Asian Journal of Research in Crop Science

5(4): 17-23, 2020; Article no.AJRCS.60043
ISSN: 2581-7167

Effect of Ferrous Iron Stress to West Sumatera Paddy Rice (*Oryza nivara*) Growth on Nutrient Culture Media

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Authors' contributions

This work was carried out in collaboration among all authors. Author ID performed the experiment, wrote the manuscript and analyzed the data. Author Yusniwati proofread the manuscript. Author RSH assisted the experiment, prepared all experiment requirement. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRCS/2020/v5i430101
Editor(s):
(1) Dr. Al-Kazafy Hassan Sabry, National Research Centre, Egypt.
Reviewers:
(1) Jane Mary Lafayette Neves Gelinski, National Council for Scientific and Technological Development, Brazil.
(2) Vasudeva Singh, Gauhati University, Assam, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/60043

Original Research Article

Received 15 June 2020
Accepted 19 August 2020
Published 22 September 2020

ABSTRACT

Aims: The research aimed to study the response of West Sumatera brown rice growth on nutrient culture media.
Study Design: Factorial design with 2 factors in completely randomized design.
Place and Duration of Study: The research was conducted in Laboratory of Tissue Culture, Laboratory of Seed Technology and Shade net house, Faculty of Agriculture, Andalas University, Padang, Indonesia from May to July 2019.
Methodology: Factorial design with 2 factors in completely randomized design was used in the research. The first factor was brown rice genotypes (Talang Babungo, Sigambiri, Pido Manggih, Labuh Baru, Balingka, Situjuah, Banuhampu, Pulen Talao, Ladang Talamao and Sikarujuik. The second factor was $FeSo_2$ concentrations (0 ppm, 250 ppm, 500 ppm and 750 ppm). The data was analysed using F test 5% and continued by Duncan's New Multiple Range Test (DNMRT) 5%.
Results: The result showed that there was interaction between brown rice genotypes and Fe concentrations to plant height and root dry weight in early growth stage. According the stress

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sensitivity index, Sigambiri and Ladang Talamao were the tolerant genotypes to high Fe stress and Talang Babungo and Banuhampu were moderately tolerant genotype in high concentration of Fe stress.

Conclusion: High Fe concentration affected the brown rice growth. This result can be a consideration to cultivate brown rice in high iron content land.

Keywords: Oryza nivara; genotypes; stress sensitivity index; tolerant.

1. INTRODUCTION

Brown rice is one of rice type that has been widely consumed by people entire the world particularly urban community [1]. Brown rice is classified to functional staple food due to anthocyanin component that plays role for human health [2]. It also contains 77.5 g carbohydrate, 7.5 g protein, 0.9 g fat, 163 mg phosphor, 16 mg calcium, 0.3 g zinc and 0.21 mg vitamin B1 in 100 g [3]. Beside that, it is reported containing antioxidant. Antioxidant was reported to play role as degenerative diseases preventing such as coronary heart, cancer, diabetes and hypertension [4]. The public awareness for health causes the brown rice demand always increases every year.

Brown rice is reported to be cultivated in several countries of Asia and America. In Indonesia, many researches reported the brown rice genotype including in West Sumatera. In West Sumatera, 19 local brown rice has been reported and those are from several districts such as Solok, South Solok, Pasaman, West Pasaman and Pesisir Selatan [5]. This result indicates that West Sumatera local brown rice is potential to develop as superior varieties.

Brown rice production is a main problem in Indonesia. One of effort that can be used to reach the goal is the dry land use that widely available in out of Java island [6]. Olaleye et al. [7] stated that the brown rice productivity decreasing is not only caused by biotic stress, but also by nutrient deficiency in land, low soil pH (acid), sulfide (H_2S), Al and Fe toxic. Fe toxic is main problem in new rice field and rice in basin land [8]. The Fe toxic could decrease productivity up to 90% [9]. It also caused nutrient deficiency, plant cell damage so that the plant growth is disrupted [10]. The symptom of Fe toxic is started from vegetative stage. The plant become stunted, reproductive stage is disrupted and this condition caused the plant produces low number of panicle. Fe toxic in susceptible genotype caused the harvest age faster for 20-25 days even the plant did not produce the spikelet [11]. Cherif et al. [12] also added the Fe toxic affected the plant height, low tiller number and low plant chlorophyll. Mehraban et al. [13] reported that 250-500 ppm of Fe concentration in nutrient solution in pH 4.5-6.0 increased the Fe in rice plant tissue and showed the Fe toxic symptom in susceptible varieties. Amnal [14] also reported that in 250 ppm of Fe concentration, variety IR64 and Danau Gaung showed the severe bronzing and increased in line to Fe concentration addition. In IR64 variety, most severe symptom occurred in 1000-1500 ppm of Fe concentration with the bronzing score 6-7, meanwhile for Grogol and Hawarabunar varieties, bronzing score was 2 and 3. Krowal Panjang, a variety that its tolerance to Fe previously was not reported, it showed the Fe toxic symptom was similar to Fe toxic symptom that underwent by Indragiri and Punggur varieties with bronzing score 4 and 5.

