Evaluation of F6 generation of upland rice promising lines for drought stress tolerance

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Short Communication: Evaluation of F_6 generation of upland rice promising lines for drought stress tolerance

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Abstract. *Mustikarini ED, Lestari T, Santi R, Prayoga GI, Cahya Z. 2022. Short Communication: Evaluation of F6 generation of upland rice promising lines for drought stress tolerance. Biodiversitas 23: 3401-3406.* Water is important in metabolic processes affecting rice crop growth and development. Drought stress can decrease rice production, necessitating the development of drought-tolerant varieties. Selection of drought-tolerant can be done during the critical period plant booting phase. This research aimed to determine promising lines of upland rice tolerant to drought stress. This research was conducted from December 2019 to May 2020 at the experimental farm of the Faculty of Agriculture, Fisheries and Biology, Universitas Bangka Belitung. The research was laid out in a completely randomized design with a single-factor treatment. The treatment was rice genotypes consisting of 10 lines and 2 check varieties; each was 3 replicates. The results showed that the drought stress in the plant booting phase of rice plants significantly affected the plant heigh character of the plant, grains number per panicle, the weight of grain per panicle, the age of flowering, and harvest time, but gave no significant effect on the number of leaves, the number of productive tillers, roots length. The upland rice lines 23A-56-30-25-1, 23A-56-30-25-12, and 23A-56-30-25-13 showed good drought stress tolerance based on leaf rolling, leaf drying, crop yield, and plant growth percentage.

Keywords: Drought stress, line, plant booting phase, tolerant, upland rice

INTRODUCTION

Water is important in metabolic processes affecting rice crop growth and development. The response of rice plants exposed to drought stress at morphological stages includes leaf rolling and reduced leaf area (Darmadi et al. 2021), reduction in the number of stomata, thus reducing transpiration rate (Kartika et al. 2020), disruption of growth, panicle initiation, flowering and decreased yields (Gaballah et al. 2021), and a significant decrease in the rate of photosynthesis at all growth stages (Zhu et al. 2020).

The response of rice plants to drought stress was preceded by the physiological response in the form of a reduction of transpiration rate to reduce water loss by closing stomata, reducing stomata number, and decreasing leaf surface area by leaf rolling (Salsinha et al. 2021). However, the most critical component that determines the survival of the rice reproductive organs is related to the supply of assimilation. The reduction in yields in drought-driven crops is due to the limited supply of assimilation produced through photosynthesis (Moonmoon and Islam 2017).

The drought will indirectly lead to a decrease in rice production. Drought resistance in rice plants is genetically controlled. The *Enhanced Response to ABAI (ERAI)* gene encodes the *β-subunit farnesyltransferase* enzyme, increasing guard cells' sensitivity to abscisic acid (ABA). ABA phytohormone plays a role in opening the stomata's closure to reduce water loss during transpiration. Drought stress causes loss of cell turgor pressure and stomatal closure so that the carbon assimilation rate decreases, resulting in a decrease in plant biomass (Salsinha et al. 2021). One gene that controls drought-resistant traits in rice plants is the WRKY gene (Sahebi et al. 2018). The use of superior varieties that are drought resistant is a prime objective in the development of upland rice.

Some selection methods that can be used to obtain upland rice genotypes that are resistant to drought checks are the use of polyethylene glycol (PEG) solution (Sunaryo et al. 2016; Sagar et al. 2020), leaf rolling and leaf drying score (IRRI 2013), evaluation of efficiency degree of drought tolerance (DTD Method) (Zu et al. 2017). The assessment in the critical period became an efficient selection in obtaining a superior drought-tolerant upland rice cultivar (Adhikari et al. 2019). The detection of plant character in response to drought stress can use root organ development (Seo et al. 2020), leaf anatomy (Zagoto and Violita 2019), leaf rolling, and leaf dryness (IRRI 2013). The selection method using stress in the critical period obtained the M5-GR150-1-9-13 line of red rice that was drought tolerant (Mustikarini et al. 2016). Drought stress applied to the booting stage showed the most significant effect on decreasing various parameters of the selection of drought-tolerant rice lines (Mustikarini et al. 2017).

