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Indices Of Drought Stress Tolerance And Sensitivity Of M3 Rice Mutant Lines (*Oryza sativa* L.) On Organic System

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ABSTRACT

One of the main problem of rice production are limited water as abiotic stress, enhancement rice variety through mutation technique by using gamma rays radiation is one of the alternatives to produce a new genetic source that tolerance to drought, and rapid methods for detecting mutant line tolerance to drought is by using indices screening. This study aimed at examining stress tolerance index, stress sensitivity index, root system and leaf rolling of the lines of mutant rice irradiated by gamma rays. The design utilized in this study was a split plot on randomized group design with drought stress (normal condition, drought stress) as the main plot and the genotypes (Inpari 10, IR 64, Sanbei, Unsyiah-1 Sanberasi, Unsyiah-3 Sanberasi, Unsyiah-5 Sanberasi) as subplots. These genotypes were those mutated by using gamma radiation of 250 Gy. The study found that the treatment of M3 mutant rice genotypes and drought stress affected the root system of rice, which was observed on day 45 and day 60 post-plantation, as well as at harvest time. Sanbei genotype had the longest root, compared to those of the other genotypes. On the other hand, the heaviest root under drought stress condition on day 45 post-plantation belonged to Unsyiah-3 Sanberasi genotype. The weight, however, was not too statistically different from those of the other genotypes of the rice mutant lines. According to the calculation of drought sensitivity index, the genotypes that showed medium tolerance to drought stress were Unsyiah-1 Sanberasi, Unsyiah-5 Sanberasi, IR 64 and Inpari 10. Although Inpari 10 genotype met the criteria for the moderately tolerant (MT), this genotype had a lower STI (0.94) compared to the other genotypes in the same group. It concluded that rice lines mutant Unsyiah –1 Sanberasi and Unsyiah- 5 Sanberasi have tolerance to drought stress compared to other genotypes.

KEYWORDS: Rice mutant lines Drought stress Root system Stress sensitivity index Stress tolerance index

INTRODUCTION

Rice is the world's most important food crop in addition to wheat [1], as more than 60% of the world's population consume rice as staple food, particularly those who live in countries situated on the tropics [2]. The consumption of rice in Indonesia increased year by year along with the increase of Indonesian's population growth [3]. Nowadays, much of rice is cultured on lands with various abiotic limiting factors, causing a major problem in the world's rice production. These abiotic factors, which affect plant metabolism, include drought, salinity, cold, and heat. However, drought is the greatest abiotic stress that causes the decrease of unhusked rice productivity. Drought stress also leads to the decrease of plant cell water potential and turgor pressure, as well as the increase of solutes concentration in the cytosol and the extracellular matrix; therefore decreases cell enlargement, inhibits cell growth, and causes reproduction failure. Moreover, drought stress influences stomata

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closure and gas exchange process, in addition to decreasing transpiration speed and carbon capture from the air [4]. Referring to these problems, it is extremely crucial to correct the plant traits by maintaining its production under varied agro-climates [5].

Variety enhancement through mutation technique by using gamma rays radiation is one of the alternatives to produce a new genetic source [6], and it is also a very successful approach to the generations of commercial varieties [7]. A mutation technique by using gamma rays is expected to improve the traits of one variety without altering the others. The mutant plants are able to uptake more water than control plants in water stress condition, and found the two best rice mutant lines namely MK-D-2 and MK-D-3 were selected in M6 generation and characterized by using physiological screening techniques [7]. Local varieties are potential varieties as genetic sources which has a hight tolerance various types of stress. Sanbei is one of local Aceh's varieties that have been reported tolerance toward drought stress [8].

