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Enhancement of Drought Stress Tolerance in Aceh's Local Rice by Mutation with Gamma Rays Irradiation

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ABSTRACT

Background: Drought stress is a major limiting factor in efforts to increase rice production, it is caused by changes in rainfall patterns and extreme weather. **Objective**: The study aimed to improve the tolerance for drought stress by mutation induced with gamma rays irradiation on Aceh's local rice. **Methodology**: The seeds of Aceh's local rice were treated with gamma rays irradiation at a dose of 250 Gy. The third generation of mutant rice was cultivated on an organic farming system using 1.0 ton-1 ha of Petroganic fertilizer. **Results**: The gamma rays irradiation increased significantly the tolerance of M_3 mutant rice for drought stress. The plant height varied greatly between lines and the highest plant height showed by line Unsyiah-3 Sanberasi. The rice line produced from mutation gamma irradiation of 250 Gy was significantly higher than the parent plant (M_0), Sanbei. The interaction of rice lines mutated gamma radiation and drought stress significantly increased the percentage of productive tillers. The highest percentage of productive tiller shown by genotype *Unsyiah-3 Sanberasi*. The interaction of rice lines results gamma radiation and drought stress was very significant to increase the yield potential. Each rice lines tested also showed a different response to drought stress by variable yield potential. In drought conditions, the highest potential yield reached 2.81 tons ha-1 was shown by line *Unsyiah-1 Simeulu* and did not differ significantly with line *Unsyiah-5 Sibahak*. **Conclusion:** This study discovered that mutation of local rice with gamma rays irradiation improved the tolerance of local rice of Aceh to drought stress and enhanced the yield potential. Thus, gamma-ray mutations can be applied to increase rice productivity in sub-optimal lands that experience abiotic stress.

KEYWORDS: irradiation, gamma rays, drought stress, Sanbei, organic, Aceh.

INTRODUCTION

The efforts to increase rice production will remain to face various constraints [1], particularly abiotic stresses [2]. One of the abiotic factors that lead to decrease production of rice plants is drought conditions [3] and it has become a major limiting factor in efforts to increase the production of rice plants in all regions of the world [4,5]. Therefore, developing crop varieties that are adapted to drought stress is essential for the sustainability of agriculture [6,7,8]. New varieties may be obtained from the genetic resources of rice originating from the local rice varieties. Local rice varieties have the ability to adapt to diverse environmental conditions [9] and is the germplasm potential as a source of genes that control important traits in plants. It was reported that local rice variety had the advantage that was tolerant to drought stress [10].

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Improved varieties by using mutation with gamma ray irradiation is an alternative way to create new genetic resources [11], and was a very successful approach for the generation of commercial cultivars [12]. The gamma irradiation technique was used to enhance the quality of rice [13]. Mutation techniques with gamma rays were expected to improve the properties of a variety without changing the nature of the rice. Induced mutagenesis and its breeding strategies are potential tools for improving both quantitative and qualitative traits in crops within a much shorter period of time than conventional breeding [14]. It has been reported that plant breeding approach with gamma irradiation mutation techniques capable of improving some properties of a variety. It was also reported a local variety Aceh, Sanbei after irradiated with a dose of 250 Gy of gamma rays caused changes in agronomic characters on the M_2 mutant rice lines, such as the number of leaves and number of tillers were increased [15]. However, further research needs to be investigated about the level of irritability of some mutant strains of rice M_3 produced from gamma ray irradiation originating from Aceh's Sanbei to drought stress in order to investigate the new lines that are tolerant to drought stress on the organic farming system. This study aims to assess the response of some mutant strains of rice M_3 to growth, productive tillers and yield of rice in drought stress conditions which are cultivated with organic systems, as well as to examine the interaction between rice lines mutated gamma radiation M_3 with drought stress treatment in order to produce a pure line as a potential new varieties are high yielding, early lifespan and drought tolerant.

