



Cultivation of Rice Abundance Super High Levels of Iron by the Method of Biofortification

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Abstract In 2013 production reached 71,279,709 Mg of dry grain milled (DGM) equivalent to 44,528,434 Mg of rice consumption. Data from the Ministry of Health, shows approximately 100 million people in Indonesia, suffer from micronutrient deficiencies (iron and iodine) because of their inability to purchase nutritious food, but only rely on the nutrition of rice. Deficiency of iron (Fe) in the child, will lead to anemia, recurrent infections, lower intelligence, emotional disturbances, and even can cause permanent brain damage. In-Indonesia about 30-60% of the child under five years old and pregnant women who suffer from the deficiency of micronutrients. The high level of the national rice consumption reaches 137 kg capita⁻¹ year⁻¹, is an opportunity to correct micronutrient deficiencies, especially iron by the method of bio-fortification, with cultivation rice plants in paddy fields of new openings that are rich in iron (Fe) soluble. The research objective is to increase productivity in the fields of new opening gripped Fe, with a target of >7 Mg ha⁻¹ with bio-fortification methods to harvest rice high iron levels with targets of >30 mg kg⁻¹. The results showed that the application of packet technology to increase production and iron levels in rice grain with the method of bio-fortification, show the results as follows: 1) The number of tillers that is highest in Inpari 29 variety in the control treatment, the number of seedlings reached 114.69 tillers; 2) Production of the highest DGM produced Mekongga varieties with coconut water treatment, namely 7,417.9 Mg; and 3) the levels of iron in grains, on all varieties with PGR treatments, show the results between 18.8-36.2 mg kg⁻¹. Information application technology package (varieties tolerant + ameliorant piles of 10 Mg ha⁻¹ + spacing of the square [(10 x 10cm) x 25 cm x 10x10cm]) so that per clumps are 4 seed + young seedling age 10 days + PGR) in SRI method can be used to increase production and iron content in the grains by the method of bio-fortification on land seized Fe.

Keywords bio-fortification, rice, iron

Introduction

Rice is the second an important food crop in the world's, which is used as a source of food after wheat, and the estimated need will continue to increase in the coming decades. The market, generally has only rice circulating iron (Fe) of about 2-3 mg kg⁻¹ [1]. According to WHO (2000), nearly 3 billion people are deficient in micronutrients, while in-Indonesia about 30-60% of child under five years old and pregnant women who suffer from the deficiency of micronutrients [1]. The high level of the national rice consumption reaches 137 kg capita⁻¹ year⁻¹ [2], is an opportunity to improve the micronutrient deficiencies, especially iron by the method of bio-fortification.

Methods of bio-fortification are the way cultivate crops on land that is a rich solution of iron (Fe), so the plants can naturally absorb many substances dissolved in the root zone of the plant, taking advantage of the tolerant plants. High levels of dissolved Fe²⁺ in the fields of the new openings, a potential that needs to be exploited, to harvest rice grain with a high iron content by the method of bio-fortification. Increased uptake of iron levels by



the plant by the method of bio-fortification, conducted by treatment with growth regulator substances from the class of auxin, because of its ability to manipulate the power of Source-Sink on plants, thereby increasing the absorption of iron in grains [3-4].

The potential of land that can be used to increase the production of rice is rice field new openings in Dharmasraya with a land area of over 2,691 ha [5], which is influenced by the solubility of Fe^{2+} and Al^{3+} high [6-8], spread out in four districts with varied land area. The average land productivity is only 2.2-2.5 Mg ha^{-1} while national production now has reached more than 4.75 Mg ha^{-1} [2].