The 6th generation lines (F6) used in the present study were produced from a cross between local rice parental lines from Bangka with Banyuasin and Inpago 8. The line rice needs to be further selected to get a new superior trait better than its elders. This study used a critical period selection method in the booting phase to find the drought-tolerant upland rice lines. The study aimed to obtain a drought-stress tolerant line. The Promising line of red rice that is drought tolerant and high yielding can be further developed into a new superior variety.

MATERIALS AND METHODS

Time and location of research

The research was conducted from December 2019 until May 2020 in the Research and Experimental Station of the Faculty of Agriculture, Fisheries, and Biology at Universitas Bangka Belitung, Indonesia.

Materials

The materials used in this research are 10 F6 rice seeds from the hybrid between the varieties of PMB-UBB1 X Inpago 8, PMB-UBB1 X Banyuasin, Inpago 8 X Balok, Inpago 8 X Banyuasin, Inpago 8 X PMB-UBB1, Balok X Banyuasin, Balok X Inpago 8, Banyuasin X Balok, Banyuasin X PMB-UBB1, Banyuasin X Inpago 8, and Inpago 8 and Inpago 12 Agritan as check varieties, polibag, chicken manure, anorganic fertilizer (Urea, SP-36, KCl).

Research design

The design used Completely Randomized Design (CRD). The treatment used in this study was a rice plant genotype consisting of 10 F6 lines and 2 check varieties. The treatment is repeated three times. The total experimental units were 36, with a sample of 10/experimental units and 360 plants. The entire sample of plants is the total population.

Procedures

Pot experiment and drought-stress treatment

Planting media was prepared by mixing 10 kg of topsoil and 75 grams of chicken manure per polybag. The manure was applied one week before planting. Planting was done by making a planting hole as deep as 3 cm**;** the spacing between polybags was 25 cm x 25 cm. The fertilizer doses were Urea 200 kg ha^{-1}, SP-36 100 kg ha^{-1}, and KCl 100 kg ha^{-1}. The next fertilizations were done using inorganic fertilizer, namely Urea, as much as 1/3 dose (at 20 DAP, 55 DAP, 65 DAP), SP-36 fertilization, and KCl were given as much as the full dose at 20 DAP (day after planting). The screen house was made 3 days before drought stress, with a size of 11 m x 6.5 m. The screen house was made of wood; the walls were made from paranet, and the roof was from plastic. Drought stress treatment was a 30% reduction in moisture content (70% field capacity). Drought treatment was given in rice plants' boot phase with no watering for 14 days. During the drought stress, irrigation was stopped to create drought stress conditions. The drought resistance assessment was based on the standard evaluation system (IRRI 2013).

Observations

Plant height was measured from the plant base to the tip of the highest panicle. Productive tiller numbers were determined 30 days after flowering for each plant. The number of leaves was obtained by counting all the leaves that grew. The calculated leaves had been perfectly formed at the time of harvest. The root length was obtained by measuring from the base of the root to the longest root. Measurement of root length was done at the time after harvest. The Numbers of filled grains was the average number of grains contained in each panicle in a single plant. The weight of filled grains per plant was obtained by weighing the entire seed within a plant. Flowering time was determined at 80% of the plants heading. The flowering time was determined on the first day of the flowering plant. The Root volume was calculated by cutting the root part of the rice plant that has been measured and cleaned. The roots of the rice plant were hardened first, then put into a measuring glass of 500 mL containing 150 mL of water, so the volume increased. The root volume calculation formula is as follows:

Root Volume (mL) = Final volume - Initial volume

The percentage of living plants was determined by calculating the number of living plants divided by the total number of plants planted multiplied by 100% in each genotype using the following formula. Observations were made at harvest.

$$\text{Percentage of living plants} = \frac{\text{number of living plants}}{\text{total of planted}} \times 100\%$$

Observation of leaf rolling and drying was done 2 weeks after the drought stress. Observations of leaf rolling and drying were carried out by observing the leaf symptoms of rice plants, then were given a score according to the symptoms that appeared. The leaf rolling and drying were obtained by observing the shape of the leaves with the scale listed in Table 1.

