, Patokbeusi, Subang, West Java 41256 Indonesia
3International Rice Research Institute, Pili Drive, UPLB, Los Baños, 4031 Laguna, Philippines
4Department of Agrotechnology, Faculty of Agriculture, Sebelas Maret University, Surakarta, Central Java 57126 Indonesia
5Corresponding author: barokahumi@yahoo.com

Abstract. WHO reported climate change already takes 150,000 casualties annually, due to the emergence of various diseases and malnutrition caused by food shortages and disasters. Rice is the staple food for almost all of Indonesian citizens, therefore Zn biofortification on rice is expected to be effective, efficient, massive, and sustainable to overcome the Zn nutritional deficiency. This study aims to identify rice with high Zn content and yield for further effort in releasing this variety. Ten lines along with two varieties as a comparison (Ciherang and Inpari 5 Merawu) were tested in Plumbon Village, Mojolaban Subdistrict, Sukoharjo Regency during February-May 2017. The experiment was designed in a Randomized Completely Block Design with four replications on a 4 m x 5 m area, with 25 cm x 25 cm plant spacing using seedling transplanting techniques of 21 days old seeds. The results showed that the plant genotypes treated had differences in yield characteristics, heading date, harvest age, panicle number, filled and un-filled grain per panicle, seed set, 1000 grains weight, Zn and Iron (Fe) content in rice grain. B13884-MR-29-I-I line (30.94 ppm Zn, 15.84 ppm Fe, 4.11 ton/ha yield) and IR 97477-115-I-CRB-0-SKI-1-SKI-0-2 (29.61 ppm Zn, 13.49 ppm Zn, 4.4 ton/ha yield) are prospective variety to be released. Ciherang had Zn content of 23.04 ppm, 11.93 ppm Fe, and yield of 4.07 t/ha.

1. Introduction
Climate changes characterized by rising air temperatures and changes in the magnitude and distribution of rainfall have had a wide impact on many aspects of human life [1]. Increased air temperature directly affect the production of cereals including rice, the staple food of the Indonesian population. Indonesia Country Study on Climate Change 1998 [2] reported the vulnerability of agricultural production systems by climate change, as in 1991 and 1994, climate anomaly caused Indonesia to import rice (600,000 tons in 1991 and more than one million tons in 1994). Rice and other cereals are very sensitive to temperature change even in small degree rise. Rice reproductive part called spikelet will become sterile if the air temperature increase, which will affect its productivity [3]. FAO states that climate change, as well as changes in disease patterns and pests, will affect how food production systems will be done in the future. It will also have a direct impact on food security, and poverty levels, especially in countries with dependence on the agricultural sector. WHO reports that
climate change caused 150,000 casualties annually due to the emergence of various diseases and malnutrition due to food shortages and disasters [4].

Indonesia needs additional food production along with increasing population [5]. Based on Statistics Bureau data, the national rice demand reaches the number of 28 million tons per year or 139 kg per capita per year or 380 grams per day while the total population of Indonesia in 2015 was 254.9 million with the poor population was 28.51 million people [6]. Poor people living in this condition will experience health problems because they cannot afford to buy rice with high nutrition such as those containing iron and zinc (lack of micronutrient) due to its expensive price.

Zinc is a component with more than 300 enzymes to repair cell damage, retain fertility, synthesize proteins, and boost immunity among many important functions in human health [7]. Symptoms of zinc deficiency in both small and large level can lead to stunted growth, eczema, hair loss, delayed sexual maturity and mental development disorders. Micronutrition study in Indonesia found that the prevalence of zinc deficiency children is 36.1%, with the highest percentage occur in West Nusa Tenggara province of 46.6% and the lowest in West Sumatera province of 11.7% while the percentage for South Sulawesi province was 22.7%.