One of effort to solve the problem in marginal land is tolerant plant use to environmental stress. An effort to increase plant growth and neutralize Fe impact is important in increasing the plant growth particularly rice cultivation in new rice fields with Fe^{2+} content > 1000 ppm. The tolerant plant to environmental stress is able to adapt morphologically and Physiologycally [15]. The research aimed to study the effect of Fe stress to West Sumatera brown rice (*Oryza nivara*) growth on nutrient culture media.

2. MATERIALS AND METHODS

2.1 Experimental Site

The research was conducted in Laboratory of Tissue Culture, Laboratory of Seed Technology and Shade Net House of Faculty of Agriculture, Andalas University, Padang, West Sumatera Indonesia from May to July 2019.

2.2 Preparation of $FeSO_4$ Stock Solution

The nutrient solution was made according Hoagland nutrient solution composition. Four solution stocks were obtained, A, B, C and D. These materials then were dissolved by distilled water and stirred until homogeneous by magnetic stirrer and then entered into bottles and storaged in refrigerator. For iron stress stock solution, it was made from 15 g of $FeSO_4 7H_2O$ and 1 liter of distillation water.

2.3 Preparation of Nutrient Culture Media and $FeSO_4^-$ Treatment

The nutrient solution was taken from stocks solution that were previously made. The nutrient culture media was made by taking 100 mL of stock solution and entered to seedbed sized 35 x 45 cm. For iron stress media, iron stock solution was added according the protocol. Then, 100 L/seedbed of distilled water was added. pH of nutrient culture medium was corrected to 4.0 by using NaOH 0.1 N. If the pH was lower than 4, the pH was increased by adding NaOH 0.1 N until the pH score became 4.

2.4 Examination of Effect Fe Stress yo West Sumatera Brown Rice

Factorial design with 2 factors in completely randomized design was used in the research. The first factor was brown rice genotype. Ten brown rice genotypes were used in the assay, they were Talang Babungo, Sigambiri, Pido Manggih, Labuh Baru, Balingka, Situjuah, Banuhampu, Pulen Talao, Ladang Talamao and Sikarujuik. The second factor was $FeSO_4$ concentrations, they were 0 ppm, 250 ppm, 500 ppm and 750 ppm. Used seeds of the research was West Sumatera brown rice collection of Department of Agriculture, Andala University. The seeds were soaked by water for 24 hours. The seeds then sowed in sandy media on seedbed for six days. During the germination, the weeds were watered everyday until the media became moist. Water culture system was used to plant the sprouts. 12 seedlings/genotype of similar sprouts were selected to plant in nutrient culture media in seed bed. Previously, the seedlings were washed by distilled water. To keep the seedlings in media surface, the seedbed was covered by styrofoam that was previously perforated 30 holes and the cotton was entered to the holes. In middle of cotton, the seedling was entered until the root reached the solution that previously made. The nutrient solution was flowed by aerator to avoid the oxidation. The maintenance was conducted from germination until 6 days after planting (dap). The observed parameters were plant height, length of root, length of root extension, root dry weight and stress sensitivity index (SSI). The SSI was measured by formula follows [16]:

$$S = (1 - Yp)/(1 - Xp/X)$$

Notes:

S = Stress sensitivity index to Fe
Yp = average of a population value that underwent stress
Y = average of a population that did not undergo the stress
Xp = average of all population value that underwent stress
X = average of all population that did not undergo the stress

2.5 Statistical Analysis

All data were tested by F test in 5% and continued by Duncan's New Multiple Range Test in 5%.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Plant height

One of indicator the effect of iron to plant is plant height. Our result showed that 10 brown rice genotype showed the different response to iron stress. The highest plant growth in no iron stress was Sigambiri and the shortest plant was Situjuah. In 250 ppm of Fe stress, Banuhampu was the best genotype and the shortest plant growth occurred in Sikarujuik. In 500 ppm of Fe stress, Sigambiri was the best genotype and Sikarujuik was the shortest genotypes for plant height. In highest concentration, Banuhampu was the best genotype fo plant height and Sikarujuik was the shortest genotype for plant height (Table 1).

3.1.2 Length of root

Brown rice genotype showed the various length of root. According the result, Sikarujuik was genotype that had the shortest length of root (15.39 cm) and the longest root occurred in Sigambiri variety (26.03 cm) (Table 2). The different length of root of brown rice was dominantly affected genetic factor (Panda et al. 2019). Iron concentration did not affect the length of root of brown rice. Brown rice root could grow in Fe stress concentration. But, the texture of root was rough and rigid and the color was reddish brown than untreated brown rice root by Fe stress that had supple root, smooth and white (Fig. 1).

