High phenolic and mineral compound of rice lines as an alternative healthy food to maintain human health under climate change

I A Rumanti1, Yudhistira Nugraha1, Trias Sitaresmi1*, Rina Hapsari Wening1
1Indonesian Center for Rice Research (ICRR), Indonesian Agency of Agriculture Research and Development, Jl. Raya Sukamandhi No 9, Subang, Jawa Barat, 41256, Indonesia
Email: indrastuti.apri@gmail.com

Abstract. Climate change promotes variances diseases in rice plant and humans. Unpredicted weather due to climate change caused an optimal condition for vectors, pathogens and hosts to be survive and reproduced themselves. Phenolic compounds have the ability to act as antioxidants that can counteract free radicals and prevent various diseases in humans. The mineral content of Zn and Fe is also very useful to anticipate anemia, stunting and immune boosting in humans being. The research was conducted by tested 14 promising lines and 2 check varieties in the farmer field. The research was followed randomized block design with 3 replications. The grains of each line and checks were analysis in the laboratory for mineral content and phenolic content. The results showed that there were several rice lines that had good yield potential with high antioxidant and mineral content, including IR83663-20-3-2-2, B5640H-MR-1-PN-1, and BH39D-MR-PN -1. IR83663-20-3-2-2 has a high Zn content in rice that is 23.76 ± 2.03 ppm, with grain yield of 5.39 t ha⁻¹, and early maturity. While the B5640H-MR-1-PN-1 line is a black rice and has a phenolic compound of 4801.00 ± 0.00 mg AAE 100-g of brown rice. The BH39D-MR-PN-1 line is a red rice which has a higher total phenolic compound up to 5743.35 ± 9.74 mg AAE 100-g of brown rice and Zinc mineral content of 23.66 ± 0.25 mg kg⁻¹. The availability of specialty rice varieties with high mineral content and phenolic compounds will provide an alternative for healthy food, especially during a pandemic virus recently. Besides this, the foodstuffs can be utilized to broad market opportunities and decrease a need of imports.

1. Introduction
The extreme weather due to climate change affect to infectious disease occurrences. Unpredicted weather due to climate change caused an optimal condition for vectors, pathogens and hosts to be survive and reproduced themselves. Temperature and precipitation are the most affecting, while sea level elevation, wind, and daylight duration are also important [1]. Climate change promotes variances diseases in rice plant and humans. Therefore, immune boosting is very important to maintain human health.

Recently, rice is not only a daily food for people and fulfill a carbohydrate need. It has shifted into food with special functions, especially related to health. Rice breeding activities were very proactive to improve the nutrition content of rice through conventional breeding or genetic engineering. Pigmented rice is one of a specialty rice that is most consume due to antioxidant content. Rice has high bioactive
chemical, such as tocopherols, tocotrienols, ferulic acid, γ-oryzanol, and phenolic compound [2]. Phenolic compounds have the ability to act as antioxidants that can counteract free radicals and prevent various diseases in humans. It reported had act as anti-carcinogenic, anti-mutagenic, metal chelating, and anti-microbial [3][4]. The most phenolic compound in pigmented rice is anthocyanin [5], which included in the flavonoid group. Anthocyanin content of rice will determine the darkness intensity of grain and closely related to antioxidant activities [6][7].

Beside antioxidants, zinc (Zn) and iron (Fe) were also important for human health. Thus, micronutrient deficiency will be affected to human health. Zinc poses a great risk to the cognitive development and physical faculty of many children, is more prevalent in developing countries [8]. The prevalence of micronutrient deficiencies, especially Fe, Zn, and Vitamin A in Indonesia is relatively high. The proportion of very short and short toddlers in Indonesia is 30.8% [9], the proportion of Zn deficiency in toddlers is 31.6%, while Vitamin A deficiency is about 14.6% [10]. Zn malnutrition is attributed to a lack of access to nutritious food which results in various health problems. Among cereals, rice is identified as having low grain Zn content and sensitivity to soil Zn deficiency [11].