Methodology:

This study conducted in the a Greenhouse, located in the Experimental Farm, Faculty of Agriculture, University of Syiah Kuala, Banda Aceh, Indonesia. The research carried out in December 2015 until May 2016. The geographical location of the Experiment Station was located at 5°34'1,63"N-95°22'23,09"E with an elevation of 3 m above sea level. Research material used were local varieties of rice varieties of Aceh, Sanbei (parent strain) that have been irradiated with gamma ray at a dose of 250 Gy. Rice seeds resulted from gamma-ray radiation was used as rice seed M_3 which has three different variants of the parent (*Unsyiah-1 Simeulu*, *Unsyiah-3 Sanberasi*, *Unsyiah-5 Sibahak*), as well as one variety that was resistant to drought stress (*Inpari 10*), and the variety is susceptible to drought stress (*IR-64*).The organic fertilizer used was PIM-Organik with the composition of the C/N ratio of 15-25%; water content 10-25%; P2O5<6%; K2O<6%. It was also used bio-decomposer, Beyonic and liquid organic fertilizer, Bio-Super Active.

The seeds were germinated on growth media with cotton and incubated at 30°C for 3 days. Then, the germinating seeds were sown in seedling trays filled with soil. Growth media was screened with a sieve of 5,000 μm in diameter hole then added to potting soil that has been provided as many as 144 pot. Before planting, the media was puddled in the pot. The media left in waterlogged conditions to encourage the formation of soil aggregates. After one week of flooding, planting media was treated with organic fertilizers PIM 6.25 g pot-1 (1.0 ton ha-1) as a base fertilizer and a bio-decomposer solution of 5 ml each pot, then flooded back for one week. The seedlings 10 days after sowing were planted into the pots, each pot was planted one plant [16]. Maintenance carried out by addicting 3.0 g/pot of the organic materials at 14 days after planting. Weeding was done by removing the weeds in the pot manually every 2 weeks or depending on the growth of weeds in the pot.

Plants were treated with two methods, normal condition and drought condition. Normal condition was created by the regular water application with 1 cm of water level. Watering was hold in accordance with the age or stage of plant growth until the reproductive phase [16]. While, drought stress was treated in two stages of vegetative and generative phase. The drought condition was applied by drying the growing medium in the pot during 2 weeks. Then, it was recovered by watering until the water puddles as high as 1 cm of the soil surface in the pot, the healing process carried out for 10 days. Continuously, in the second stage of drying plant media back left in dry conditions for one week, when the rice plant entered the reproductive phase [17].

Experimental design and statistical analysis: The research was designed as Split Plot. The pattern randomized as block design factorial 2x6 with conditions of drought stress as the main plots and rice strains as subplots. Irrigation condition consists of two level items, namely normal condition and drought conditions. Factors of rice strain levels consists of six items, namely: *Inpari 10*, *IR64*, *Sanbei*, *Unsyiah-1 Simeulu*, *Unsyiah-3 Sanberasi*, *Unsyiah-5 Sibahak*. Data were analyzed by F test and if it showed a significant effect continued by Honestly Significant Difference (HSD) at 5% [18].

Results:

Effect of gamma rays on plant height of mutant rice:

Based on the analysis of variance (F-test) showed that the use of rice mutant line (M_3) and the treatment of drought stress effected on morpho-physiological performances of rice plant (Table 1 and Table 2). Statistical analysis showed that drought stress did not affect plant height, but gamma rays irradiation was significantly increased the plant height observed at 30 and 45 days after planting, as well as plant height at harvest time. Plant height of the rice mutants was significantly higher ($p<0.05$) than the parent plant, *Sanbei*. The plant height varied greatly among lines on the observation at the age of 30 days after planting. The highest plant showed by line *Unsyiah-3 Sanberasi*, and it was 10.46% higher than *Sanbei*, 7.80% higher than *Unsyiah-1 Simeulu*, 4.88%

higher than *Inpari 10*, and 10.07% higher than *IR 64*, and 2.2% higher than *Unsyiah-5 Sibahak*. Plant height of the rice mutants at age 45 days after planting were significantly higher ($p<0.05$) than the parent plant of *Sanbei*. The highest plant also indicated by *Unsyiah-3 Sanberasi* and it was 12.38% higher than *Sanbei*, 8.01% higher than *Unsyiah-1 Simeulu*, 7.81% higher than *Inpari 10*, 13.10% higher than *IR 64*, and 3.62% higher than *Unsyiah-5 Sibahak*. While, the plant height at harvest time, the highest value was also demonstrated by line *Unsyiah-3 Sanberasi*, and it was 24.11% higher than *Sanbei*, 23.38% higher than Unsyiah-1 Simeulu, 13.21% higher than Unsyiah-5 Sibahak, 31.12% higher than Inpari 10, and 34.22% higher than IR 64.

