GROWTH AND PATTERNS OF DISTRIBUTION OF Fe^{2+} IONS IN SOME HYBRID RICE VARIETIES

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ABSTRACT

Increased production, many face increasingly complex challenges. The problem, which is not less important is the limited rice varieties tolerant to iron stress. The aim of the study was to obtain growth and distribution patterns of Fe^{2+} ions in several hybrid rice varieties. Experiment in Koto Baru, Sitiung I, Dharmasraya Regency, West Sumatra. The experiment used a completely randomized design, with five replications. The single factor is rice varieties, namely: Mekongga, Inpari 24, Inpari 27 and Inpari 28. The paddy fields used contain $104.69 \text{ mg kg}^{-1} \text{ Fe}^{2+}$, these concentrations greatly affect growth which causes poisoning. The production of milled dry grain in all varieties remained high even though it was iron-strapped, because the number of productive tillers formed in the SBSU system was more than 21 stems per clump, with production of grain weight $> 5.4 \text{ Mg ha}^{-1}$. The pattern of iron distribution occurs through absorption and translocation in the vegetative and generative phases to roots, stems, leaves, grain, and rice. The difference in iron levels in each part shows the ability of each variety to adapt. To obtain rice grains rich in iron and able to grow and produce well in iron-clamped conditions, it is recommended that aquaculture using the SBSU system with Inpari 27 varieties be able to produce iron content in rice grains of more than 27 mg kg^{-1} with production of grain weight 6.83 Mg ha^{-1} .

Keywords: Iron; Rice; SBSU System; Sitiung

INTRODUCTION

The projection of rice production in Indonesia in 2018, according to BPS is estimated to reach more than 56.54 million Mg of grain weight equivalent to 32.42 million Mg of rice with a standard area of 7.1 million ha of rice. The level of rice consumption in Indonesia is $111.58 \text{ kg capita}^{-1} \text{ year}^{-1}$, increasing production continues to be sought to increase the income and welfare of the community and improve national food security [1, 2]. Increasing rice production in the future will face many increasingly complex challenges. The occurrence of

competition and land use change for agriculture with various other development needs such as transportation infrastructure, offices, reservoirs, housing and aggravated by weather conditions and the environment associated with nutrient stress, climate, weeds, pests and diseases. Another problem, which is not less important is the limited rice varieties that are tolerant of environmental stress, especially iron stress [3, 4, 5].

There are many methods that are often used by researchers to determine the adaptability of a plant variety to stress. Methods that are often used include: 1). Ability to symbiosis with soil microorganisms [6, 7], 2). Root growth [8, 9], 3). Detoxification of organic acids [10, 11], and 4. Utilizing plants that are tolerant of environmental stress [12, 13, 14].

The availability of fertile land for agriculture is very limited, the available land is marginal land with various problems. In acid mineral soils, iron toxicity often occurs, which causes root damage and low nutrient availability. Efforts to improve root function and neutralize the bad effects of iron are becoming increasingly important, for the growth of rice plants. To overcome these obstacles is the use of species tolerant to stress [20, 21]. Tolerant plants have the ability to develop various mechanisms to adapt. The adaptation mechanism developed by plants can occur morphologically and physiologically [15, 16, 17]. Development of rice varieties tolerant of iron stress can be done through screening and genetic engineering.

In addition, cultivation methods are very helpful in increasing the growth, production and adaptability of rice plants to iron stress. In the cultivation with the Sistem Bujur Sangkar Utama (SBSU) method the potential of tillers formed reached 3.6 million stems, whereas with the conventional method only 1.6 million stems. There was an increase in the number of tillers by 125%, when compared to conventional methods which had been applied by farmers [16, 24].

Rice germplasm has a very narrow genetic variability for the iron content of the endosperm (rice grains). Generally, plants use a reduction mechanism or chelate to get iron from the soil. The reduction mechanism, used by most plant species, involves the production of Ferric Chelate Reductase which reduces Fe^{3+} to Fe^{2+} on the root surface. Fe^{2+} is dissolved then transported into root cells by special transporters, namely, Iron-Regulated Metal

Transporter 1 [34, 35]. Efforts to produce tolerant varieties, need to be supported by information about growth, production and distribution patterns of iron in hybrid rice varieties.