Data analysis

The data were first subjected to a normality test, then followed by an ANOVA at a 95% confidence level, the post hoc Duncan's Multiple Range Test (DMRT) at a 95% confidence level. Finally, correlation analysis was conducted to see their relationship using Pearson correlation (Pearson Product Moment).

Table 1. The scale level of leaf rolling and leaf drying of rice plants against drought stress was based on the Standard Evaluation System (IRRI 2013)

Scale	Category	Leaf rolling	Leaf drying
0	Highly tolerant	Leaves healthy	No symptoms
1	Tolerant	Leaves start to fold (shallow)	Slight tip drying
3	Moderately tolerant	Leaves folding (deep V-shape)	Tip drying extended up to ¼
5	Moderate	Leaves fully cupped (U-shape)	One-fourth to ½ of all leaves dried
7	Moderately susceptible	Leaf margins touching (O-shape)	More than 2/3 of all leaves were fully dried
9	Susceptible	Leaves tightly rolled (V-shape)	All plants were dead. Length in most leaves fully dried

RESULTS AND DISCUSSION

Leaf rolling, leaf drying, and the percentage of survival plants (%)

The drought tolerance evaluation is one of the efforts to obtain drought-tolerant rice genotypes. The tolerance evaluation aims to obtain a drought-tolerant F6 rice line. Lines that are tolerant to drought stress can be identified based on the character of leaf rolling and leaf drying. The tested upland rice genotypes showed different symptoms in leaf rolling and leaf drying due to drought stress. The upland rice genotypes tested had two criteria, 1 (tolerant) and 5 (moderate), on the character of leaf rolling. Line 23a-56-30-25-13 showed a leaf rolling score of 1 (tolerant). Which is more tolerant than check varieties. Nine rice lines showed a leaf rolling score of 9. The tested upland rice genotype showed that leaf drying was categorized into two levels. Six lines showed a leaf drying score of 1 (tolerant). Four lines showed a leaf drying score of 5, which is more drought susceptible than the check variety. Drought-tolerant lines showed the appearance of leaves that are still fresh with the drying of small leaves (the tip of the leaves dries). The results showed that the percentage of living plants in each genotype of upland rice plants tested was different. Four lines showed a 100% percent of living plants comparable to the Inpago 8 variety. The leaf rolling and leaf drying criteria of the F6 rice line are presented in (Table 2).

DMRT test results showed that lines 23a-56-30-25-1 performed differently in the high character of the plant, the amount of grain content per panicle, and the weight of the content grains per panicle compared to the other 9 lines and 2 check varieties. The flowering time of Inpago 8 differed significantly compared to all lines tested. The leaves number, productive tillers number, root length, and root volume of all lines did not differ significantly with check variety (Inpago 8 and Inpago 12 Agritan) (Table 3).

Correlation of upland rice character

Rice tolerance to drought is related to grain density characteristics, the number of filled grains, the length of the roots, and root volume. The number of productive tillers, the number of leaves, and the number of filled grains had a significant positive correlation with the weight of filled grains. The filled grain number was significantly positively correlated with plant height, the number of productive tillers, the number of leaves, the volume of roots, and the weight of filled grains. Root length had a positive (+) correlation with productive tillers number and root volume. Root volume character was significantly and positively correlated with the number of productive tillers, the number of leaves, and the length of the roots (Table 4).

Discussion

Drought stress treatment given over 14 days led to a decrease in some characters. The results showed that tested genotypes' leaf rolling and leaf drying differed significantly. Rice affected by drought stress indicates leaf rolling (Singh et al. 2017). Drought tolerance is a complex phenomenon involving many adaptation mechanisms, one of which is leaf rolling induced by the effects of water availability and photosynthetic activity under stressful conditions (Ben-Amar et al. 2020). The process that plants experience when gripped by drought after leaf rolling is leaf drying. Rice affected by drought stress indicates leaf aging (Swapna and Shylaraj 2017). The process occurs because an increase in the reactive oxide type causes leaf aging and drying (Krieger-Liszkay et al. 2019).