Table 1: Summary of the analysis of variance of plant morphology effected by mutant rice lines (M3) and treatment of drought stress in the organic farming system.

Parameters	F test value		
	Drought	Rice Lines	Interaction
Plant height (cm)	0.12 ^{ns}	29.79**	1.98 ^{ns}
Number of tillers	0.16 ^{ns}	0.15 ^{ns}	0.06 ^{ns}
Yield potential (ton ha-1)	2.80 ^{ns}	5.26**	3.89*

Description: *: Influential evident at the level of 0.05; **: Influential very real at the level of 0.01; ns: non significantly at 0.05 level.

Table 2: Average plant height in rice lines (M3) resulted from gamma ray irradiation and drought stress treatment at age 30, 45 days after planting (DAP) as well as at the time of harvest.

Treatment	Plant height (cm)		
	30 DAP	45 DAP	Harvest Time
Effect of drought stress			
Normal condition	69.46	72.18	94.64
Drought condition	69.20	70.84	93.72
$BNT_{(0.05)}$	-	-	-
Effect of Genotypes			
Inpari 10	70.04 bc	71.91 bc	85.00 ab
IR 64	66.61 a	68.29 a	82.17 a
Sanbei	66.14 a	68.36 a	91.17 b
Unsyiah-1 Simeulu	68.11 ab	71.77 b	92.05 b
Unsyiah-3 Sanberasi	73.27 d	77.25 d	113.37 d
Unsyiah-5 Sibahak	71.80 cd	74.77 cd	101.33 c
$BNT_{(0.05)}$	2.60	2.87	8.79

Description: Values followed by the same letter in the same column was no significant effect on the level of 5% chance (Test of LSD).

Effect of rice mutant line and drought stress on productive tillers:

The interaction of rice lines mutated gamma radiation and drought stress significantly increased ($p<0.05$) the percentage of productive tillers (Table 3). In normal condition, *Unsyiah-3 Sanberasi* showed the highest percentage of productive tillers. It was 4.63% higher than *Sanbei*, 23.75% higher than *Unsyiah-1 Simeulu*, and 17.53% higher than *Unsyiah-5 Sibahak*. In drought stress condition, Sanbei showed the highest percentage of productive tillers, and it was 0.38-9.64% higher than mutant lines. Each of these lines tested showed differences in percentage of productive tillers because of differences response of mutant lines to drought stress. Meanwhile, the average percentage of lowest productive tillers in drought stress conditions indicated by Inpari 10 genotypes, and it was not significantly different with line *Sanbei*, *Unsyiah-3 Sanberasi*, *Unsyiah-5 Sibahak*. In the normal condition (control), the highest percentage of productive tiller shown by *Inpari 10* but did not differ statistically with genotype *Sanbei* and *Unsyiah-3 Sanberasi*. In the condition of drought stress, line *Unsyiah-1 Simeulu* showed the percentage of tillers higher than the normal (control), while it was a different thing shown by genotype *Inpari 10*, where the condition of drought stress decreased the percentage of productive tillers.

Table 3: The average percentage of productive tillers due to the interaction effect between line rice mutated gamma radiation (M_3) and drought stress treatment

Condition	Percentage of productive tillers (%)					
	Inpari 10	*IR 64*	*Sanbei*	*Unsyiah-1 Simeulu*	*Unsyiah-3 Sanberasi*	*Unsyiah-5 Sibahak*
Normal	98.15 Db	83.33 BCa	89.29 CDa	71.61 Aa	93.92 CDa	77.46 ABa
Drought stress	76.68 Aa	90.86 Ba	90.91 Ba	90.56 Bb	85.86 ABa	82.15 ABa
$BNT_{(0.05)}$	11,79					

Description: Values followed by the same letter (Capital letters on the same line, the lowercase letters in the same column) showed no significant difference in the level of the opportunities 0.05 (Test of LSD)