Research on the growth, production and pattern of distribution of iron in hybrid rice varieties is still very limited. The results of this study are very important for the engineering of tolerant rice varieties [22], especially tolerant to iron stress. In addition, also to meet the needs of iron-rich rice and increase production through the development of cultivation methods.

MATERIALS AND METHODS

The experiment was carried out in the new openings field gripped by Fe^{2+} , in Block E Jorong IV Sitiung I, Koto Baru, Dharmasraya Regency. Single factor experiments using a completely randomized design, with five replications. The single factor is hybrid rice varieties, namely: Mekongga, Inpari 24, Inpari 27, and Inpari 28.

One week before the paddy field is hijacked, a seven-day recall will be carried out and add 10 Mg ha^{-1} of cow manure as a source of organic material. Furthermore, the paddy field was incubated for two weeks, after which it was hijacked again and continued by harrowing until the land was ready for planting. The size of the plot used in this experiment is $6 \text{ m} \times 3 \text{ m}$.

The rice seed is soaked in Decis solution, with a concentration of 3 g L^{-1} and 1 ml L^{-1} for 20 minutes, after which it is rinsed thoroughly and soaked for 24 hours. Germination is done by wrapping rice seeds with wet paper and placed in a basin. Planting is carried out in accordance with the prescribed treatment. Maintenance activities carried out are fertilizing Urea $1/3$ dose, SP 36 and KCl at the beginning of planting. Furthermore, giving Urea $1/3$ dose at the age of 6 weeks and $1/3$ again will enter the generative phase. Weeding is done at the age of 2 weeks and aged 6 weeks after planting. Irrigation is carried out alternately, and water is stagnant when primordial flowers.

Observations were made in each unit of experiment, three samples for each unit of agronomic and physiological characteristics. The rice plant samples were dried with an oven, then blended to analyze Fe^{2+} levels on roots, stems, leaves, grain and rice grains. The sample was weighed 0.5 gram, then it was reconstructed with concentrated H_2SO_4 , HClO_4 , and

HNO₃ and then heated until the extract was clear. Furthermore, the sample was diluted to 50 ml, then Fe²⁺ levels were measured using the AAS Farian AA 240 tool with the SNI 6989.4.2009 method, in the LLPT Region X Laboratory.

RESULTS AND DISCUSSION

Growth

The height of rice plants as shown in Table 1 ranges from 64-71 cm, lower than the description, which is between 81-106 cm. This shows that the high growth of rice varieties (Mekongga, Inpari 24, Inpari 27 and Inpari 28) has been hampered due to the presence of Fe²⁺ stresses. The inhibition of the growth of the rice plant was thought to be due to the presence of barriers in the formation, photosynthetic translocation, and growth hormones that function to encourage the growth of rice plants [5, 21, 32].

Table 1 shows that, the growth of tillers formed using the SBSU System was able to increase the number of tillers by 62-76% (45-65 stems) with the percentage of productive tillers 34-49% (21-24 stems), whereas in conventional cultivation methods, the number tillers formed around 13-16 stems [18]. The number of productive tillers is formed because in the SBSU system there are four sub clusters that allow plants to avoid early competition in the vegetative phase so that each subgroup can develop well [24].

With the SBSU method the potential of the tillers formed can reach 3.6 million tillers/stems, while the conventional method is only 1.6 million tillers/stems. There is a 125% increase, compared to conventional methods which have been applied by farmers (Figure 1). The advantages of this system are: 1) more seedling growth, 2) no competition for growth in the vegetative phase due to the distance between seedlings in one subgroup, 3) higher population per hectare, 4) increasing production per hectare, 5) Age faster harvests of around 90 days, and 6) cultivated with System of Rice Intenfication [2].

The longest panicle length is in the Inpari 24 (24 cm), Inpari 27 (23.8 cm), Inpari 28 (23.3 cm) and Mekongga (22.2 cm) varieties, while the number of panicle branches in each variety does not show significant differences, namely 8.2 to 8.6 fruit. Panicle formation is an important stage in rice productivity associated with grain formation [36]. The grain weight per clump in the four rice varieties ranged between 58-67.3 g. In theory, with around 90,000

clumps per ha of SBSU system cultivation [16], the grain weight to be obtained will range from 5.22 - 6.06 Mg ha⁻¹. This is not different from the production obtained in the production parameters of grain weight ha⁻¹ (Table 2).