Table 2. The scale of leaf rolling and leaf drying at two weeks of age after exposure to drought stress and the percentage of live plants (%)

Lines	Character scale		Percentage of living plants (%)
	Leaf rolling	Leaf drying	
19i-06-09-23-27	9	1	100
19i-06-09-23-3	9	1	93.33
19i-06-30-17-17	9	5	86.66
19i-06-30-17-27	9	1	93.33
21b-57-21-21-1	9	5	70
21b-57-21-21-25	9	1	73.33
23a-56-24-22-13	9	5	96.66
23a-56-30-25-1	9	5	100
23a-56-30-25-12	9	1	100
23a-56-30-25-13	1	1	100
Inpago 8	9	1	100
Inpago 12	9	1	96.66

Note: Scale of leaf rolling and leaf drying: highly tolerant (0), tolerant (1), rather tolerant (3), moderately tolerant (5), moderately susceptible (7), susceptible (9) (IRRI 2013)

Table 3. The average genotypes character of rice plants after drought stress treatment in the generative phase

Lines	Character							
	Height plant (cm)	Filled grains number per panicle (grain)	Filled grains weight per panicle (grain)	Flowering date (DAP)	Leaves number (strands)	Productive tillers numbers (stem)	Root lenght (cm)	Root volume (mL)
19i-06-09-23-27	67.85cde	16.50d	0.94c	76.14bc	52.70a	16.80a	39.40a	34.33a
19i-06-09-23-3	67.28cde	66.22cd	1.91bc	76.94bc	52.88a	17.47a	47.41a	56.33a
19i-06-30-17-17	66.03de	0.00d	0.00c	83.76ab	52.76a	13.40a	36.05a	26.43a
19i-06-30-17-27	69.98cd	98.47bcd	2.50bc	72.68c	64.25a	20.84a	35.37a	22.67a
21b-57-21-21-1	65.19de	0.00d	0.00c	82.43ab	33.61a	11.33a	34.06a	21.80a
21b-57-21-21-25	62.79de	0.00d	0.00c	72.54c	45.67a	18.65a	43.70a	47.80a
23a-56-24-22-13	55.82e	61.78cd	1.70bc	79.96bc	58.24a	14.79a	43.14a	35.26a
23a-56-30-25-1	**92.26a**	**333.50a**	**7.52a**	71.60c	70.10a	18.23a	40.50a	41.33a
23a-56-30-25-12	78.95bc	247.22abc	5.76ab	72.06c	78.57a	20.27a	43.63a	62.83a
23a-56-30-25-13	85.43ab	276.80ab	5.73ab	**71.47c**	57.07a	16.77a	44.90a	48.33a
Inpago 8	89.23ab	0.00d	0.00c	90.70a	61.13a	9.56a	40.06a	44.00a
Inpago 12	83.68ab	172.09abcd	3.30abc	77.84bc	49.14a	13.47a	42.67a	50.67a

Note: The numbers followed by the same letter in the same column are not significantly different based on the Duncan Multiple Range Test (DMRT) level of 95%. DAP (day after planting)

Table 4. Correlation of upland rice character at harvest time

Character	Character							
	Height plant	Productive tillers numbers	Leaves number	Root length	Root volume	Filled grains number per panicle	Filled grains weight per panicle	Flowering age
Height plant	-							
Productive tillers numbers	-0.051							
Leaves number	0.342*	0.710**						
Root lenght	0.066	0.356*	0.302					
Root volume	0.176	0.372*	0.374*	0.871**				
Filled grains number per penicle	0.543**	0.474**	0.484**	0.312	0.427**			
Filled grains weight per penicle	0.231	0.420*	0.350*	0.202	0.297	0.595**		
Flowering age	-0.082	-0.760**	-0.489**	-0.266	-0.339*	-0.545**	-0.413*	-

Note: *significant at 5% (P<0.05), **significant at 1% level (P<0.01). Correlation values of 0.00-0.20 (no correlation), 0.21-0.40 (low correlation), 0.41-0.60 (moderate correlation), 0.61-0.80 (high correlation), 0.81-1.00 (very high correlation)