Effect of rice mutant line and drought stress on yield potential:

Based on the analysis of variance (F test) the interaction of rice lines results gamma radiation and drought stress was very significant ($p<0.01$) to increase the yield potential of the mutant lines. The average yield potential due to the influence of the mutated line interaction of gamma radiation (M_3) and drought stress is presented in Table 4. Each rice lines tested also showed a different response to drought stress by variable yield potential. In drought conditions, the highest potential yield reached 2.81 tons ha-1 was shown by line *Unsyiah-1 Simeulu*. It was 38.62% higher than *Sanbei* as parent plant and 45.12% than *Inpari 10* as a control for drought tolerance genotype. However, the yield potential of *Unsyiah-1 Simeulu* did not differ significantly with line *Unsyiah-5 Sibahak*, which reached 2.52 ton ha-1. While, in normal conditions with the highest yield potential shown by line *Sanbei*, and it was highly significant with other lines. The observations showed that the line Sanbei was the highest yield potential in the treatment of normal condition.

Table 4: The Average yield potential due to the interaction effect between line rice mutated by gamma ray irradiation (M_3) and drought stress treatment

Condition	Yield potential (ton ha^{-1})					
	Inpari 10	*IR 64*	*Sanbei*	*Unsyiah-1 Simeulu*	*Unsyiah-3 Sanberasi*	*Unsyiah-5 Sibahak*
Normal	1.62 Aa	1.88 ABa	3.16 Cb	2.37 Ba	1.87 ABb	1.85 ABa
Drought stress	1.52 ABa	2.06 BCa	1.7 Ba	2.77 Da	1.02 Aa	2.32 CDa
LSD $_{(0.05)}$	0.58					

Description: Values followed by the same letter (Capital letters on the same line, the lowercase letters in the same column) showed no significant difference in the level of the opportunities 0.05 (Test of LSD)

Discussion:

It was discovered that mutation of local rice with gamma rays irradiation improved the tolerance of local rice of Aceh to drought stress. Gamma-ray mutations can be applied to increase the rice productivity, especially enhanced the yield potential. The another study showed that a positive signal exists in the effect of radiation on drought tolerance (19,20). The gamma radiation affected the morphological and agronomic characters of local variety of rice and the radiation suppressed the rice growth i.e., height of plant and number of tillers [20]. Plants respond to survive under water-deficit conditions via a series of molecular processes culminating in stress tolerance. The drought-inducible genes with various functions have been identified by molecular and genomic analyses in rice [21]. The improvement of a drought-tolerant genotype under different drought conditions is necessary to enhance rice productivity and food security [22].

In this study, it was found that plant height of each rice lines were varied widely (Table 2). Mutation technique have shown to be very useful in rice improvement, especially for characters controlled by closely linked genes that are difficult to break by gene recombination [23]. Used irradiation by gamma-ray exhibited different genetic variability such as semi-dwarf, early heading and high yield plants [23,24]. The variation of plant height in each line was caused due to internal factors and external factors affecting the growth of the rice plant. Internal factors are genetic factors of a crop, while external factors are environmental factors to grow plants [25]. The previous research showed that the morpho-agronomic performances of rice lines clearly referred to a high genetic variation of the local rice [9]. Each of these lines has genetic factors and different characters that control the properties of the line. Similar findings of another study showed that the internal factors (genetic) affected the photosynthetic rate, respiration, assimilation, formation of pigments such as chlorophyll and carotene, enzyme activation and differentiation [25,26].

The lines of mutant rice (M_3) produced from mutation gamma irradiation of 250 Gy (*Unsyiah-1 Simeulu*, *Unsyiah-3 Sanberasi*, *Unsyiah-5 Sibahak*) were higher than the parent plant (M_0), namely line *Sanbei* (Table 2). It was proved that gamma radiation can change the nature of the gene in rice plants of the third generation. Genetic changes in cells caused by radiation of gamma rays that can cause changes in plant phenotype [27]. Mutations with gamma radiation can increase genetic diversity, based on the results of this study in which morphological characters indicated by the mutant lines diverse and different from the parent. In consistent with other research, it was found three strains of rice that the mutation results in the third generation (M_3), which has a plant height was higher than the parent plants are subjected to drought stress.