The results of the soil analysis experiments show the location of the chemical characteristics vary widely from very low to very high, namely: N total (0.11% is low), available P (3.29 mg kg^{-1} is very low), with the arrangement of cations (K^+ 0.05 mol kg^{-1} is low, Na 0.13 mol kg^{-1} is low, Mg 0.20 mol kg^{-1} is very low, Ca 0.08 mol kg^{-1} is very low), whereas Al^{3+} 4.78 mg kg^{-1} medium, and Fe^{2+} 104.69 mg kg^{-1} high [9]. Levels ion ferrous high as a result of flooding led to a reduction of Fe^{3+} to Fe^{2+} , in addition to the other problems that often arises is the low fertility of the soil [8,10], which causes the inhibition of plant growth and development, especially on the varieties which are sensitive to nutrient stress [7, 11-14].

To increase production and high-iron rice harvest yield, productivity improvement program needed new openings with wetland rice cultivation technology engineering methods of bio-fortification. Information on the cultivation of rice super high abundance of iron in the bio-fortification method has not been widely reported by the investigators that the study of rice cultivation super high abundance of iron needs to be done.

This experiment is an attempt to increase the productivity of wetland new openings to the scenes Fe, with a target of $>7 \text{ Mg ha}^{-1}$, increase the absorption of iron in the rice grain with targets of $>30 \text{ mg kg}^{-1}$; and to determine the type of plant growth regulator auxin class appropriate to increase the absorption of iron in the rice grain. The results of this study are very important as information for research on increasing production, breeding technology development of stress tolerance ferrous especially rice paddies new openings, and to address micronutrient deficiencies in society.

Materials and Method

The experiment was conducted from February to August 2014, the opening of new wetland located in the Subdistrict of Koto Baru, District of Dharmasraya. This experiment used two-factors of a factorial design in a Completely Randomize Design with three replications. The first factor was the rice variety (V) as follows: $V_1 =$ Inpari 24; $V_2 =$ SBY; $V_3 =$ Inpari 26; $V_4 =$ Inpari 27; $V_5 =$ Inpari 28; $V_6 =$ Inpari 29; $V_7 =$ Inpari 30; $V_8 =$ Ciapus; $V_9 =$ Mekongga; and $V_{10} =$ Anak Daro. The second factor was a plant growth regulator (Z), namely: $Z_0 =$ Control; $Z_1 =$ Synthetic PGR (Auxin); $Z_2 =$ Natural PGR (coconut water).

The combination package technology used in these experiments, namely: (1). tolerant varieties Fe and Al; (2). Ameliorant piles of cow 10 Mg ha^{-1} ; (3). Spacing square [(10 x 10 cm) x 25 cm x (10x10 cm)]; and (4). One seedling per planting point and seedlings age 10 days [8, 13, 15-16].

Observations agronomic characters performed on each unit of the experiment as much as three samples of each unit such as plant height, the number of tillers, panicle number, panicle length, percentage of grain pithy, the weight of 1000 grain; and dry grain mill (DGM) ha^{-1} . Levels of iron (Fe) grains measured with an AA Varian 240 with the method of SNI 6989.4.2009 in the Integrated Laboratory Kopertis Region X Padang.

Rice seeds, prior to germinating soaked in a solution of Decis, with a concentration of 3 g l^{-1} and 1 ml l^{-1} for 30 minutes, then rinsed thoroughly and soaked for 2 x 24 hours. Rice field flooded with water for 7 days, then added ameliorant the form of piles of the cow as much as 10 Mg ha^{-1} as a source of organic material. After that, the soil depth of 25-30 cm by means of hand tractors, and proceed with the printing of experimental plots with a size of 6.0 x 3.0 meters, a total of 60 experimental plots. Then the wetland incubated for 2 weeks after planting in SRI method.

Germination of seed paddy in a rectangular basin with a sprinkling of rice seeds that have been soaked, on top of a layer of rice paper and covered with rice paper that has been moistened. The condition is on hold until the sprouts 10 days old and ready to be planted in the field. The maintenance practices is the third Urea fertilizer dose, SP-36 and KCl in the early planting (250 kg NPK Phonska) 170 kg Urea, 36 kg P_2O_5 and 60 kg K_2O). A further provision of Urea third dose at age 6 weeks and another third will enter the generative phase. Weeding is done at 2 weeks of age and the age of 6 weeks after planting. Watering is done intermediates, and standing



water when primordial interest. Harvesting is done after the flag leaf yellowing 80% of the total population and dried grain panicle.