3.1.3 Dry weight of root and stress sensitivity index (SSI)

Our study showed that there was interaction between brown rice genotype and Fe stress. In 0 ppm of Fe concentration, the highest dry weight occurred in Ladang Talamao genotype and the lowest weight occurred in Sikarujuik genotype. In 250 ppm, Balingka was the highest dry weight and Labuh Baru was the lowest one. Genotype Sigambiri was the highest dry weight in 500 ppm concentration and Sikarujuik was the lowest one. In highest concentration, 750 ppm, Pido Manggih was the highest dry weight and the Sikarujuik was the lowest one (Table 3).

3.2 Discussion

The different response of brown rice genotype was caused by the characteristic of each genotype. The characteristic of plant is dominantly regulated by genetic factor [17]. According the result, Sikarujuik was the most susceptible genotype to Fe stress due to it underwent drastic decline in each Fe concentration. Stein et al. [18] stated that Fe toxic on rice plant was started from vegetative phase and it affected the plant height. The plant became stunted and reproductive development phase was disrupted. This condition caused the rice plant produced small number of panicle and empty grain.

Nutrient culture media use could accelerate and simplified the plant tolerance examination to Fe stress in water media that contained nutrients. This condition accelerated the plant selection [19]. According the result, we can conclude Pido Manggih, Balingka, Situjuah and Banuhampu were more tolerant genotypes than among tested genotypes.

The result indicates that Fe only affected the morphology of genotype but it did not affect the length of root growth of brown rice. Root underwent Fe stress, there was iron sediment in upper root layer and caused the texture of brown rice root became rough, rigid and reddish brown in color [20]. Green and Etherington [21] stated that iron stress symptom is marked by the appearance of iron sediment Fe^{3+} in top of brownish root.

Table 1. Plant height of brown rice genotypes after treated by Fe stress in 2 weeks after planting (cm)

Genotype	$FeSO_4$ concentration			
	0 ppm	**250 ppm**	**500 ppm**	**750 ppm**
Talang Babungo	32.95 a AB	25.15 b B	22.90 b ABC	23.45 b BC
Sigambiri	38.95 a A	25.75 b B	28.70 b A	25.25 b BC
Pido Manggih	38.15 a A	23.75 b BC	25.80 b AB	29.00 b AB
Labuh Baru	33.20 a AB	21.95 b BC	22.20 b ABC	23.60 b BC
Balingka	33.30 a AB	24.65 b B	21.95 b ABC	27.80 ab ABC
Situjuah	30.20 a B	26.30 ab B	22.70 b ABC	27.45 ab ABC
Banuhampu	35.50 a AB	34.65 a A	22.40 b ABC	33.20 a A
Pulen Talao	34.05 a AB	20.65 b BC	21.20 b BC	21.00 b CD
Ladang Talamao	36.60 a AB	24.15 b B	21.75 b ABC	25.65 b BC
Sikarujuik	35.30 a AB	17.40 b C	16.60 b C	15.05 b D
Coefficient of variation = 11.17%				

Note: Similar letters indicate significantly different according Duncan's New Multipe Range Test 5%

Length of root of several brown rice genotype that was applied by iron stress was longer than untreated by iron stress. It indicates there is no effect of iron concentration to root growth of brown rice. In concentration 250 ppm of iron, Balingka, Situjuah and Banuhampu had longer root than untreated plant by Fe. In 500 ppm concentration, generally, length of root of brown rice was longer than untreated plant except Balingka and Banuhampu genotypes.

Generally, the treated brown rice genotype by Fe concentration showed the the higher length of root than untreated genotype. It caused the dry weight to of brown rice genotype was also higher than untreated genotype. Fageria et al. [22] stated that dry weight indicated the ability of plant to absorb water due to the root of plant that had higher dry of weight was bigger than lower dry of weight. According to root performance, treated genotype by Fe stress showed the rough root and the size was big and there was Fe sediment in root system. This condition caused the treated genotype by Fe stress was higher than untreated by Fe. The involved mechanism in plant resistance to Fe toxic was the iron layer formation in root surface. In stress condition, the high Fe stress caused the more sediment [20].