An efforts to overcome the lack of micronutrients supply is urgent to bring the provision of supplementation (fertilization) and biofortification sustainably. Provision of supplementation through zinc fertilization of rice can increase zinc levels in rice [8]. However, this method is less effective due to nutritional loss from runoff, leaching, and the evaporation process. Therefore, a new strategy to overcome malnutrition of micronutrient is by the mean of biofortification. Biofortification provides a cost-effective and sustainable solution in tackling the lack of nutrients supply [9]. This method is one of the plant breeding strategies to increase the zinc content in rice while improving the nutrition capacity with relatively low cost. Breeding materials are conventionally formed (hybridization and selection) or non-conventional (another culture and gene transformation). High zinc levels rice produced by these, inexpensive cost, production can be consumed directly by the middle to lower community as a source of energy and sources of nutrients [10]. These results provide rice with high micronutrient content. Rice with high micronutrient content can improve the micronutrient supply for consumers which will overcome the micronutrient deficiency.

In this regard, IRRI (International Rice Research Institute) and Indonesian Center of Rice Research (ICRR) has assembled rice lines that have high zinc content. Selection and yield test stages have been done to obtain high-zinc rice lines. Multi-location tests are being conducted in 16 locations in various parts of Indonesia to obtain the adaptive high-zinc variety to meet the national needs. The purpose of this study was to identify high-zinc rice genotypes with high yield which will be proposed for the release of new rice variety.

2. Methods

The experiment of high-zinc rice lines was carried out in 2017 during the rainy season (February to May 2017) in Plumbon Village, Mojolaban Subdistrict, Sukoharjo Regency, Indonesia with an altitude of ± 120 meters above sea level. A total of 10 lines of high zinc content from IRRI and ICRR along with two comparative varieties (Inpari 5 Merawu and Ciherang) were tested. The experiment was designed in a Randomized Completely Block Design with four replications in 4 m x 5 m plots with planting space of 25 cm x 25 cm. Transplanting was applied to 21 days old seedlings. Observations including the agronomic characteristics such as plant height, number of productive vegetative and generative phases, heading date, harvest age, panicle number, filled and un-filled grain per panicle, weight of grain per seed, seed set, 1000 grain weight, yield, Zinc content and Fe content in brown rice grains with XRF tool. The cultivation technique was carried out in accordance with the guidelines of integrated crop management. The observed data were analyzed using crop start statistic software and the mean difference between lines were analyzed using the LSD method at the threshold level of error at 5%.
3. Result and discussion

Variance analysis showed differences between genotypes in agronomic character and genotype results of line observed (Table 1). The results showed that the tested genotypes had differences on yield, heading date, harvest age, panicle number, filled and un-filled grain per panicle, the weight of filled grain per clumps, seed set, 1000 grain weight, Zn and Iron (Fe) content in brown rice grains. This indicates that the differences occur between genotypes, not simply because of environmental influences. The appearance of a variety or line will be influenced by two factors: the genetic properties it carries and the environment where it is cultivated; if the environment is uniform, the different appearance of the plant character will be influenced only by the genetic properties.

Table 1. Variance analysis of results, zinc and Fe content and agronomic properties of 10 high zinc rice lines along with 2 check varieties, Sukoharjo, MH 2017.

| No. | Properties                | F Value | Significance |
|-----|---------------------------|---------|--------------|
| 1   | Yield                     | 8.15    | 0.000**      |
| 2   | Zink Content              | 12.94   | 0.000**      |
| 3   | Fe Content                | 3.75    | 0.002**      |
| 4   | Vegetative Plant Height   | 0.89    | 0.556        |
| 5   | Generative Plant Height   | 0.63    | 0.788        |
| 6   | Number of Vegetative Tillers | 1.42   | 0.21         |
| 7   | Number of Generative Tillers | 1.18  | 0.336        |
| 8   | Heading date              | 27.01   | 0.000**      |
| 9   | Harvest date              | 70.36   | 0.000**      |
| 10  | Panicle number/plant      | 3.89    | 0.001**      |
| 11  | Filled grain/panicle      | 4.25    | 0.001**      |
| 12  | Unfilled grain/panicle    | 6.14    | 0.000**      |
| 13  | Weight filled grain/plant | 1.59    | 0.015*       |
| 14  | Seed Set                  | 6.21    | 0.000**      |
| 15  | 1000 grain weight         | 13.25   | 0.000**      |