Fe-deficiency will induce anemia which can cause impaired cognitive and physical development in children and reduction of daily productivity in adults [12]. In order to prevent iron deficiency, the WHO and UNICEF recommend intervention strategies including increased iron intake via dietary diversification, supplementation, and/or fortification. The improved nutritional will appropriate control of infectious diseases [13].

Biofortification or biological fortification of a major staple food such as rice is one way to increase the micro-nutrient content of foodstuffs which play an important role in overcoming malnutrition and consumer health problems [14]. It will also cost-effective in reducing Zn and Fe malnutrition among the poor, whose diet mainly depends on rice. Biofortification is a strategy that complements other strategies i.e., food diversification, supplementation and fortification. Therefore, the breeding process due to increasing the nutritional value of rice were conducted and reported in the manuscript. The research was conducted to identify the best promising lines in term of high productivity, mineral and phenolic content in its grains.

2. Materials and Methods

2.1. Field test
During wet season of 2016, 14 improved rice lines and 2 check varieties (INPARI 5 MERAWU and AEK SIBUNDONG) were transplanted in the irrigated rice field. The experiment was lay outing followed randomized complete block design with 3 replications. 18-21 days old of seedling were planted in 4m x 5m of plots with a spacing of 25 cm x 25 cm. The fertilizers were used 250 kg ha⁻¹ of Urea, 100 kg ha⁻¹ of SP36, and 100 kg ha⁻¹ of KCl. Stem borer and other pests were controlled by integrated pest control principles. Several characters such as flowering time, plant height, productive tillers, filled and unfilled grain per panicle, 1000 grain weight and yield were observed. Data were statistically analyzed using SAS statistical software (9.1). Analysis of Variance (ANOVA) was conducted for all traits and mean separation was done using Least Significant Difference (LSD) at P=0.05.

2.2. Measurements of Zn and Fe content
Measurements were conducted using brown rice as a sample. The sample preparation was carried out using 50 dry rices, it conducting using Satake Testing Husker and polypropylene roller to keep it free from Zn contamination. Contamination was also avoided by using freshly harvested rice samples. Furthermore, the samples are sorted and only the head rice is tested. Measurements were carried out using the XRF machine from Oxford instrument X-Supreme which has been validated based on the ICP method.
2.3. Measurements of phenolic compound
Total Soluble Phenolic Compounds (TSPCs) were analysed using spectrophotometer. 80 μL of rice flour were diluted with 2 ml of distilled water, and add by 200 μL Folin–Ciocalteu reagent 0.25 N. After 3 minutes, the solution added by 1 ml of Na2CO3 7.5%. The final solution then incubated in the dark room for 2 hours. The absorbance was measured using a spectrophotometer at a wavelength of 765 nm. Abs blank was measured using methanol 80%. Curve standard was develop using ascorbic acid and the result will be mg ascorbic acid equivalent (AAE).

3. Results and discussion

3.1. Performances of Improving rice lines
Climate change are induced unpredicted weather such as temperature increase and uncertain rain, it created stresses to rice plants. The location was meet a long dry season and seem the rice plant have no enough water to it growth. It was probably caused lower rice productivity compare to normal condition. The yield was range from 4.13 to 6.07 kg ha⁻¹. It is quite low for irrigated rice lines, but the check varieties were showed the low yield as well, compared to thus yield potency. The yield potency of Aek sibundong was 6.5 kg ha⁻¹, while Inpari 5 Merawu is 7.2 kg ha⁻¹. But in this experiment, Inpari 5 Merawu has produce 6.0 kg ha⁻¹ only. BH39D-MR-11-1-1-6 and BP9494C-1-1-2-3 produced similar yield with Inpari 5 Merawu, 6.07 kg ha⁻¹ and 6.00 kg ha⁻¹, respectively.