Results and Discussion

The experimental results showed that there was variation in the growth and production of all varieties of rice that are treated with growth regulator substances of synthetic and natural (coconut water) of all observed parameters such as plant height, number of tillers, number of panicles, and a panicle length (Table 1), the number of spikelet, number of grain panicle⁻¹, weight of 1000 grain; and DGM ha⁻¹ (Table 2), and levels of iron (Fe) in grains (Table 3).

Table 1: Plant height, tillers number, panicle number and panicle length several varieties of rice cultivated by the method of bio-fortification

Type of PGR	Rice Variety									
	Inpari 24	SBY	Inpari 26	Inpari 27	Inpari 28	Inpari 29	Inpari 30	Ciapus	Mekongga	Anak Daro
Plant Height (cm)										
Control	105.13 e	99.53 g	89.27 q	93.53 j	89.31 q	114.69 a	85.87 u	92.69 lm	91.33 n	105.73 d
Atonik (5ppm)	100.43 f	96.01 h	89.15 q	87.65 r	85.77 u	85.15 v	89.33 q	92.51 m	90.43 o	112.19 c
Coconut Water	95.99 h	94.57 i	89.61 p	87.27 s	93.27 k	82.27 x	84.65 w	92.83 l	86.73 t	113.35 b
Σ Tillers (pieces)										
Control	92.8 e	94.0 c	78.4 w	93.0 d	89.0 J	91.6 g	88.4 k	83.6 q	82.8 s	86.0 n
Atonik (5ppm)	95.0 a	91.6 g	92.4 f	93.0 d	90.2 h	83.0 r	94.8 b	85.0 o	89.4 i	89.3 i
Coconut Water	92.8 e	92.4 f	80.0 u	81.2 t	78.8 v	75.0 x	87.0 l	83.8 p	83.6 q	86.2 m
Σ Panicle (pieces)										
Control	43.3 c	45.8 b	37.2 f	38.7 e	34.6 ij	23.7 t	28.7 q	30.6 o	37.4 f	28.5 q
Atonik (5ppm)	50.4 a	34.9 hi	25.5 s	35.0 h	37.4 f	32.2 kl	25.3 s	31.5 n	29.9 p	26.2 r
Coconut Water	40.3 d	31.6 mn	28.9 q	32.0 lm	32.4 k	35.9 g	23.1 u	34.5 j	32.6 k	25.2 s
Panicle Length (cm)										
Control	18.69 fgh	17.52 h	24.99 ab	24.63 abc	19.14 fg	21.75 d	20.04 ef	18.96 fg	10.77 j	18.42 gh
Atonik (5ppm)	18.87 fgh	21.03 de	23.73 bc	24.54 abc	21.48 d	21.84 d	19.32 fg	17.52 h	18.96 fg	15.56 i
Coconut Water	18.24 gh	23.28 c	23.28 c	25.80 a	18.06 gh	21.39 de	19.05 fg	17.52 h	20.04 ef	18.42 gh

Mean followed by different letters on the same variables in each treatment showed significantly different at 5% level by Tukey test.

The differences of growth, showed differences in the adaptability of each of the rice varieties to stress Fe. But all these varieties are able to grow and produce well, as shown in Tables 1 and 2. This indicates that the growth of rice plants affected by the function of genotype and environment, thus causing variations in growth and



production can not be separated from the specific plant responses the diverse environments so that the interaction between genotype by environment [17], to be considered in determining the varieties of rice with the ability to absorb iron from the environment in response genotype of each variety.

