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Screening of Ferro Stress Tolerant Rice Varieties based on Agronomy and Physiology Characters using Hydroponic Method

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Abstract: The availability of fertile land for agriculture is minimal, as a result, marginal land is problematic to meet food needs. The problem with cultivating rice on this land is ferrous ion poisoning which causes nutrient deficiencies, plant cell damage, air deficits and growth barriers. This research expands the survival rate, growth and several agronomic and physiological characteristics under ferrous stress conditions. The aim is to produce important information about the effect of iron on the agronomic and physiological characteristics of several rice varieties cultivated hydroponically. The experiment was carried out in Air Pecah Lubuk Minturun, Padang City, West Sumatra from March to July 2022. The two- factor factorial experiment used a completely randomized design, with three replications. The first factor was rice variety, namely: Mekongga, Inpari 24, Inpari 27, and Inpari 28. The second factor was ferrous ion content consisting of three levels, 0.0 mg kg^{-1} Fe (control), 75 mg kg^{-1} Fe, and 150 mg kg^{-1}. The Mekongga rice variety has the lowest agronomic and physiological adaptability compared to the Inpari 24, 27, and 28 varieties in hydroponic cultivation. The highest MDG production under ferrous stress conditions in hydroponic cultivation was obtained to the Inpari 28 variety, namely 3,465 Mg ha^{-1} with iron contents in grain husks, and rice grains of 35 mg kg^{-1} and 34 mg kg^{-1} respectively.

Keywords: Adaptation; Ferrous grip; marginal land; screening; iron

Introduction

The availability of fertile land for agriculture is minimal, as a result, to meet food needs, the available land is marginal land which is problematic. To be able to utilize these lands, it is necessary to select and engineer plant varieties that are tolerant and able to adapt well [1]. The main problem in cultivating rice on this land is Fe^{2+} (ferrous) ion poisoning which causes nutrient deficiencies, plant cell damage, water deficit, and growth barriers. This resistance increases further when submerged in water, which causes an increase in ferrous ions which are toxic, and K, P, Ca, and Zn nutrients are deficient [2;3].

The methods used by researchers to determine the level of tolerance of plant varieties to various stresses include looking at the ability of plants to form a symbiosis with microorganisms [4;5;6], agronomic and physiological plant growth and detoxification mechanisms by organic acids, either by accumulation or exudation [2;4].

Rice germplasm has very narrow genetic variability for endosperm iron content [7;8], therefore conventional breeding has failed to increase endosperm iron content in rice varieties. Genetic engineering approaches are used for rice biofortification, and these approaches rely on information related to the genetic networks that control iron absorption, transport, and storage. Plants generally use reduction- or chelation-based mechanisms to obtain iron from the soil [1;2;9;10;11;12].

Efforts to bind micronutrients such as ferrous naturally by rice plants tolerant to ferrous stress can be carried out using the biofortification method. These ferrous ions will become part of the rice plant, especially in the rice grain content [1;13;14]. The hydroponic cultivation method makes it easier to fertilize, use water, and maintain and control the treatment carried out on experimental rice plants. So hydroponic cultivation makes it easier to control iron treatment for biofortification activities in rice plants.

Field testing and breeding into consumer-preferred varieties is necessary to demonstrate the agronomic resistance and nutritional benefits of high ferrous traits [13]. Therefore, the influence of various levels of ion stress on plant growth and development requires an in-depth study for iron biofortification.

Although ferrous ions can cause disturbances in plant metabolic processes, up to a certain threshold their effects can be tolerated by tolerant plants [6;13]. Plant tolerance to these stresses is an important factor for adaptability [15;16].

Identification of agronomic and physiological characters in plant growth due to increasing ferrous concentrations in nutrient solutions is an important parameter for selecting genotypes based on their level of tolerance to ferrous stress [2;9;13;17]. Serious problems in hydroponic rice cultivation with Fero stress will cause nutrient deficiencies, and damage to plant cells, causing stunted plant growth [2;10]. These growth barriers increase, especially in conditions of low solubility of essential nutrients, resulting in a deficiency of K, P, Ca, and Zn nutrients [4;18].

This study investigated survival rates, growth, and several agronomic and physiological characteristics under ferrous stress conditions. The research aims to produce important information about the influence of ferrous ions on the agronomic and physiological characteristics of several rice varieties cultivated hydroponically.