The weight of 1000 varieties of grains (Mekongga, Inpari 24, Inpari 27 and Inpari 28) which are cultivated in iron-choked conditions are 26.5 g; 28.0 g; 27.5 g and 28.1 g. The grain weight is higher than the description except for the Mekongga variety. According to the description, Mekongga (27-28 g); Inpari 24 (26 g); Inpari 27 (26.7 g) and Inpari 28 (27.4 g). The high weight of 1,000 grains shows that the rice varieties are able to adapt to stress, through a mechanism of greater iron translocation to the roots in the generative phase so that the growth of panicles and grain can develop well (Table 2). The highest production of grain weight from the four varieties is Inpari 27, which is 6.83 Mg ha⁻¹, then Mekongga (6.34 Mg ha⁻¹), Inpari 28 (5.93 Mg ha⁻¹) and Inpari 24 (5.36 Mg ha⁻¹). The production is higher than the description, namely 5.7- 6.7 Mg ha⁻¹ [18]. The high production of grain weight in this experiment even though rice plants experienced iron stress, because in cultivation with the SBSU system they were able to produce higher productive tillers compared to conventional methods [24].

Iron Distribution

The paddy fields used in this experiment contain 104.69 mg kg⁻¹ Fe²⁺, the concentration greatly affects plant growth which causes poisoning. The poisoning process occurs after paddy fields are inundated, causing the process of oxide Fe³⁺ to be reduced to Fe²⁺ compounds. The color of the soil changes to gray and Fe²⁺ in large amounts dissolved in the soil. Soluble Fe²⁺ concentrations vary from 0.1 mg kg⁻¹ to 600 mg kg⁻¹ after irrigation occurs. In acid sulphate soils, the concentration of Fe²⁺ can reach more than 5,000 mg kg⁻¹ after several weeks of irrigation. In acid soils with high levels of organic matter and high oxides it can produce ferrous concentrations to toxic levels in ultisol, oxisol, acid sulphate, and tidal peat soil [23, 24].

The results of the analysis of iron content in parts of roots, stems, leaves, grain, and rice showed diversity in each variety and part of the plant. The presence of such diversity shows, there is a difference in tolerance of rice plants to iron stress (Tables 3 and 4) [21].

Rice germplasm has a very narrow genetic variability for endosperm iron content. Generally, plants use a reduction or chelation mechanism to get iron from the soil. The reduction mechanism, used by most plant species, involves the production of Ferric Chelate Reductase which reduces Fe^{3+} to Fe^{2+} on the root surface. Fe^{2+} is dissolved then transported into root cells by special transporters, namely, Iron-Regulated Metal Transporter 1 [34].

In Table 3, it can be seen that the iron content contained in the roots of the generative phase has increased compared to the vegetative phase. Iron levels increase in varieties; Mekongga, Inpari 24, Inpari 27 and Inpari 28, respectively for; 1.41; 1.11; 2.82; and 1.50 times compared to iron levels in the vegetative phase. In these data it can be seen that the increase in ferrous levels in Inpari 27 and Inpari 28 is greater than that of Mekongga and Inpari 24. This indicates that in the varieties of Inpari 27 and Inpari 28, iron levels increase more at the root part especially in the generative phase. The results of the study on transgenic rice plants gripped by iron, also showed that the rice plants were more translocating iron to the roots and leaves, this is thought to be a mechanism developed by plants to be able to adapt to iron stress. Roots respond by modulating metabolism, gene expression and protein activity, which results in changes in cell wall composition, transport processes, cell size and shape, and root architecture [29]. The increase in iron content is thought to be a plant mechanism to influence the adaptability of the rice varieties to iron stress [25, 26].

Figure 2A shows that 4 seedlings were planted according to the SBSU System with the distance of each seed being 7.5 x 7.5 cm, and the distance between the clumps was 25-30 cm. The cultivation pattern with this method can produce tillers around 60-120 stems [16]. This happens, because rice plants avoid competition in the vegetative phase, especially in the formation of tillers so that they can produce more numbers of tillers even in iron-clad conditions.

The development of iron-rooted rice plants is still actively growing, we can see this in Figure 2B. The results of the analysis, the roots contain the most iron compared to other parts, such as stems, leaves, grain and grain of rice (Tables 3 and 4). In the generative phase of iron accumulation in roots increases higher, the mechanism is thought to be used by rice plants to adapt to iron stress by detoxifying stress, externally and internally. Tolerant rice varieties have genetic abilities in the management of nutrients and environmental influences

to adapt to nutrient stress [3, 5]. Proteins play a different role in detoxification which is coded localized to all root cells [33].