| Code | Rice lines/check varieties | Parameters | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
|------|---------------------------|------------|----|----|----|----|----|----|----|
| A    | Fe7                       | 108.47     | 17.0 | 112.3 | 117.0 | 29.4 | 25.53 | 4.90 |
| B    | Fe27                      | 100.82     | 16.0 | 105.5 | 112.9 | 26.8 | 26.80 | 5.55 |
| C    | IR832286-22-1-2-1-1       | 121.38     | 17.2 | 113.3 | 117.4 | 38.1 | 26.04 | 5.39 |
| D    | FFZI                      | 100.61     | 17.2 | 112.9 | 125.1 | 34.7 | 23.99 | 4.86 |
| E    | PKBC-179-168-88-36        | 114.51     | 18.1 | 108.4 | 96.4  | 25.0 | 28.10 | 5.31 |
| F    | S/PKC-179-168-88-36       | 114.51     | 18.6 | 107.3 | 95.8  | 25.4 | 27.81 | 4.13 |
| G    | B10970C-MR-4-2-1-1-Si-3-2-4-2-1-PN-4 | 94.06 | 16.6 | 105.7 | 109.3 | 39.2 | 28.10 | 4.66 |
| H    | B5640H-MR-1-PN-1          | 122.16     | 15.3 | 118.3 | 117.4 | 37.6 | 24.99 | 4.39 |
| Y    | BH39D-MR-11-1-1-6         | 119.18     | 15.9 | 113.1 | 129.6 | 34.7 | 27.03 | 6.07 |
| K    | Bio196-D3                 | 108.47     | 17.2 | 108.9 | 108.4 | 37.8 | 25.37 | 4.98 |
| L    | Bio197-D5                 | 104.79     | 17.5 | 106.9 | 104.3 | 31.7 | 25.36 | 5.60 |
| M    | AGH-BMH                   | 93.63      | 18.1 | 106.6 | 103.3 | 32.4 | 24.10 | 5.15 |
| N    | BP9454F-17-3-5-B          | 101.93     | 16.8 | 111.3 | 123.7 | 46.9 | 25.67 | 4.57 |
| P    | BP9494C-1-1-2-3           | 104.68     | 16.9 | 112.3 | 114.1 | 37.0 | 27.20 | 6.00 |
| R    | INPARI 5 MERAWU           | 107.18     | 17.2 | 111.2 | 106.4 | 42.6 | 28.01 | 6.00 |
| S    | Aek Sibundong             | 111.50     | 16.8 | 113.3 | 111.8 | 37.5 | 26.97 | 4.79 |
|      | Average                   | 107.99     | 17.0 | 110.4 | 112.0 | 34.8 | 26.32 | 5.15 |
|      | CV (%)                    | 6.29       | 13.2 | 1.9  | 15.0  | 30.8 | 5.83  | 11.7 |
|      | LSD (5%)                  | 11.41      | 4.1  | 3.6  | 26.5  | 17.9 | 2.52  | 0.14 |

Note: 1 plant height (cm), 2 productive tiller number, 3 time to harvest (das), 4 filled grain per panicle, 5 unfilled grain per panicle, 6 1000 grain weight (g), 7 yield (t ha⁻¹)

The average number of productive tillers ranged from 15.3 to 18.6 tillers per hill (Table 1). BH39D-MR-11-1-1-6 has medium category productive tillers, which is 15.9 tiller per hill. The check
varieties Inpari 5 Merawu and Aek Sibundong also have the equal number of tillers, 17.2 and 16.8 tiller per hill. The number of productive tillers was reported to have a positive and significant correlation with grain yield in rice [15]. Therefore, productive tillers are widely used as character selection indirectly related to grain yield characters.

The filled grain per panicle is representative of good assimilate allocation in rice lines, which is allocated precisely on target so that it affects grain yield. BH39D-MR-11-1-1-6 has highest filled grain and produced highest grain yield. It means that the rice line was effective distribute assimilate into grain and the yield components.