Numerous variables have been used by researchers in determining a plant's tolerance level towards drought stress, and the criteria have been proposed to select genotypes based on their performance in stress and non-stress condition [9]. The indices of drought are quantitive measures that characterizes the level of tolerance to environmental stress by using assimilation data [10]. The relative yield performance from genotypes that growth in drought-stress and non stress condition can be use an indicator to identify tolerance level towards drought stress [11]. The indices used in this study are drought stress tolerance index (STI) [12] and sensitivity index (SI) [13]. According to Ali *et al*., [14] that grain yield under stress and non-stress environments were highly correlated with the stress tolerance index (STI), and found that STI were considered the best indices for the selection of the relatively tolerant lines. On the other hand, leaf rolling is considered as an alternative way to determine leaf water status visually and highly correlated with retaining leaf potential [15].

Root efficiency is one of the factors contributing to the absorption of nutrients and water from soil [16]. Root is also the organ which plays an essential role as the plant responds to drought stress. The depth of upland rice's root systems has been studied as drought adaptation [17]. The roots found in the deeper layer of the soil are still surrounded by moisture, enabling them to continue to grow. For this reason, the root system will multiply it self by optimizing groundwater exposure [18]. Davatgar *et al*., [15] reported that root weight was highest under water stress condition at 50% of flowering followed by severe stress at mid-tillering compared to root under well water plants. Therefore, this study was aimed at examining stress tolerance index, stress sensitivity index, and leaf rolling of the lines of mutant rice irradiated by gamma rays.

Methodology:

This study was conducted in a greenhouse located in the Experimental Garden of the Faculty of Agriculture, Syiah Kuala University in Darussalam, Banda Aceh. The coordinates of this location is 5°34'1,63"N - 95°22'23,09"W. The study was conducted from December 2015 to June 2016. The planting materials used in this study were varieties of Acehnese local rice seed such as the Sanbei variety and Sanbei that had been radiated with a dose of 250 Gy gamma rays. The gamma-radiated rice seeds used in this study were M_3 seeds which had three different variances from their predecessors and two comparison varieties: drought stress tolerant variety (Inpari 10) and drought stress susceptible variety (IR64). The medium used was entisol soil, while the fertilizers were PIM organic fertilizer, Bio Super Active organic liquid fertilizer, and Beyonic biodecomposer.

The no-drying treatment of rice was done by keeping the planted rice in normal condition by means of watering and maintaining a 1-2 cm layer of water above the ground until the plant entered the reproductive phase [19]. Meanwhile, the drought stress treatment was done in two phases: vegetative and generative phase. In the vegetative phase, the drying process began when the plant was 30 days old, and the rewatering took place two weeks afterwards. In the generative phase, drying process began 14 days after the recovery period from the the drying process of the former phase. The drying process in the generative phase was carried out for 7 days [20].

The design utilized in this study was the Split Plot Design, with drought stress acting as the main plot (control, drought) and the genotypes (Inpari 10, IR 64, Unsyiah-1 Sanberasi, Unsyiah 3 Sanberasi and Unsyiah 5 Sanberasi) acting as subplots.

Root system was measured based on the length of the roots and their dry weight. The measurement of the root length was done three times: after the completion of the vegetative phase, after the completion of the generative phase, and at harvest time. The root length was measured from the tip of the plant to the longest root. The dry weight of the root, however, was obtained by weighing the roots that had been dried in the oven at 60^0C for 3x24 hours, hence maintaining a constant weight.

The leaf rolling was visually observed and categorized based on the standard evaluation system for rice by giving a score of 1 to 9 referring to the following Table 1.

The sensitivity index was calculated by using the formula of [13] below:

$$S = \frac{1 - Y}{Yp} / \frac{1 - X}{Xp}$$

Where S = sensitivity index, Yp = the mean yield of unhusked genotype under stress condition, Y = the mean yield of unhusked genotype under non-stress condition, Xp = the mean yield of all genotypes under stress conditions, X = the mean yield of all genotypes under non-stress conditions. The criteria to determine the degree of stress tolerance was: if the S value was lesser than 0.5 ($S \leq 0.5$), the genotype was sensitive; if the S value was between 0.5 and 1 ($0.5 < S \leq 1$), the genotype was moderately sensitive; and if the S value was greater than 1 ($S > 1$), the genotype was considered resistant.