In the vegetative phase the iron content absorbed by the stem is higher when compared to the generative phase. Decrease in iron content in Mekongga, Inpari 24, Inpari 27 and Inpari 28 respectively, namely; 0.18; 0.33; 0.39 and 0.09 times. This is inversely proportional to the iron content in the roots. For leaf parameters iron content absorbed by plants when entering the generative phase has increased compared to the vegetative phase. In all rice varieties the increase in iron content varies, namely; Mekongga (100%), Inpari 24 (63%), Inpari 27 (84%) and Inpari 28 (50%), compared to iron content in the vegetative phase (Tables 3 and 4). The difference in the pattern of iron content absorption in the vegetative to generative phase, shows that there are differences in the adaptation mechanisms of each rice variety in the face of iron stress, this causes a difference in the tolerance level of each variety in the face of stress [5, 27].

The highest iron content in the Mekongga variety grain, which is 56.40 mg kg^{-1} , whereas the lowest in Inpari 24 varieties is significantly different from other varieties (Table 4). Whereas, the highest iron content in rice parameters occurs in Mekongga varieties, which is 44.78 mg kg^{-1} , significantly different from other varieties namely, Inpari 24 (29.47 mg kg^{-1}), Inpari 27 (27.60 mg kg^{-1}) and Inpari 28 (23.42 mg kg^{-1}).

In Table 4, it can be seen that all rice varieties absorb iron to be translocated to grain and rice grains. In Inpari 24 varieties iron absorption in grain is lower than other varieties, namely 40.33 mg kg^{-1} , but iron absorption is higher in rice grains which is equal to 29.47 mg kg^{-1} , except Mekongga which is 44.78 mg kg^{-1} and not different real with Inpari 27 and 28 respectively 27.60 and 23.42 mg kg^{-1} . The highest absorption of iron content in grain and rice grains is in the Mekongga variety, which is equal to 56.40 mg kg^{-1} and 44.78 mg kg^{-1} . The genetic engineering approach has targeted increasing iron absorption, translocation and storage in rice endosperm [34].

In all parameters observed (Tables 1, 2, 3 and 4), it can be seen that the mechanism of all hybrid rice varieties adapt to iron stress in the vegetative and generative phases, lies in its ability to regulate iron distribution patterns absorbed by roots, stems, leaves, grain and rice in the vegetative and generative phases. In all the varieties observed, it can be seen that

the pattern of distribution of Fe will increase from the vegetative phase to the generative phase, especially in the root and leaf parameters, but decreases in the stem parameters.

The mechanism of plant tolerance to stress can occur both externally and internally [4, 19, 28]. The internal mechanism occurs by regulating the absorption and translocation of iron in the roots, stems, leaves, grain and grains of rice. In plants tolerant to nutrient stress more are translocated to the root portion [29] especially in the generative growth phase. Adaptation through an external mechanism requires an avoidance mechanism, while the internal mechanism requires high tissue tolerance to iron stress or avoidance of high iron concentrations in the tissue [11]. High iron content as a result of irrigating wetlands causes reduction of Fe^{3+} to Fe^{2+} which causes poisoning. Another problem, which often arises is the low level of soil fertility due to nutrient binding by heavy metals [30]. Rice fields that experience iron poisoning, will cause delays in plant growth and development, especially in sensitive varieties.

The results of research on several tolerant plant varieties such as corn, soybeans, wheat, ground cover legumes and rice showed the ability of plant roots to continue to grow and develop well even in nutrient-choked conditions [6, 9]. Adaptability of plants to stress, can also occur through the metabolism of nitrate (NO_3^-), ammonium (NH_4^+), nitrite (NO_2^-), phenolic acid as indicated by differences in levels of NO_3^- , NH_4^+ and NO_2^- between sensitive and tolerant species [13]. The main difference from the adaptation mechanism is in the area where the binding occurs, whether in the symplast or apoplast. Both of these mechanisms can occur simultaneously, although in different degrees according to the type of plant and its ability to adapt [31, 32].