3.2. Nutritional content of rice lines

The rice lines have Fe content equal to Inpari 5 Merawu, but it has higher Zn content in the pericarp compare to Inpari 5 Merawu (Table 2). Inpari 5 Merawu is national variety which is released as specialty rice with Fe and Zn content relatively higher than Ciberang. There were 2 lines with white pericarp and 2 lines with red pericarp which were equal Zn content with Inpari 5 Merawu. The white pericarps were Fe7 and IR832286-22-1-2-1-1, while BH39D-MR-11-1-1-6 and Bio196-D3 have red pericarp. Their Zn content were about 23.40 ± 1.84, 23.76 ± 2.03, 23.66 ± 0.25 and 23.61 ± 2.25 mg/kg, respectively. BH39D-MR-11-1-1-6 showed the higher Zn content among others, while Inpari 5 Merawu has Zn content about 22.56 ± 1.42 mg kg⁻¹. Human body is absolutely need zinc as a micronutrient that have beneficial a lot for health [16]. Several physiological functions are depending to zinc intake, such as cellular and humoral immunity, cell growth and division, antioxidant activities and sexual development.

Zinc is play important role as a mediator of growth hormone activity, and it will important for synthesis and degradation of carbohydrates, fats, proteins, nucleic acids and embryo formation. Nutritional Adequacy Ratio (NAR) for zinc is 9-12 mg day⁻¹ for women and 12-17 mg day⁻¹ for men (depending on age group). It is mean that the human body have to adequate zinc intake. The body will very susceptible to disease, if the zinc intake was not sufficient [17].

### Table 2. Nutrition content of rice lines

| Code | Rice lines/check varieties | Rice colour | Total Phenolic compound (mg AAE* 100 g⁻¹) | Fe & Zn content |
|------|---------------------------|-------------|------------------------------------------|-----------------|
| A    | Fe7                       | White       | 1108,10 ± 52,87                          | 13.66 ± 2.92, 23.40 ± 1.84 |
| B    | Fe27                      | White       | 600,51 ± 5,40                            | 13.97 ± 3.05, 20.64 ± 1.67 |
| C    | IR832286-22-1-2-1-1       | White       | 825,60 ± 53,95                           | 14.01 ± 0.45, 23.76 ± 2.03 |
| D    | FFZI                      | White       | 741,67 ± 43,16                           | 12.92 ± 1.16, 21.54 ± 3.01 |
| E    | PKBC-179-168-88-36        | Red         | 5491,55 ± 253,59                         | 14.74 ± 0.44, 21.38 ± 1.10 |
| F    | S/PKC-179-168-88-36       | Red         | 4778,11 ± 107,91                         | 14.32 ± 1.60, 22.08 ± 0.59 |
| G    | B10970C-MR-4-2-1-1-1-Si-3-2-4-2-1-PN-4 | Red | 4087,57 ± 80,93                           | 12.92 ± 0.56, 22.60 ± 1.23 |
| H    | BS640H-MR-1-1-1-1-1       | Black       | 4801,00 ± 0,00                           | 13.51 ± 2.32, 20.14 ± 0.30 |
| Y    | BH39D-MR-11-1-1-1-1       | Red         | 5743,35 ± 124,10                         | 13.14 ± 1.36, 23.66 ± 0.25 |
| K    | Bio196-D3                 | Red         | 5480,10 ± 107,91                         | 13.32 ± 1.41, 23.61 ± 2.25 |
| L    | Bio197-D5                 | Red         | 4751,40 ± 415,45                         | 14.41 ± 2.08, 22.97 ± 1.76 |
| M    | AGH-BMH                   | White       | 310,17 ± 9,65                            | 14.29 ± 2.83, 21.54 ± 1.44 |
| N    | BP9454F-17-3-5-B          | White       | 840,86 ± 64,75                           | 14.17 ± 3.81, 22.77 ± 2.99 |
| P    | BP9494C-1-1-2-3           | White       | 646,29 ± 199,63                          | 14.64 ± 2.30, 21.04 ± 1.08 |
| R    | INPARI 5 MERAWU           | White       | 608,14 ± 80,93                           | 14.46 ± 2.45, 22.56 ± 1.42 |
| S    | Aek Sibundong             | Red         | 4785,74 ± 10,79                          | 13.67 ± 2.86, 21.11 ± 1.64 |