The highest and lowest growth of rice plants high obtained on varieties Inpari 29 (control) 114.69 cm and natural plant growth regulator treatment (82.27 cm). At the height parameters of plants showed that the treatment plant growth regulator may hamper the development of higher plants, in some varieties such as Inpari 24, SBY, Inpari 27 Inpari 29, and Mekongga but on some other varieties, treatment PGR able to increase as height as in Inpari 28 and Anak Daro. High rice crop will affect the shape of the canopy of the plant, which will relate to the process of photosynthesis so as to increase growth and production related to assimilating as the results of photosynthesis [17].

The number of tillers increased for some varieties like Inpari 24 Inpari 26 Inpari 30 Ciapus, Mekongga and Anak Daro treated with PGR, but the other varieties of plant growth regulator treatments are no different from controls. The highest increase in the number of tillers in combination treatments with PGR Inpari 24 synthetic, ie 95 tillers, while the lowest number of seedlings in treatment Inpari 29 with coconut water, which is 75 tillers. The number of tillers formed presumably because of the application of a square spacing of the system [(10 x 10 cm) x 25 cm x (10x10 cm)] for one clump of four seeds (Figure 2B). Implementation within the plant, could encourage optimal accretion seedling growth and avert such plants from the competition early in the absorption of nutrients, water and light.

In conventional farming (local farmers), the number of tillers that formed about 20 tillers, contrary to the treatment plant growth regulator is able to increase the number of tillers 4x more than the conventional cultivation (Figure 1A). Differences increase in the number of tillers, presumably because of differences in the use of packet technologies are applied in the system of rice cultivation. Description of the national yield rice released by various institutions and researchers indicate the number of productive tillers produced per clump of 10-20 stems or panicles [18-20].



Figure 1: Growth tillers one clump with five seeds (farmers) produce 15-20 tillers (A), and one clump (4 seeds) with spacing [(10x10cm) x 25cm x (10x10cm)] produces 95-105 tillers (B)

The highest number of panicles occur in Inpari 24 with synthetic plant growth regulator treatment, ie 50.4 panicles, while the lowest for the number of panicles Inpari PGR 29 without treatment (control) with 23.7 panicles. The high panicle formation in rice seedling is an indicator of the high productive tillers [20]. The number of panicles produced in these experiments reached 2-3 times higher, compared to the number of panicles formed in several national yielding rice varieties. National varieties are cultivated generally only produces panicles per hill about 10-20 [21].

The difference in the number of tillers in both the way of cultivation, for the cultivation of conventional seeds number consists of 4-6 in one clump (growing point) at the age of about 21 days, while the technology package used seedlings about 12 days and in one clump composed 4 seedlings in space (Figure 1B), with this method the plants are able to grow optimally protected from competition at an early stage so that the number of panicles



formed more. Superior rice varieties should have long panicles, with the amount of grain that more and composition of the bottom leaf-shaped horizontal and vertical top so that photosynthesis occurs at its optimum . Rice panicle longest in this experiment was obtained in treatment PGR coconut water, which is 25.80 cm in Inpari 27, not different with the result of the synthetic plant growth regulator treatments and control on the same variety, and no different from the controls on Inpari 26 is 24.99 cm. While the shortest panicle finds in varieties of Anak Daro with synthetic plant growth regulator treatment, ie 15.56 cm (Table 2).

The number of panicles mostly in Inpari 24 with the synthetic plant growth regulator treatments (47.8 pieces), while the least number of panicles on Inpari 30 with PGR treatment of coconut water is 20.1 units. The results of field observations (*in situ*) showed that the number of panicles formed on the rice plant local farmers, is significantly lower at around 5-10 panicles.

Table 2: Number of spikelet, number of grains per panicle, 1000 grain weight, and DGM ha⁻¹ several varieties of rice cultivated by the method of bio-fortification.