Root dry weight variable, is an important indicator to see tolerance to stress and its ability to adapt to acid soils. Damage to root hood cells occurs because plants experience Ca deficiency which plays an important role in the development of plant cell walls [29]. In tolerant species there is a layer of mucus (mucilage) which acts to absorb most of the iron in the rhizosphere so that plants avoid damage to the roots. The results of this study indicate that, it is thought that in rice plants there are also similarities in tolerance mechanisms for environmental stress conditions [24], such as Fe^{2+} stresses. This research is very important for the development of engineering of breeding engineering against stresses, especially

varieties that are cultivated on iron-gripped land in the context of extensification and intensification of rice as a food source. The genetic engineering approach targets increasing iron absorption, translocation and storage in rice endosperms.

CONCLUSIONS

Grain weight production in all varieties is still high even in iron-clogged conditions, because the number of productive tillers formed on the SBSU system is more than 21 stems per clump, with production of grain weight $> 5.4 \text{ Mg ha}^{-1}$. The pattern of iron distribution in four hybrid rice varieties occurs through the mechanism of absorption and iron translocation in the vegetative and generative phases, including roots, stems, leaves, grain, and rice. The difference in iron content in each part shows the ability of each variety to adapt to iron stress. In order to obtain rice grains rich in iron and able to grow and produce well, it is recommended that aquaculture use the SBSU Inpari 27 system which is capable of producing iron content in rice grains of more than 27 mg kg^{-1} with grain weight production of 6.83 Mg ha^{-1} .

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Figure 1. Schematic description of the method of rice cultivation using the SBSU System, in each clump there are four sub-clumps of rice which then develop into one large clump with a number of tillers 60-120

Figure 2. Planting with the SBSU system using 2-week-old seedlings (A), and root growth of rice plants experiencing stress Fe^{2+} (B)

Table 1. Data on plant height, number of tillers, productive tillers, and percentage of productive tillers

Rice Cultivars	Parameters			
	Plant Height (cm)	Total Tiller (stem)	Productive Tillers (sticks)	Productive tillers (%)
Mekongga	71 a	65 a	22 a	34 b
Inpari 24	71 a	63 a	23 a	39 ab
Inpari 27	64 b	59 ab	24 a	42 ab
Inpari 28	64 b	45 b	21 a	49 a

The numbers followed by different letters on the same parameters in each treatment showed a significant difference at the 5% level of the LSD test

Table 2. Data on panicle length, number of branches, 1000 grain weight, Grain Weight/Clump and Grain Weight

Rice Cultivars	Parameters				
	Panicle length (cm)	Number of panicles (stalks)	1000 Grain Weight (g)	Grain Weight/Clump (g)	Grain Weight (Mg ha ⁻¹)
Mekongga	22.2 b	8.5 a	26.5 a	59.1 a	6.34 a
Inpari 24	24.0 a	8.3 a	28.0 a	67.3 a	5.36 a
Inpari 27	23.8 a	8.2 a	27.5 a	58.0 a	6.83 a
Inpari 28	23.3 ab	8.6 a	28.1 a	67.3 a	5.93 a

The numbers followed by different letters on the same parameters in each treatment showed a significant difference at the 5% level of the LSD test

Table 3. Iron levels in the roots, stems, and leaves of several lowland rice varieties in the vegetative and generative phases

Rice Cultivars	Iron content (mg kg ⁻¹)					
	Root		Stem		Leaf	
	Vegetative	Generative	Vegetative	Generative	Vegetative	Generative
Mekongga	2.86 ab	6.89 bc	0.051 ab	0.042 a	0.027 b	0.054 b
Inpari 24	3.42 a	7.23 b	0.046 ab	0.031 b	0.040 a	0.065 a
Inpari 27	2.51 b	9.59 a	0.054 a	0.033 b	0.032 b	0.059 ab
Inpari 28	2.61 b	6.52 c	0.044 b	0.040 a	0.026 b	0.039 c

The numbers followed by different letters on the same parameters in each treatment showed a significant difference at the 5% level of the LSD test

Table 4. The pattern of distribution of iron in grain and rice in several lowland rice varieties in the generative phase

Rice Cultivars	Iron content (mg kg ⁻¹)	
	Grain	Rice Grains
Mekongga	56.40 a	44.78 a
Inpari 24	40.33 b	29.47 b
Inpari 27	51.30 a	27.60 b
Inpari 28	54.56 a	23.42 b

The numbers followed by different letters on the same parameters in each treatment showed a significant difference at the 5% level of the LSD test