Phenolic, zinc and iron analysis were conducted in brown rice
The filled grain per panicle is representative of good assimilate allocation in rice lines, which is allocated precisely on target so that it affects grain yield. BH39D-MR-11-1-1-6 has the highest filled grain and produced the highest grain yield. It means that the rice line was effective in distributing assimilate into grain and the yield components.

BH39D-MR-11-1-1-6 and Bio196-D3 were pigmented rice (red) that associated with antioxidants which are believed to be able to prevent free radicals, and it is useful for human health. There were 7 pigmented rice that had high phenolic compound in the endosperm. Six lines have red pigments such as PKBC-179-168-88-36, S/PKC-179-168-88-36, B10970C-MR-4-2-1-1-Si-3-2-4-2-1-PN-4, BH39D-MR-11-1-1-6, Bio196-D3, Bio197-D5, and 1 black pigment i.e., B5640H-MR-1-PN-1. All pigmented rice lines showed a high phenolic compound which is related to antioxidant reaction. The red rice lines have phenolic compound around 4751.40 ± 415.45 to 5743.35 ± 124.10 AAE 100-g of brown rice. B5640H-MR-1-PN-1 as black rice line has 4801.00 ± 0.00 AAE 100-g of phenolic compound.

Aleurone part of red rice producing anthocyanins genes, which gave a red or purple color, while aleurones and endosperm of black rice can produce anthocyanin with high intensity and affected to rice color, then the color is deep purple or almost black [21]. The results showed that both black and red rice were having a similar high phenolic compound (Table 2). They have a different secondary metabolite, whereas the main secondary metabolites in red rice are proanthocyanidins, while in black rice are anthocyanins [22]. Anthocyanins and proanthocyanidins in rice pigmented are able to play a role in a variety of biological activity in the body [23]. The mechanism is through increasing the insulin production in B cells and reducing the blood sugar response, reducing the oxidation of B lipid acid, reducing triglyceride and LDL cholesterol [24].

Fortification strategy was not always successful, because iron supplementation can cause increased severity of infectious diseases in the presence of malaria [19], while iron fortification of food is difficult since bioavailable iron compounds often cause changes in colour and flavour [20]. Based on those facts, it seems biofortification strategy through breeding is a good opportunity to fulfil the need of thus micronutrients. The mineral content in rice, such as Fe, Zn and phenolic compounds, were reported will not affect the taste of rice, then hopefully it will not affect to consumer preferences [14]. The special mineral content in brown rice will also giving opportunity for export program. People in developed countries that have a high purchasing power for specialty rice products, especially related to dietary programs in health maintenance.

4. Conclusion

Several rice lines that had good yield potential with high antioxidant and mineral content, including IR83663-20-3-2-2, B5640H-MR-1-PN-1, and BH39D-MR-PN-1. BH39D-MR-11-1-1-6 was best rice line with red endosperm, high zinc and phenolic compound. The lines are promising to be released as new rice functionally varieties to provide the healthy rice nationally.

References
[1] WHO 2003 Climate change and infectious diseases. [https://www.who.int/globalchange/environment/en/chapter6.pdf]
[2] Chakuton K. Puangpropintag D. and Nakornriab M. 2012. *J Asian J of Plant Sci* 11: 285-293
[3] Anli R E. and Vural N. 2009 Antioxidant phenolic substances of Turkish red wines from different wine regions. *Molecules* 14: 289-297
[4] Proestos C. Bakogianis A. Psarianos C. Koutinas A A. Kanellaki M. and Komaitis M. 2005. High performance liquid chromatography analysis of phenolic substances in Greek wines. *J. of Food Control* 16: 319-323
[5] Yodmanee S. Karrila T T. and Pakdeechanuan P. 2011. Physical, chemical and antioxidant properties of pigmented rice grown in Southern Thailand. *International Food Research J.* 18: 901-906
[6] Yang D S. Lee K S. Jeong O Y. Kim K J. and Kays S J. 2008 Characterization of volatile aroma compounds in cooked black rice. *J of Agricultural and Food Chemistry* 56: 235-240.