The formula for stress tolerance index [12] used was:

$$ITC = \frac{HC \times HP}{HP^2}$$

where HC = the mean yield of unhusked genotype under stress condition, and HP = the mean yield of unhusked genotype under normal condition.

The data obtained in this study were analyzed by using an F-test. As a significant effect was evidenced by the test, a Duncan New Multiple Range test (DNMRT) was applied at 5% rate ($DNMRT_{0.05}$).

Table 1: The scoring for leaf rolling [20]

Score	Leaf Rolling Criteria
0	Leaves healthy/no rolling
1	Leaves start to fold
3	Leaves folding into a V-shape
5	Leaves fully cupped into a U-shape
7	Leaf margins touching; making an O-shape
9	Leaves tightly rolled

Results:

Effect of Genotypes and Stress on Root Length of Rice:

Results of the analysis of variance showed that drought stress treatment had no significant effect on the length of root observed at day 45 and 65 post-plantation, nor at harvest time. Meanwhile, the genotypes of rice mutated by gamma-radiation demonstated a significant effect on the length of root observed at harvest time, but not at day 45 and 65 post-plantation. The root length affected by the genotypes of M_3 mutant rice lines is presented in Figure 1. The longest root was shown by Sanbei genotype, although the length was not too significantly different from that of Unsyiah-3 Sanberasi genotype. Inpari 10 genotype showed the shortest root length, followed by IR64 and Unsyiah-5 Sanberasi.

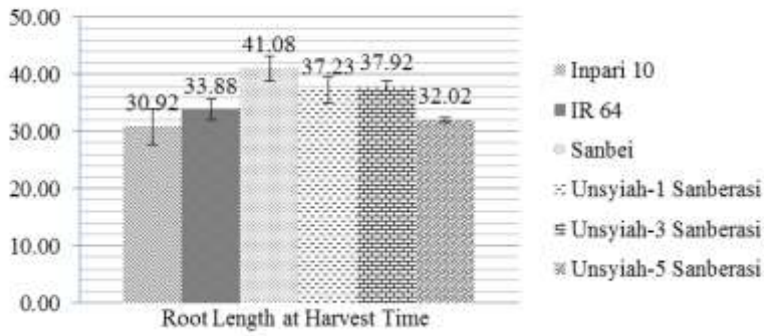

Fig. 1: Treatment effect of genotypes of M_3 mutant rice lines on root length at harvest time

Effect of Genotypes and Drought Stress on Dry Weight of Root:

Results of the analysis of variance showed that the interactions between rice genotypes and treatment of drought stress affected the dry weight of root observed following the first drying stage (day 45 post-plantation) and the second drying stage (day 65 post-plantation). However, no significant effect was seen on the dry weight of root observed at harvest time. The average dry weight of root as a result of interactions between the gamma-radiated rice genotypes and drought stress is presented in Table 2 and Table 3 below.

Table 2: The average dry weight of root at day 45 after plantation as a result of interactions between the genotypes and drought stress

Treatment	Dry Weight of Root at day 45 post-plantation (g)					
	Inpari 10	IR 64	Sanbei	Unsyiah-1 Sanberasi	Unsyiah-3 Sanberasi	Unsyiah-5 Sanberasi
Control	4,48 ± 1,51 Ab	6,71 ± 1,14 BCb	7,24 ± 2,51 Cb	4,86 ± 1,16 ABb	7,28 ± 1,24 Cb	8,51 ± 2,23 Cb
Drought stress	1,65 ± 0,61 Aa	3,12 ± 0,25 ABa	1,79 ± 0,18 Aba	2,30 ± 0,50 Aba	3,46 ± 0,92 Ba	1,74 ± 0,55 ABa

Note : Numbers followed by the same letters (An uppercase letter on the same row, a lowercase letter on the same column) indicate no significant difference at 0.05 probability (DNMRT 5%)

Table 2 shows that there are some differences in the dry weight of root of each tested genotype observed at day 45 post-plantation due to the different treatments given. The treatment of drought stress caused a decrease in the dry weight of root in each of the tested genotypes, which was significantly different from the plants in the control group. Under drought stress, the heaviest dry weight of root belonged to Unsyiah-3 Sanberasi genotype, which was not too significantly different from those of Sanbei, Unsyiah-1 Sanberasi, and Unsyiah-5 Sanberasi genotypes. Meanwhile, under normal condition, the heaviest dry weight was found in Unsyiah-5 Sanberasi, closely followed by Sanbei and Unsyiah-3 Sanberasi genotypes.