Type of PGR	Rice Variety									
	Inpari 24	SBY	Inpari 26	Inpari 27	Inpari 28	Inpari 29	Inpari 30	Ciapus	Mekong ga	Anak Daro
Σ Spikilet (pieces)										
Control	6.3 b	6.9 b	9.5 ab	9.60 ab	6.9 B	10.1 ab	8.8 ab	8.3 ab	11.0 ab	8.8 ab
Atonik (5ppm)	7.1 b	7.6 b	7.6 b	8.0 ab	7.3 B	9.9 ab	7.3 b	8.3 ab	10.9 ab	8.9 ab
Air	6.6 b	12.2 a	8.2 ab	9.0 ab	6.8 B	10.3 ab	9.0 ab	8.0 ab	11.0 ab	9.3 ab
Kelapa										
Σ Grains Panicle ⁻¹ (grain)										
Control	105.77 gh	101.03 ijk	138.43 ab	137.58 b	99.23 jkl	118.83 d	102.83 hij	95.53 l	141.83 a	109.83 fg
Atonik (5ppm)	104.43 hi	75.53 o	104.73 hi	112.83 ef	105.13 h	115.83 de	85.43 n	85.67 n	97.83 kl	105.97 gh
Air	101.03 ijk	68.23 p	112.43 ef	126.73 c	97.13 kl	122.93 c	85.43 n	90.33 m	89.03 mn	96.63 l
Kelapa										
1.000 Grain Weight (g)										
Control	25.89 j	32.57 c	24.58 l	29.03 f	32.35 c	26.01 j	24.70 l	25.62 jk	39.44 a	24.11 m
Atonik (5ppm)	30.24 e	26.88 i	23.47 n	29.19 f	25.80 j	28.14 g	23.97 m	27.44 h	34.13 b	25.35 k
Air	25.38 k	23.82 mn	23.88 m	28.41 g	27.12 hi	31.22 d	30.38 e	24.72 l	39.51 a	21.48 o
Kelapa										
Dry Grain Milled Production (DGM Mg ha ⁻¹)										
Control	6,767 b	6,881 b	5,912 d	5,418 fg	4,891 h	6,346 c	4,404 ij	3,068 p	5,552 ef	5,606 e
Atonik (5ppm)	5,974 d	4,375 ijk	4,535 i	3,903 m	4,170 l	4,314 jkl	2,024 r	4,444 ij	3,455 n	4,216 kl
Air	5,262 g	3,884 m	3,887 m	2,751 q	1,920 r	3,953 m	2,850 q	3,406 no	7,418 a	3,266 o
Kelapa										

Mean followed by different letters on the same variables in each treatment showed significantly different at 5% level by Tukey test.

Spikelet highest in varieties of SBY with coconut water treatment plant growth regulator that is 12.2 pieces, significantly different from the Inpari 24 and 28, whereas the other varieties were not significantly different (Table 2).



In the control treatment showed, several parameters were observed giving better results compared to the treatment plant growth regulator such as synthetic and coconut water, the parameters; plant height, panicle length, the number of grain panicle⁻¹, and the weight of 1000 grains. This is presumably because one packet technology is the use of manure (cow) mixed with cow urine as much as 10 Mg ha⁻¹, is sufficient to meet the needs of rice plants cultivated. Plants need PGR in very small quantities, which is just in mg l⁻¹. Cow urine contains plant growth regulator auxin from the group, which plays a role in stimulating cell renewal, cell enlargement, cell division, and differentiation [22-24].

Mekongga varieties (control) shows the number of grain panicle⁻¹ high of 141.83 grain are not significantly different with Inpari 26 (control) 138.43 grain, while the number of grain panicle⁻¹ lowest on the varieties of SBY-treated with coconut water, which is 68.23 grain (Table 3). Rice plants can grow well even in drought stress Fe, because the varieties used tolerant varieties that can adapt to drought stress. In addition, packages of applied technology could encourage the growth of rice plants because of the availability of nutrients. Application of the optimum planting distance (4 points cropping) to encourage the early vegetative growth is better, so the plants protected from competition at an early stage [9]. The technology package, to encourage vegetative growth is more intensive because the seeds are still very young. The intensive vegetative growth, also driven by the use of plant growth regulator that is able to increase cell division, cell elongation and morphogenesis.