The research showed that each line had different responses to drought stress treatment based on the percentage of productive tillers. Similarly, the variable plant height, leaf width, number of productive tiller percentage that varied greatly influenced by the interaction between genetic properties and environmental conditions. In the previous research, it was found that the tillering in addition influenced by genetic factors [9]. In conditions of drought stress, percentage of productive tillers of *Inpari 10* had decreased. It was because the period of vegetative growth and generative plant experiencing water stress, where water plants need to carry out various processes, such as forming and filling the cells of organs, regulating turgidities cells to run the mechanism motion organ (opening and closing of stomata), solvents solid materials, substances reactant in photosynthesis and as the temperature control of all organs of plants [28]. Besides water, it also plays an important role in the translocation of nutrients from the roots throughout the plant, so the lack of water will result in a decrease in photosynthesis which resulted in poor growth and development of plants [25,29]. So, morphological characters were significantly correlated with resistance to drought stress was the number of tillers, where the higher the number of tillers, the more sensitive to drought stress. The percentage of productive tiller on line *Unsyiah-1 Simeulu* increased after crops obtain the treatment of drought stress. This is presumably because the recovery ability *Unsyiah-1 Simeulu* high enough after being subjected to drought stress on the generative phase and vegetative phase.

This study discovered that the use of gamma ray irradiation significantly increased the yield potential of mutant lines. This results assumed that the mutant lines were able to distribute and storage the results of assimilates effectively. This finding is consistent with other research that found leaves and other green tissue is a source of origin of the result of assimilation. Part of the proceeds of assimilation retained in tissues for cell maintenance, and other translocated to the utilization of vegetative and reproductive growth [25]. The harvest index describes the ratio between the weight of dry matter yields and crop biology, economy and highly dependent on the magnitude of the translocation photosynthate [30]. The higher index as harvest is the higher the number of seeds produced. On the condition of drought stress liner weight biomass correlated to the root biomass, due to the drought stress conditions, largely the result photosynthate allocated for root growth, so that the roots reach into broader and deeper to absorb water in deeper soil layers [31]. Based on the other research explains that the depth and the dry weight root best on the condition of drought stress was positively correlated with the ability of plants to maintain production of biomass and making a real contribution in reducing yield loss [32].

The results of the study proved that irradiation with 250 Gy gamma rays has been able to improve the genetic character of local rice, which is to increase rice resistance to drought stress. This is demonstrated by mutant lines that are better tolerant of drought stress. Thus, gamma-ray mutations can be applied to increase rice productivity in sub-optimal lands that experience abiotic stress. Nevertheless, this study still has limitations because it is implemented in pots as a growing medium in a greenhouse, so it is necessary to carry out a direct research tested in paddy fields that are experiencing drought stress. The success of improving the the local rice has triggered the researcher and others to help improve the agronomic properties of their local rice through technology of nuclear, because it is believed that radiation mutation breeding can improve the weakness of varieties local rice without changing other preferred properties. This will certainly benefit farmers because of shorter planting times, better quality and quantity of rice production. Induction of mutations using irradiation produces the most mutants (about 75%) when compared to other treatments such as chemical mutagen. The advantage of using gamma rays is that the doses used are more accurate and the penetration of irradiation into cells is homogeneous [33,34].

Conclusion:

Gamma rays irradiation was significantly enhanced the tolerance of Aceh’s local rice to drought stress. The induced mutation was significantly increased the plant height of M_3 mutant rice. The highest plant height showed by *Unsyiah-3 Sanberasi*. The plant height of mutant lines was significantly higher than the parent plant, Sanbei. The interaction of rice lines mutated gamma radiation and drought stress also significantly increased the percentage of productive tillers of mutant lines. The highest percentage of productive tiller highest showed by *Unsyiah 3 Sanberasi*. However, the genotype *Unsyiah-1 Simeulu* with the highest potential yield reached 2.81 tons ha-1 showed the best response in adapting drought stress. It was discovered that the mutant lines that are better tolerant of drought stress. Thus, gamma-ray mutations can be applied to increase rice productivity in sub-optimal lands that experience abiotic stress in the future. It is important to evaluate a direct research tested in paddy fields that are experiencing drought stress.

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