Materials and Methods

The experiment was carried out in Air Pecah Lubuk Minturun, Padang City, West Sumatra from March to July 2023. The experiment was carried out using the hydroponic

method using a four inch diameter pipe with a water source from irrigation water around the experimental rice fields. The two-factor factorial experiment used a completely randomized design, with three replications. The first factor was rice variety, namely: Mekongga, Inpari 24, Inpari 27, and Inpari 28. The second factor was ferrous ion content consisting of three levels, 0.0 mg Fe kg^{-1} (control), 75 mg Fe kg^{-1}, and 150 mg Fe kg^{-1}.

Rice seeds are kept for 2 x 24 hours, after they germinate, they are planted in the planting hole cups on the paralon with a planting distance of 30 x 30 cm. The nutrient solution for hydroponic water culture media uses Merck A & B nutrients which contain macro and micronutrients. The three-day-old sprouts were then grown in a plastic cup with holes in the plastic cup with foam on the inside. Three rice seeds were planted in each cup. On each paralon stem, there are 15 cups where rice plants can grow. Plants were maintained until 21 days old, without iron stress treatment. Iron ($FeSO_4$) treatment is only carried out in the third week after planting and then the treatment is given every 10 days until the plants are ready to harvest. The nutrient solution was changed every 14 days, and during the experiment, the nutrient solution was ventilated using an aerator. Harvesting is done after the leaves and grain of the rice plant are golden yellow with the grains being hard when pressed.

Results and Discussion

The Fe^{2+} levels contained in the nutrient solution in hydroponic cultivation used in this experiment have influenced the growth of all rice varieties. The growth of these rice varieties shows diversity in all observation parameters (Tables 1, 2, 3, and 4), this occurs because each rice variety has different genetic potential in responding to stress and the environment [4;8;10;19].

The results of the analysis of agronomic character parameters in several ferro-stressed rice varieties are presented respectively in Tables 1, 2, 3, and 4. These results show that there is diversity in growth and production in several ferro-stressed rice varieties in all observation parameters such as plant height, number of leaves, number of tillers $clump^{-1}$, number of productive tillers clump-1, age at flowering, panicle length, number of grains $panicles^{-1}$, percentage of filled grains $panicle^{-1}$, weight of grains $clump^{-1}$, weight of 1,000 filled grains, weight of milled dry grain (MDG) ha^{-1}, grain husks Fe content, and rice grain Fe content. This diversity shows that there are differences in the tolerance of rice plant adaptation mechanisms to iron stress.

The height of rice plants under ferrous stress conditions shows differences in the responsiveness of each variety. The Mekongga and Inpari 24 varieties caused a decrease in plant height, whereas in the Inpari 27 and 28 varieties, there was an increase in plant height with increasing Fero stress. The Inpari 28 variety in Fero stress conditions reached a height of 82 cm (+5%) which was the highest compared to other varieties, while the Mekongga variety was the lowest, namely 64 cm (-7%). This condition also occurred in the number of leaves parameter, wherein the Mekongga variety there was a decrease of 28% and Inpari 28 an increase of 14% (Table

1). The parameter number of leaves in clump^{-1} in all rice varieties shows an increase in the number of leaves along with increasing ferrous ion concentration, except for the Mekongga variety. The lowest number of leaves was 61 in the Menkongga variety and the highest number of leaves in the Inpari 27 variety, namely 95 leaves. The percentage increase in the number of leaves in the Inpari 24, Inpari 27, and Inpari 28 varieties respectively increased by 2% (2 pieces), 6% (5 pieces), and 14% (11 pieces) compared to conditions without stress, while Mekongga experienced a decrease by 28% (-24 strands) in conditions of 150 mg kg-1 iron stress (Table 1).

Table 1 also shows that the parameter number of clump^{-1} seedlings is significantly different. In Inpari 24 it tends to be stable under high ferrous stress, namely 24 tillers, while in the Inpari 27 and 28 varieties there is an increase in the number of tillers, respectively by 12% (3 stems) and 21% (5 stems) compared to conditions without ferrous stress, on the contrary the Mekongga variety experienced a decrease of 22% (-6 stems). In the parameters of productive tillers of clump^{-1} with conditions without stress and ferrous stress, the Mekongga variety had the lowest number of productive tillers, namely only 7-9 stems. Under conditions of ferrous stress, the Inpari 27 and 28 varieties experienced an increase in the number of productive clump^{-1} tillers by 31% (4 stems) while the Inpari 24 variety tended to be stable with a number of 18 tillers.