** = significantly different at $\alpha=0.01$; * = significantly different at $\alpha = 0.05$

Observation on the yield variables of high zinc rice line in Sukoharjo shows significant differences (Table 2) as shown in IR 95133:1-B-16-14-10-GBS (5.12 ton/ha) and IR 97477-115-1-CRB-0-SKI-3-SKI-0-2 (4.84 ton/ha) which gives a higher yield than the best of Ciherang check variety yield (4.07 ton/ha). IR 97477-115-1-CRB-0-SKI-3-SKI-0-2 shows highest results compared to all checks. This is because the strain has a lot of panicles (17 panicles) and more resistant to pest attack (majorly aphid and stem borer). The more the number of panicles formed, the more results will be obtained. However, IR 99284-15-3-2 (2.59 ton/ha) and IR 99284-15-3-2 (2.73 ton/ha) performed a lower yield than the best of Ciherang check variety yield (4.07 ton/ha). This is because IR 99284-15-3-2 at the time of the initial vegetative phase and the final vegetative were hit by aphid attack while the other lines are relatively resistant to aphid attack. Efforts to overcome by means of pesticide application have been done, but the strain still shows less growth compared to another line. B13884E-MR-30-2 line shows a lower yield than the best of Ciherang check variety because it has fewer numbers of tillers.
Table 2. Average yield, Zinc and Fe content of 10 high zinc rice line with 2 check varieties, Sukoharjo, MH 2017

| No. | Genotype lines       | Yield | Heading date | Harvest date | Panicle number/plant | Filled grain/panicle | Filled grain weight/plant | Unfilled grain/panicle | Seed Set | 1000 grain weight | Zn content | Fe content |
|-----|----------------------|-------|--------------|--------------|----------------------|---------------------|----------------------------|------------------------|----------|-------------------|------------|------------|
| 1   | IR 95133:1-B-16-14-10GBS | 5.12  | 81           | 105          | 14                   | 89                  | 28.06                      | 31                     | 73.59    | 22.97            | 25.03      | 13.11      |
| 2   | IR 99270-34-2-1       | 4.19  | 93           | 1/9          | 17                   | 54                  | 21.47                      | 52                     | 52.78    | 23.63            | 24.13      | 13.23      |
| 3   | IR 97477-115-1-CRB-0-SKI-1-SKI-0-2 | 4.40  | 84           | 107          | 18                   | 92                  | 33.79                      | 24                     | 79.66    | 20.92            | 29.61      | 13.49      |
| 4   | IR 99284-15-3-2       | 2.59  | 90           | 117          | 17                   | 75                  | 29.72                      | 36                     | 65.36    | 23.92            | 27.45      | 13.43      |
| 5   | IR 97477-115-1-CRB-0-SKI-3-SKI-0-2 | 4.84  | 86           | 107          | 17                   | 82                  | 31.18                      | 33                     | 71.19    | 22.88            | 27.59      | 12.70      |
| 6   | IR 97646-108-1-CRB-0-SKI-1-SKI-2-2 | 4.59  | 84           | 108          | 16                   | 95                  | 32.64                      | 22                     | 80.71    | 22.44            | 26.79      | 12.48      |
| 7   | IR 99680-3-CRB-0-SKI-1-SKI-2-5 | 3.98  | 84           | 106          | 20                   | 79                  | 36.03                      | 27                     | 74.31    | 22.83            | 27.13      | 12.84      |
| 8   | B13884E-MR-22-3-1     | 3.62  | 81           | 104          | 16                   | 98                  | 37.85                      | 24                     | 80.72    | 24.67            | 25.73      | 13.40      |
| 9   | B13884-MR-29-1-1      | 4.11  | 73           | 97           | 15                   | 113                 | 44.22                      | 24                     | 82.05    | 25.97            | 30.94      | 15.84      |
| 10  | B13884E-MR-30-2       | 2.73  | 87           | 108          | 13                   | 113                 | 30.69                      | 29                     | 79.21    | 21.39            | 28.25      | 13.23      |
| 11  | Inpari 5 Merawu       | 4.01  | 81           | 105          | 13                   | 80                  | 28.64                      | 35                     | 68.74    | 27.46            | 23.61      | 12.88      |
| 12  | Ciherang              | 4.07  | 84           | 106          | 12                   | 91                  | 28.67                      | 32                     | 73.83    | 25.85            | 23.04      | 11.93      |