Table 2. Length of root of brown rice genotypes after treated by Fe stress in 2 weeks after planting (cm)

Genotype	$FeSO_4$ Concentration				Average	
	0 ppm	250 ppm	500 ppm	750 ppm		
Sigambiri	30.50	22.60	26.05	24.95	26.03	A
Talang Babungo	24.00	22.70	24.30	20.20	22.80	AB
Ladang Talamao	27.00	20.65	25.40	17.05	22.53	AB
Balingka	19.75	24.75	22.05	22.45	22.25	AB
Pido Manggih	21.75	19.50	22.35	19.90	20.88	AB
Banuhampu	19.75	23.15	22.95	17.25	20.78	AB
Situjuah	17.55	21.50	23.80	20.00	20.71	AB
Labuh Baru	20.00	15.75	25.95	16.25	19.49	BC
Pulen Talao	21.00	15.35	19.20	19.10	18.66	BC
Sikarujuik	16.25	15.10	17.00	13.20	15.39	C
Coefficient of variation = 23.29%						

Note: similar letters indicate significantly different according Duncan's New Multipe Range Test 5%

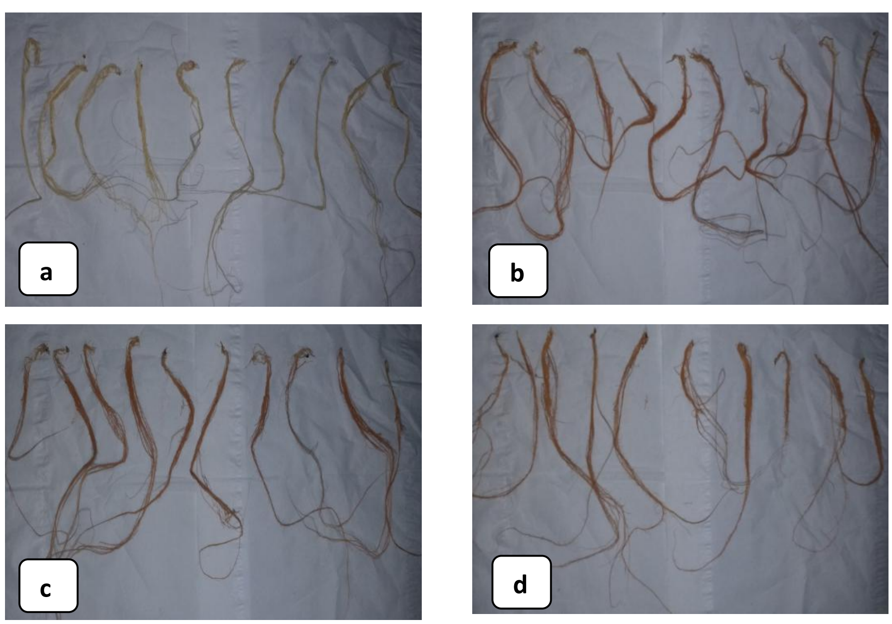

Fig. 1. Length of root of 10 brown rice genotypes performance in several Fe concentration in 2 weeks after planting (a= 0 ppm; b = 250 ppm; c = 500 ppm; d = 750 ppm)

Table 3. Stress sensitivity index (SSI) of root of brown rice genotypes after treated by Fe stress in 2 weeks after planting

Genotype	Stress Sensitivity Index					
	250 ppm	Note	500 ppm	Note	750 ppm	Note
Talang Babungo	0.0502	T	0.301	T	0.531	MT
Sigambiri	0.412	T	0.495	T	0.479	T
Pido Manggih	1.130	S	1.713	S	2.038	S
Labuh Baru	0.204	T	2.136	S	1.066	S
Balingka	4.721	S	2.355	S	3.401	S
Situjuah	2.438	S	1.828	S	1.122	S
Banuhampu	0.586	MT	0.627	MT	0.536	MT
Pulen Talao	0.978	MT	0.794	MT	1.053	S
Ladang Talamao	0.314	T	0.363	T	0.320	T
Sikarujuik	2.431	S	1.240	S	1.389	S

Note: Tolerant (T) if SSI value < 0.5; Moderately Tolerant (MT) if SSI value 0.5 < ISC ≤ 1; Susceptible (S) if SSI value ≥ 1

Root is most responsive part of plant to stress including iron [23]. The root poisoning affects the growth of plant due to this part is responsible to absorb water and nutrients that required by plant. Disruption of this process causes the plant poisoning. The plant poisoning by iron stress in vegetative phase decreases the plant height, tiller, chlorophyll and dry weight of root [22]. The benefit of brown rice examination to Fe stress in nutrient culture is to obtain the tolerant genotype to iron stress that can be widely cultivated in land that contains iron [24].

4. CONCLUSION

This study has proven that there is interaction between brown rice genotypes and several Fe concentrations. According to the stress sensitivity index, four brown rice genotypes (Talang Babungo, Sigambiri, Banuhampu and Ladang Talamao) are recommended to grow in high iron land. However, the examination of iron stress effect to yield of brown rice are required to obtain superior genotypes that resistant to iron stress with high production.

ACKNOWLEDGEMENTS

Authors would like to thank to everyone who helped and supported the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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