The same result is also demonstrated by the dry weight of root after the second drying stage (day 65 post-plantation). The dry weight of root of each genotype under drought stress condition differed significantly from the weight of those growing under normal condition. Under normal condition, the heaviest dry weight of root was shown by Unsyiah-1 Sanberasi genotype, which was not too significantly different from that of Sanbei genotype. Meanwhile, under drought stress condition, there was no significant difference between the dry weight of roots of each tested genotypes.

The different rice genotypes also affected the dry weight of root observed at the time of harvest. The following figure (Figure 2) presents the dry weight of root which was resulted from different treatments given to the genotypes and observed at harvest time. From figure 2, we can see that the Sanbei genotype had the heaviest dry weight of root, although the value was not too significantly different from those of Unsyiah-1 Sanberasi, Unsyiah-3 Sanberasi, and Inpari 10. Meanwhile, among the other genotypes, the lightest dry weight of root was shown by IR64 genotype. Nonetheless, its root's dry weight was not too significantly different from those of Inpari 10, Unsyiah-1 Sanberasi, and Unsyiah-3 Sanberasi.

Table 3: The average dry weight of root at day 65 after plantation as a result of interactions between the genotypes and drought stress

Treatment	Dry Weight of Root at day 65 after plantation (g)					
	Inpari 10	IR 64	Sanbei	Unsyiah-1 Sanberasi	Unsyiah-3 Sanberasi	Unsyiah-5 Sanberasi
Control	10,41 ± 3,25 Ab	13,52 ± 3,24 BCb	16,28 ± 3,67 CDb	18,49 ± 2,33 Db	14,03 ± 4,44 BCb	11,87 ± 1,84 ABb
Drought Stress	8,18 ± 1,84 Aa	6,54 ± 0,66 Aa	7,54 ± 3,04 Aa	7,37±2,11 Aa	6,52 ± 1,14 Aa	5,78 ± 1,81 Aa

Note : Numbers followed by the same letters (An uppercase letter on the same row, a lowercase letter on the same column) indicate no significant difference at 0.05 probability (DNMRT 5%)

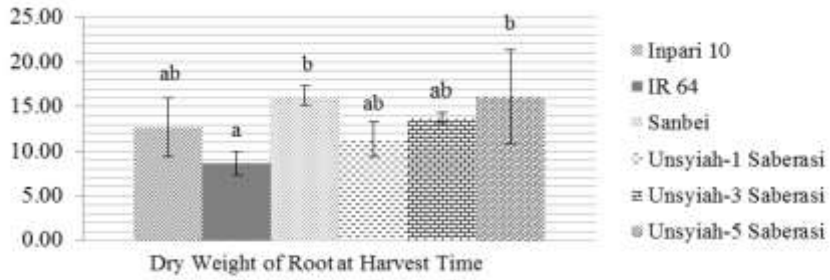

Fig. 2: Dry weight of root at harvest time as a result of interactions between genotypes and drought stress

Effect of Genotypes and Drought Stress on Leaf Rolling, Stress Tolerance Index, and Drought Sensitivity Index:

The observation of leaf rolling, stress tolerance index, and stress sensitivity index is presented in Table 4. Based on the observation, none of the tested genotypes showed the same leaf rolling score when observed on the drying period of both vegetative and generative phases. In the vegetative phase (day 7 post-drying), both IR64 and Unsyiah-3 Sanberasi genotypes scored 9 in the leaf rolling score [20], which means that their leaves were completely rolled. The leaves of Sanbei and Unsyiah-5 Sanberasi, on the other hand, folded into a V-shape (score of 3). On day 14 post-drying, all tested genotypes showed the leaf rolling of the score of 9. In the generative phase, IR64 and Unsyiah-3 Sanberasi also showed the leaf rolling of the score of 9. Meanwhile, Inpari 10 genotype scored 5 in the leaf rolling score, meaning that its leaves were fully cupped into a U-shape. The other genotypes showed the leaf rolling of the score of 7, which means that the leaf margins were touching one another and forming an O-shape.