LSD 5% 0.76 3 2 4 23 13.07 10 9.70 1.54 1.93 1.40
CV (%) 13.20 2.30 1.30 15.80 17.50 27.90 22.10 9.00 4.50 5.00 7.40

Description:
Yellow print = significantly higher than the best check
Green print = significantly lower than the best check
Red Print = best check
B13884-MR-29-1-1 line resulted heading and harvest date 73 and 97 Days After Planting (DAP) respectively, earlier than Inpari 5 Merawu of 81 DAP and 105 DAP. IR 99270-34-2-1 line (93 DAP and 119 DAP), IR 99284-15-3-2 (90 DAP and 117 DAP), and B13884-MR-30-2 (87 DAP and 108 DAP) showed longer heading and harvesting date time than Inpari 5 Merawu, 81 DAP and 105 DAP, respectively. Similarly, the IR 97477-115-1 CRB-0-SKI-3-SKI-0-2 (86 DAP) line having a heading date longer than Inpari 5 Merawu (90 DAP) and IR 97646-108-1-CRB-0-SKI-1-SKI-2-2 (108 DAP) which has a longer harvest date than Inpari 5 Merawu of 105 DAP. The lines that have a faster harvest date (maturity) will be harvested faster compared to the line that has a longer physiological lifetime (length). Differences in flowering age are caused by genetic differences in varieties and line, characterized by different vegetative phase and different plant responses to the growing site [12].

IR 99270-34-2-1 (17 panicles), IR 97477-115-1-CRB-0-SKI-1-SKI-0-2 (18 panicles), IR 99284-15-3-2 (17 panicles), IR 97477-115-1-CRB-0-SKI-3-SKI-0-2 (17 panicles) and IR 99680-3-CRB-0-SKI-1-SKI-2-5 (20 panicles) have more number of panicles than the best check of Inpari 5 Merawu (13 panicles). However, IR 99270-34-2-1 (52 grains) have more number of filled grain per panicle than the best check of Ciherang (32 grain) but the number of filled grains per panicle is less than the best check of Ciherang (91 grains). IR 97646-108-1-CRB-0-SKI-1-SKI-2-2 (22 grains) line has less number of unfilled grains per panicle than Ciherang (32 grains), unlike the B13884-MR-29-1-1 line which has a higher weight of filled grain per plant as much as 44.22 grams from the best check Ciherang (28.67 grams). However, for seed set properties, IR 99270-34-2-1 (52.78%) had the lower fertility of the best check of Ciherang (73.83%). The percentage of unfilled grain is affected by environmental conditions before, during or after flowering, especially by climate (rainfall and number of rainy days) [13]. In addition, the difference in the number of failed grain per panicle produced from each genotype is due to genetic factors of each genotype. The number of filled grain per panicle is influenced by genetic factors [14]. Besides, the environmental factors play a role in the high number of filled grain per panicle, because the sunny weather conditions can increase the rate of photosynthesis, the energy of light used to overhaul the water and the gas of carbon dioxide is converted into food, the resulting photosynthate will be stored in stem and leaf tissue, then it will be translocated to grain in the maturation stage. All of the tested lines have 1000 grains weight smaller than the best check of Inpari 5 Merawu (27.46 grams). This is because the form of grain lines tested in the form of long and somewhat lean is different from the form of grain Inpari 5 Merawu which has a long and slightly fat grain.