[7] Kaneda I., Kubo, F. dan Sakurai, H. 2006. Antioxidative compounds in the extracts of black rice brans. *J of Health Science* 52 (5): 495-511.

[8] [Wessells] K. R. & Brown K. H. 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

[9] Kementrian Kesehatan. 2018. Hasil utama Riskesdas 2018. Badan Penelitian dan Pengembangan Kesehatan Kementrian Kesehatan. 200 hal

[10] Herman, S. 2007. Studi Masalah Gizi Mikro di Indonesia (Perhatian Khusus pada Kurang Vitamin A, Anemia dan Seng). Laporan Penelitian. Bogor. Puslitbang Gizi

[11] Sarah E. Johnson-Beebout,1 Johnvie Bayang Goloran,a,1 Francis H. C. Rubianes,1 Jack D. C. Jacob,2 and Oliver B. Castillo 2016 Zn uptake behavior of rice genotypes and its implication on grain Zn biofortification *Sci Rep.* 6: 38301

[12] Black, R. E., Victora, C. G., Walker, S. P., Bhutta, Z. A., Christian, P. and M. de Onis. 2013. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet* 382, 427–451. doi: 10.1016/S0140-6736(13)60937-X

[13] WHO. 2001. Iron Deficiency Anaemia: Assessment, Prevention and Control: A Guide for Programme Managers. Available at: http://apps.who.int/iris/bitstream/10665/66914/1/WHO_NHD_01.3.pdf?ua=1

[14] Indrasari, SD., dan Kristamtini. 2018. Biofortifikasi mineral Fe dan Zn pada beras: perbaikan mutu gizi bahan pangan melalui pemuliaan tanaman. *J Litbang Pertanian* 37 (1): 9-16

[15] Kartina, N., Wibowo, BP., Rumanti, IA., Satoto. 2017. Korelasi hasil gabah dan komponen hasil padi hibrida. *J penelitian pertanian tanaman pangan* 1 (1):11-19.

[16] Wijayanti S 2004 Potential and prospect of indigenous Indonesian functional food (In) Presented at Seminar Nasional: Pangan Fungsional Indigenous Indonesia. Potensi, Regulasi, Keamanan, Efikasi dan Peluang Pasar (Bandung: Litbang Pertanian)

[17] Hidayat A 1999 Zink (zinc) essential for health *J of Medicine Trisakti* 18(1): 19-27

[18] Meng F, Wei Y, Yang X 2005 Review Iron content and bioavailability in rice *J Trace Elem Med Biol.* 18(4):333-8

[19] Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, Dhingra U, Kabole I, Deb S, Othman MK, Kabole FM 2006 Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet*. 367(9505): 133-43.

[20] Hurrell RF 2002 Review: Fortification: overcoming technical and practical barriers. *J Nutr.* 132(4 Suppl): 806S-12S.

[21] Wanti S, Andriani MAM, Parmanto NHR 2015 Pengaruh berbagai jenis beras terhadap aktivitas antioksidan pada angkak oleh monascus purpureus. *Biofarmasi* 13 (1): 1–5.

[22] Goufo P, Trindade H 2013 Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. *Food Sci. & Nutrition* 2 (2): 75–104.

[23] Arifin AD, Yuliana ND, Rafi M 2019 Antioxidant activity of pigmented rice and its impact on health. *Pangan* 28 (1): 11–22

[24] Kruger MJ, Davies N, Myburgh KH, Lecour S 2014 Proanthocyanidins, anthocyanins and cardiovascular diseases. *Food Research Internat J.* 59: 41–52.