Table 4: Effect on Genotypes and Drought Stress on Leaf Rolling, Stress Tolerance Index, and Drought Sensitivity Index

Genotypes	Leaf Rolling Score			STI	SI	Criteria
	Vegetative Phase		Generative Phase			
	Day 7 PD	Day 14 PD	Day 7 PD			
Inpari 10	5	9	5	0,94	0,93	MT
IR 64	9	9	9	1,10	0,80	MT
Sanbei	3	9	7	0,54	1,38	SS
Unsyiah-1 Sanberasi	5	9	7	1,19	0,77	MT
Unsyiah-3 Sanberasi	9	9	9	0,55	2,84	SS
Unsyiah-5 Sanberasi	3	9	7	1,26	0,70	MT

Description : PD: Post-drying; STI: Drought Stress Tolerance Index; SI: Sensitivity Index; T: Tolerant; MT: Moderately Tolerant; SS: Sensitive.

Discussion:

The results showed that each genotype had its own genetic factors and characters controlling its traits, which is indicated by the lengths and the dry weights of the roots. Those varied root lengths and dry weights were caused by internal and external factors of the plants themselves, namely genetic factor and growing environment factor. The internal factors, which were related to the activities of the meristem cells, contributed to the extension of root growth and plant stems. According to Arterburn and Dhingra [21] genetic traits influence plants' speed of cell division and hormonal responses, boost deep rooting formation and the efficiency of nutrient utilization, as well as many other considerable factors controlled by genetics. Gardner *et al.*, [22] added that internal factors (genetic) also influence the rates of photosynthesis, respiration, distribution of the products of N assimilation, formation of pigments such as chlorophyll and carotene, as well as enzyme activation and differentiation. Meanwhile, environmental factors that affect plant growth and development consist of climate, soil, and biological factors [23].

The results found that Sanbei genotype had the longest root. The previous research also showed that Sanbei genotype was local rice varieties had the longest radicula compared to other varieties (Cirata, Inpari 7, Ciherang, IR 64, Situ Patenggang, Situ Bagendit, Limboto, Towuti, Rommakokot, Pade Mas, Salah Manyang, Bo Santeut, Sigupai, Si Tadun, Ramoes, and Siputeh) [24]. Based on the analysis of variance (F test), drought stress treatment had no significant effect on the length of root observed at day 45 and 65 post-plantation, nor at harvest time, it was allegedly due to the planting was done in a pot. Efendi [25] reported that drought stress treatment had no significant effect on the length of root of upland rice grown in polybags. So, with limited media although plants develop resistence to drought stress by root elongation, but root elongation not showed significantly. Nevertheless, this root elongation of Sanbei genotype was a result of the genotypes resistance to drought. This is due to the fact that a drought-resistant plant elongates its root under water-deficit conditions to allow the roots to absorb water from deeper soil layers with enough water availability, which is impossible to do with short roots [26]. The depth root growth capability was the most accepted target for improving resistence to drought stress of plant [27].

According the analysis of variance showed that there are some differences in the dry weight of root of each tested genotype observed at day 45 and 65 post-plantation due to the different treatments given. The results showed that mean of dry weight of root decreased significantly after the plants were subjected to drought stress in vegetative (day 45 post-plantation) and generative stage (day 65 post-plantation). Laila and Waluyo [28] reported that rice plant IR 64 varieties that growth under drought stress showed the lower of mean dry weight of roots. Another study also reported that similar results, dry weight of rice hybrid root decreased significantly after the plants were subjected to drought stress in vegetative and generative stage [29]. It indicated that drought stress treatment during vegetative and generative stage due to inhibited root growth and root distribution and affected to the decreasing mean dry weight of root. Efendi [25] also added that the higher intensity of drought stress followed by decreases the dry weight of root.