Table 3: Levels of iron (Fe) grains some rice varieties in cultivation with bio-fortification in SRI method.

Type of PGR	Rice Variety									
	Inpari 24	SBY	Inpari 26	Inpari 27	Inpari 28	Inpari 29	Inpari 30	Ciapus	Mekongga	Anak Daro
	Iron Content of Rice Grains (mg kg ⁻¹)									
Control	21.1 hijk	26.8 cdefg	24.1 efghij	20.3 jk	22.4 ghijk	31.1 abc	29.3 bcde	20.5 jk	35.7 a	23.1 ghijk
Atonik (5ppm)	24.0 fghijk	20.3 jk	23.0 ghijk	22.0 ghijk	29.0 bcdef	33.8 ab	23.2 ghijk	21.0 hijk	30.2 bcd	20.6 ijk
Coconut Water	24.2 efghij	26.2 cdefgh	23.8 fghijk	36.0 a	36.2 a	22.1 ghijk	25.2 defghij	20.0 jk	25.8 defghi	18.8 k

Mean followed by different letters on the same variables in each treatment showed significantly different at 5% level by Tukey test.

Iron levels in some varieties of rice were cultivated by the method of bio-fortification showed an iron content varying from 18.8 to 36.2 mg kg⁻¹. Iron levels are lowest for the varieties of Anak Daro by treatment with coconut water (18.8 mg kg⁻¹), while the iron levels are highest in rice grain Inpari 28 (36.2 mg kg⁻¹) was not significantly different from the Inpari 27 with water treatment pure coconut (36.0 mg kg⁻¹), as well as varieties of Mekongga control (35.7 mg kg⁻¹), Inpari 29 with atonik treatment (33.8 mg kg⁻¹) and control (31.1 mg kg⁻¹). Iron levels Inpari 28 (36.2 mg kg⁻¹) were treated with coconut water increased by 62% compared with controls (22.4 mg kg⁻¹), while the local varieties of Anak Daro (18.8 mg kg⁻¹) which was treated with coconut water decreased by 19% compared with controls (23.6 mg kg⁻¹). Iron levels in all varieties are cultivated on land seized by Fe (Table 3) is much higher, when compared with the levels of iron grains in the market is only about 2-3 mg kg⁻¹ (Inez in Anonymous, 2012). The low iron content, presumably because rice varieties are cultivated on lands fertile rice fields with dissolved iron levels are very low.

Cultivated varieties (Table 3) is a stress-tolerant varieties Fe [15], so these varieties able to adapt well. In addition, also carried out engineering culture by applying a combination of several technology package, consisting of: tolerant varieties Fe and Al³⁺ ameliorant piles of 10 Mg ha⁻¹ + Spacing square [(10 x 10 cm) x 25 cm x (10x10cm)] + One seedling per point of planting were seedlings age 10 days [8, 13, 15-16].

Conclusions

The results showed that the application of packet technology to increase production and iron levels in rice grain with the method of bio-fortification, which shows the results:



1. The number of tillers that are highest in Inpari 29 in the control treatment, the number of seedlings reached 114.69 tillers.
2. Production of the highest DGM produced by Mekongga varieties with coconut water treatment, ie 7.418 Mg ha⁻¹.
3. The content of iron in grains, on all varieties with PGR treatments show the results between 18.8-36.2 mg kg⁻¹.

Information application technology package (varieties tolerant + ameliorant piles of 10 Mg ha⁻¹ + spacing of squares [10 x 10cm] x 25 cm x 10x10cm) + young seedling age 10 days + PGR) with the SRI method can be used to increase production and the iron content in the grains by the method of bio-fortification on land seized Fe.

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