In terms of number of tillers clump^{-1}, all varieties under ferrous stress were significantly different, but the number of tillers clump^{-1} in Mekongga decreased by 22%, while Inpari 24 tended to be stable at high ferrous stress, namely 24 tillers. On the other hand, Inpari 27, and Inpari 28 showed an increase in the number of tillers clump^{-1}, respectively by 12% and 21%, even under ferrous stress conditions. Differences can be seen in non-stressed and stressed conditions, this is thought to influence the mechanisms by which plants adapt morphologically. Meanwhile, the productive tiller parameter of clump^{-1} under normal conditions for the Mekongga variety is the lowest, namely only 9 productive tillers, this shows 157% and 143% lower when compared to the Inpari 24, Inpari 27, and Inpari 28 varieties (Table 1).

Ferrous stress did not significantly affect flowering time in all varieties in stressed or non-stressed conditions, however, in the Mekongga variety, there was a tendency to flower later than other varieties, namely 73 HST. The Inpari 24 variety flowered more quickly in conditions without stress (67 days) and in stressed conditions, it took longer, namely 70 days. In the Inpari 27 variety, the flowering age is 67 days, both under stress and without stress, whereas in Inpari 28 the flowering age is 2 days faster (67 days) compared to without ferrous stress, namely 69 days (Table 1).

The differences in the growth of agronomic characters in each rice plant variety subjected to ferrous stress (Table 1) indicate that these differences indicate the different adaptability of each variety (Figure 1). One method to overcome this problem is to use plants that are tolerant to environmental stress [4;6;13]. Efforts to increase plant growth and neutralize the negative effects of ferrous ions are becoming increasingly important for increasing plant growth, especially in cultivating rice plants on ferro-stressed land. Plants that are tolerant to environmental stress can adapt morphologically and physiologically [2;6;20], thus enabling plants to grow and produce well.

The results of the analysis in Table 2 show that there is variation in panicle length for each rice variety in response to Fero stress. The shortest panicle length occurred in the Mekongga variety, which was similar to other varieties in both non-stressed and stressed conditions, namely 17-21 cm. The Inpari 24, 27, and 28 varieties did not show any significant difference in panicle length under conditions of stress and without ferrous stress (26-28 cm) with the longest panicle of the Inpari 24 variety, namely 28 cm. In Figure 1 you can see the differences in plant growth in the rice varieties Inpari 28 (A), Inpari 27 (B), Inpari 25 (C), and Mekongga (D).

Table 1: The plant height, number leaves per clump, tillers per clump, productive tillers per clump, and flowering age of several rice varieties in hydroponic ferro treatment

Fe Concentration (mg kg^{-1})	Mekongga	Inpari 24	Inpari 27	Inpari 28
Plant Height (cm)				
0	69cd	81a	70bcd	78abc
75	79abc	82a	80ab	81a
150	64d	80ab	80ab	82a
The Number Leaves (sheet clump^{-1})				
0	85ab	85ab	90ab	80ab
75	88ab	74bc	86ab	84ab

150	61c	87ab	95a	91ab
The Number Tillers (tillers clump^{-1})				
0	27ab	24ab	26ab	23b
75	26ab	21b	26ab	23b
150	21b	24ab	29a	28a
Productive Tillers (tillers clump^{-1})				
0	9bc	18a	13ab	13ab
75	7c	17ab	14ab	14ab
150	7c	18a	17ab	17ab
Flowering Age (days)				
0	71a	68a	67a	69a
75	70a	68a	67a	69a
150	73a	70a	67a	67a

Row and column numbers followed by the same lower case letters are not significantly different according to the DNMRT test 1%.