Amos and Walter [30] pointed out that under drought stress conditions, the weight of biomass correlates linearly to the root biomass because most of the photosynthates from shoots are allocated for root growth, hence the root range is becoming wider and deeper for the purpose of absorbing water from deeper soil layers. In addition, referring to the study of [30], the depth and the dry weight of root under drought stress conditions not only correlate positively to the plant's ability to maintain the production of shoot biomass but also give an actual contribution in cutting down crop losses.

According to the calculation of stress tolerance index and drought sensitivity index presented in Table 4, it can be seen that each tested genotype showed different results of STI and drought sensitivity index. Genotypes that demonstrated medium tolerance to drought were the Inpari 10, IR64, Unsyiah-1 Sanberasi and Unsyiah-5 Sanberasi. The rice genotype that showed the highest tolerance index was Unsyiah-5 Sanberasi, followed by

Unsyiah-1 Sanberasi and IR64. The higher the stress tolerance index percentage, the greater the genotype's tolerance level to stress [9, 32]. In this study, although the Inpari 10 met the criteria for the moderately tolerant (MT), this genotype had a lower STI (0.94) compared to the other genotypes in the same group. These results are constantly with the results of study [11] that drought tolerance indices were varied indicating genotypic variability. Based on stress tolerance index, Unsyiah- 5 Sanberasi met the higher value compared to other genotypes, but the results of sensitivity index calculating showed that Inpari 10, IR64, Unsyiah-1 Sanberasi and Unsyiah-5 Sanberasi had a medium tolerance to drought stress .

In this case also showed that the amount of unhusked rice formed by Sanbei and Unsyiah-3 Sanberasi genotypes under drought stress condition was lower than that under controlled condition. Therefore, both genotypes' crop yield potential declined. This low crop yield potential of Sanbei genotype under drought stress treatment caused its drought stress sensitivity index to fall into sensitive (SS) category. This presumably occurred for two reasons. First, Sanbei genotype formed a great root system, which was indicated by having the longest root (Figure 1) and the heaviest root (Figure 2). Therefore, under drought stress condition, the assimilate partitioning of the genotype's growth was more likely to be distributed to form roots as a defense mechanism to protect the plant from drought stress [33]. Second, the genotype was planted in a flowerpot, hence the medium was very limited. Even though Sanbei genotype developed a resistance to drought by elongating its roots, it did not demonstrate any significant differences among the other genotypes with poor root systems.

It was discovered that, the indices of drought will be useful to rapidly screen selection of plant that tolerance to drought. In this study also found that mutation method allows to obtain a new genotypes that were tolerance to drought stress and had better growth performance than the parents. Genetic and molecular analyses are needed to ensure specific genes that control the tolerant properties of drought stress in rice mutant lines Unsyiah-1 Sanberasi and Unsyiah-5 Sanberasi.

Conclussion:

The drought stress treatment not showed significant effect on the length of root observed at day 45 and 65 post-plantation, nor at harvest time. Meanwhile, the genotypes of rice mutated by gamma-radiation demonstated a significant effect on the length of root observed at harvest time, but not at day 45 and 65 post-plantation. Based on observation Sanbei genotype had the longest root, compared to those of the other genotypes.

There are interaction between drought treatment and genotypes that affected the dry weight of root observed following the first drying stage (day 45 post-plantation) and the second drying stage (day 65 post-plantation). The dry weight of root decreased significantly after the plants were subjected to drought stress. On the other hand, the heaviest root under drought stress condition on day 45 post-plantation belonged to Unsyiah-3 Sanberasi genotype. The weight, however, was not too statistically different from those of the other genotypes of the rice mutant lines. Based on the observation of drought indices showed that rice lines mutant Sanberasi Unsyiah-1 and Sanberasi Unsyiah 5 have tolerance to drought stress.

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