All line tested had zinc content in rice higher than the best check of Inpari 5 Merawu (23.61 ppm). Only IR 95133: 1-B-16-14-10-GBS (25.03 ppm) and IR 99270-34-2-1 (24.13 ppm) line showed no significant difference in their zinc content compared to Inpari 5 Merawu. B13884-MR-29-1-1 line contains the highest zinc content (30.94 ppm) and iron content (15.84 ppm) compared to the other lines and the best checks. Rice contains beneficial active components for physiological functions of the body in order to maintain healthiness [15]. Rice with high zinc content required to meet the needs of micro-minerals in the body [16]. Zinc is included in the group of micronutrients that the body absolutely needs. Zinc intake provides great benefits to the human body. The physiological functions that depend on zinc are cell growth and division, antioxidants, sexual development, cellular and humoral immunity, dark adaptation, tasting, and appetite. Zinc is especially needed for growth acceleration process. This is not only due to the effects of cell replication and nucleic acid metabolism but also as a mediator of growth hormone activity. Thus, zinc plays an important role in the synthesis and degradation of carbohydrates, fats, proteins, nucleic acids and embryo formation. Currently, Nutritional Adequacy Ratio (NAR) for zinc is 9-12 mg/day for women and 12-17 mg/day for men (depending on age group). If the intake of zinc in the body was not sufficient, it will be more susceptible to disease attacks. Even according to statistical data, the increasing death rate, in particular, were caused majorly by the lack of zinc intake in children and adults [17].

B13884-MR-29-1-1 is prospective to be proposed as a new rice variety with high nutritional value because this line has high zinc content, iron, and high yields beyond the study checks. Rice with high zinc content is very useful in improving the nutrition of the community. In addition, consuming high-

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zinc rice can prevent low appetite, dermatitis, growth disorder, slow sexual maturity, infertility, and immunodeficiency. Severe Zn deficiency is characterized by decreased immune response function and increased incidence of infection [18]. In addition, this strain also has the advantage of having a heading date and shorter harvest date than other genotypes and has the highest filled grain weight per plant compared to other genotypes. Early maturing rice has high economic value because of its rapid growth, able to compete with weeds and requires relatively little water during growth thereby reducing management costs [19 in 20]. Early-age rice can also increase the intensity of planting and land use.

IR 97477-115-1-CRB-0-SKI-1-SKI-0-2 is also perspective to be proposed to be new rice variety with high Zn and yield characteristics. As is shown that the line has a zinc content of 29.61 ppm, Fe 13.49 ppm and the result of 4.4 ton/ha, higher than the reference check.

4. Conclusion
Climate changes characterized by rising air temperatures and changes in the magnitude and distribution of rainfall causing decreased productivity of agricultural products, especially rice. In addition, climate change causes the emergence of pests and diseases that directly affect food security and the level of poverty. This is worsened by malnutrition problem due to food shortages consequently to cause death. Therefore, it is necessary to create high-zinc rice varieties to overcome these problems. The results showed that the plant genotypes determined the yield characteristics, heading date, harvest age, panicle number, filled and unfilled grain per panicle, seed set, weight per 1000 grains, Zinc (Zn) and Iron (Fe) content in rice grain. B13884-MR-29-1-1 (30.94 ppm Zn, 15.84 ppm Fe, 4.11 ton/ha yield) and IR 97477-115-1-CRB-0-SKI-1-SKI-0-2 (29.61 ppm Zn, 13.49 ppm Fe, 4.4 ton/ha yield) are prospective to be proposed as new high yielding varieties with high Zn and Fe content.

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