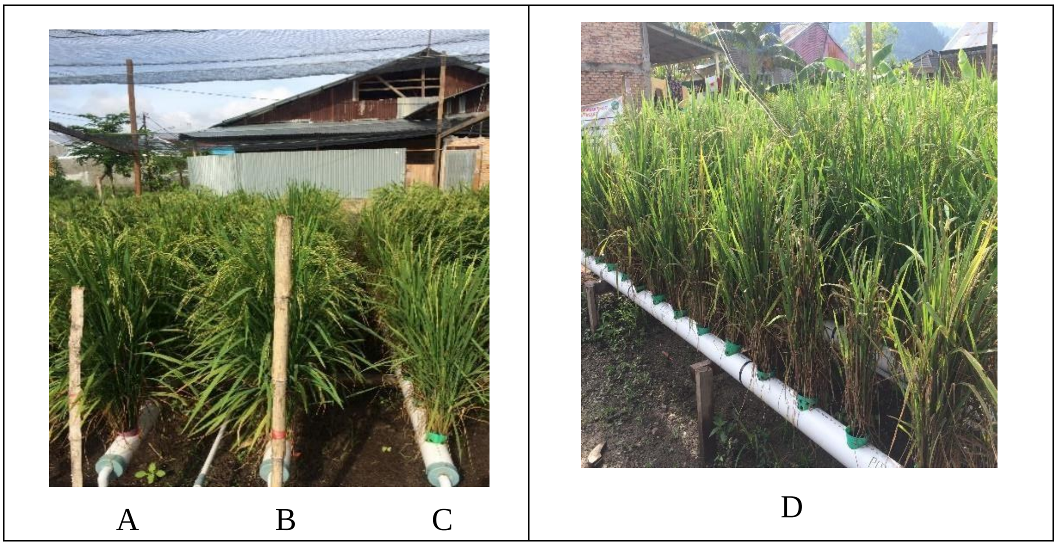

Figure 1: Growth of Inpari 28 (A), Inpari 27 (B), Inpari 24 (C), and Mekongga varieties affected by ferrous ions in hydroponic cultivation.

The number of grains panicle^{-1} in all varieties experiencing ferrous stress increased, the lowest number of panicles was in the Mekongga variety, namely 35 panicles in non- stressed conditions but increased to 63 and 44 panicles in ferro-stressed conditions. In ferro- stressed conditions, the number of grains per panicle in the three varieties (Inpari 24, 27, and 28) was not significantly different, namely 73-74 grains in panicle^{-1}, an increase of 46% (23 grains), 3% (2 grains), and 11% (7 grains) respectively (Table 2).

The percentage of pithy grain in the Mekongga variety in non-stressed and stressed conditions was the lowest compared to other varieties, namely only 6-9% of the number of grains panicle-1. This condition is very different from the Inpari 24, 27, and 28 varieties. The Inpari 24, and 28 varieties experienced a decrease of 11%, and 8% in stressed conditions respectively, whereas Inpari 27 experienced an increase of 2.7% (Table 2).

Table 2: The panicle length, the number of grains per panicle, and filled grains per panicle of several rice varieties in hydroponic ferro treatment

Fe Concentration (mg kg^{-1})	Mekongga	Inpari 24	Inpari 27	Inpari 28
Panicle Length (cm)				
0	20c	28a	27a	27a
75	17c	26ab	26ab	26ab
150	21bc	27a	27a	27a
The Number of Grains (grains panicle^{-1})				
0	35e	50de	72ab	66bc

75	63bc	58cd	64bc	56cd
150	44de	73a	74a	73a
Percentage of Filled Grains Panicle^{-1} (%)				
0	6cd	81ab	73bc	88a
75	9e	71cd	65d	80ab
150	8e	72cd	75bc	81ab

Environmental stress, including ferrous ions, causes real crop yield losses. At high concentrations, these stresses cause cell death, although at moderate levels they trigger adaptive responses [3]. The strategy used by most higher plant species involves the production of Ferric Chelate Reductase which reduces ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) on the root surface. Dissolved Fe^{2+} ions are transported to root cells by the Iron Regulated Metal Transporter 1 (IRT 1) transporter. IRT 1 is a member of the zinc, iron, and protein regulatory transporter [7;9;11;12;21;22].

The root dry weight variable is an important indicator to see tolerance to stress and its ability to adapt to acidic soil. Damage to root cap cells occurs due to Ca deficiency, which plays an important role in the development of plant cell walls. Intolerant varieties there is a mucus layer which plays a role in absorbing most of the Al in the rhizosphere so that the plant avoids root damage. The results of this research show that in rice plants there are also similar mechanisms of tolerance to environmental stress conditions, such as Fe^{2+} stress. The mechanism of tolerance to stress occurs internally and externally [6;20]. The exclusion mechanism requires a method of avoiding internal water deficit, while the inclusion mechanism requires high tissue tolerance to Fe^{2+} stress [4].

Table 3: Weight of rice filled grains per clump, weight of 1,000 filled grains, and MDG production of several rice varieties in hydroponic ferrous treatment

Fe Concentration (mg kg^{-1})	Mekongga	Inpari 24	Inpari 27	Inpari 28
Weight of Filled Grain (g clump^{-1})				
0	4,7d	25,3bc	32,9b	40,7a
75	0,8e	21,8c	28,1b	29,1b
150	0,9e	32,9b	40,5a	47,9a
Weight of 1,000 Filled Grains (g)				
0	16d	25bc	25bc	28ab
75	12e	23bc	26ab	25bc
150	14de	25bc	27ab	30a
MDG Production (Mg ha^{-1})				
0	0,397e	2,646bc	1,937d	2,352c
75	0,391e	1,671d	1,808d	2,091bc
150	0,267e	2,470c	2,89bc	3,465a

Row and column numbers followed by the same lower case letters are not significantly different according to the DNMRT test 1%.

The results of the analysis of the parameters for the weight of rice grain clump^{-1}, the weight of 1,000 grains, and the production of MDG clump^{-1} in the four rice varieties are presented in Table 3. This table shows that there is variation in the weight parameters of rice grain clump^{-1} in all rice varieties. The Inpari 24 variety's weight under stress conditions of 75 mg kg^{-1} Fe decreased by 37% (-7 grams), but under stress conditions of 150 mg kg^{-1} Fe its weight only decreased by 5% (-1 gram), namely 18 grams. A different thing happened to the Inpari 27 and 28 varieties, where under stress conditions of 150 mg kg^{-1} Fe, each variety increased its grain weight respectively by 47% (7 grams), and 44% (8 grams) but at stress 75 mg kg^{-1} Fe weight decreased. In the 1,000 grains weight parameters of the Mekongga variety, Inpari 27, and Inpari 28 experienced an increase in the weight of 1,000 grains by 14%, 8%, and 7% respectively, while Inpari 24 weighed 25 grams in the stressed and unstressed conditions.

Table 4: Ferro content of grain husks and rice grains of several rice varieties in hydroponic ferro treatment

Fe Concentration (mg kg^{-1})	Mekongga	Inpari 24	Inpari 27	Inpari 28
Grains Husks Fe Content (mg kg^{-1})				
0	21b	39ab	30ab	35ab
75	18b	29b	35ab	28b
150	51a	30ab	20b	35ab
Rice Grains Fe Content (mg kg^{-1})				
0	27abc	15c	22abc	45a
75	28abc	34a	23abc	19bc
150	22abc	32ab	22abc	34a

The ferrous content absorbed by each rice variety and translocated to the grain husk and rice grains showed differences. The Mekongga variety under stress conditions absorbed the highest amount of ferrous, namely 51 mg kg^{-1}, an increase of 143% compared to without stress. This condition is different from other varieties, where in stressful conditions the Inpari 24, 27, and 28 varieties reduce the amount of ferrous uptake, respectively by 23% (-9), 33% (-10), and 35% (0). The ferrous content in rice grains in the Mekongga variety in conditions without stress (27 mg kg^{-1}) is lower than in stressed conditions, namely 22 mg kg^{-1}, likewise in the Inpari 28 variety from 45 mg kg^{-1} it decreases to 34 mg kg^{-1}, while the Inpari 27 variety in both conditions remained 22 mg kg^{-1}. A different thing happened to the Inpari 24 variety where in conditions without stress, the ferrous content was 15 mg kg^{-1}, and after stress occurred it increased to 32 mg kg^{-1}. The iron content in rice grains of all varieties cultivated hydroponically is much higher when compared to the iron content in rice grains on the market, which is around 2-3 mg kg^{-1} but generally does not contain iron [7;9;23].

Rice varieties adapt to ferrous stress in the vegetative and generative phases which lies in their ability to regulate iron distribution patterns. In the internal mechanism, the adaptation process occurs through regulation of ferrous absorption and translocation. This method requires high tissue tolerance to ferrous stress, while the external mechanism requires the avoidance of high iron concentrations in the tissue [1;9].

High levels of iron in susceptible varieties indicate that the plant is poisoned. Potassium deficient plants are often high in iron and show severe symptoms of toxicity. This shows that the screening method can be applied to select rice plants tolerant to iron stress. The ability to adapt can influence nutrient status, which plays a role in influencing the tolerance of rice plants to iron stress [2;10;24]. Tolerant rice plants can grow well even in ferro-stressed conditions.

Conclusion

Rice plants that are tolerant to ferrous stress (Fe^{2+}) show better growth in agronomic and physiological characteristics compared to sensitive varieties. The Mekongga rice variety has the lowest agronomic and physiological adaptability compared to the Inpari 24, 27, and 28 varieties in hydroponic cultivation. The ability to adapt to ferrous stress can be seen in the development of agronomic and physiological characters. The highest MDG production under ferrous stress conditions was 3,465 Mg ha^{-1} in the Inpari 28 variety in hydroponic cultivation with iron levels in grain husks and rice grains of 35 mg kg^{-1} and 34 mg kg^{-1} respectively.

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