The results showed that six lines have the leaf drying criteria of 1 (tolerant) better than others (Table 2). The line can still grow in a drought condition even through the disrupted metabolic process. Line 23a-56-30-25-13 showed a 1 leaf rolling criteria, which is more tolerant than other tested lines. Line 23a-56-30-25-13 has a higher tolerance level than all lines tested. The leaf rolling and leaf drying levels under drought were influenced by the morphology of the leaves of each rice genotype (Cal et al. 2019). Different genetic responses in each line cause differences in the level of damage caused by leaf rolling, thought to be related to the water content in the foliage (Opalofia et al. 2018). Drought stress causes changes in chlorophyll pigment, leaf rolling causes a decrease in the rate of photosynthesis (Salsinha et al. 2021), the ability of the transpiration rate to keep the potential of leaf water remains high in times of water shortage (Afrianingsih et al. 2018). Resistant genotypes can avoid water stress and increase the ability of roots to absorb water from the soil (Gaballah et al. 2021). Tolerance to drought in rice plants is closely related to the resistance genes present in these plants.

The results showed a highly significant effect on the character of filled grain number and filled grain weight. The number of grains formed due to the checks given varies at each line tested. The results showed that 23a-56-30-25-1 resulted in the highest filled grain number and weight compared to other rice genotypes. Lines 23a-56-30-25-12 and 23a-56-30-25-13 showed a high filled grain number and weight. The three lines were tolerant to drought stress for their high filled grain yield in drought stress conditions (Table 2). The relative water content of tolerant genotypes was higher than that of susceptible genotypes, so the tolerant genotypes could still produce filled grains (Barik et al. 2019). Drought stress at the grain filling stage can reduce crop yields (Angie et al. 2019).

Drought-stress treatment caused no low number of filled grains, and even some lines produced no filled grain (Table 2). The drought tolerance test was carried out in the booting phase of the plant so that the plant suffered a water deficit at the filling phase, thus causing empty grains**.** The seed filling phase requires lots of water. Drought stress increased rice grains to increase sterility, especially in the rice panicle filling phase, causing low seed production

(Moonmoon and Islam 2017). The genotype having high empty grain is caused by the lack of water supply, resulting in a delay in flowering time which will shorten the grain filling period (Afrianingsih et al. 2018). Drought stress causes a decrease in the character of filled grain per panicle (Hosain et al. 2020). Drought stress can affect the number and weight of filled grains. The results showed that drought stress significantly affected flowering time. Line 23a-56-30-25-13 showed a faster flowering time than other upland rice genotypes (Table 2). The flowering time is faster, presumably due to the efficient use of water. The response to drought checks includes the ability of plants to continue growing in water stress conditions by lowering leaf area and shortening the growing cycle.

The plant height, root length, root volume, leaf number and productive tillers number were not significantly different among genotype treatments in the generative phase. Drought stress in the generative phase did not affect differences in plant height because plant height growth occurred in the vegetative phase (Darmadi et al. 2021). Root organs are no different because each rice plant will maintain water content by increasing water absorption in the soil. Roots are the first organ to be affected by water stress because they play a role in water absorption in the soil (Koevoets et al. 2016). The response to drought stress is seen in plant roots which play a role in the absorption of water and nutrients from the soil (Kim et al. 2020). Rice plants that are tolerant to drought stress have volume and root length. Drought stress at the flower formation stage reduced the number of panicle grains (Sihombing et al. 2017). The characteristics of the generative phase of rice plants are the elongation of the top segment on the stem, the reduction in the number of tillers that will form, the emergence of flag leaves, and flowering (Moldenbauer et al. 2018).

The rice plant's tolerance to drought is also closely related to the filled grain weight, filled grain number, root length, and root volume characters. Correlation results showed that filled grain weight positively correlated with the number of productive tillers, the number of leaves, and the number of filled grain (Table 4). The higher the number of productive tillers, the number of leaves, and the number of filled grain, the higher the weight of the grains produced. The high number of productive tillers will also produce high grain yields (Sugiarto et al. 2018). Rice plants that can produce grain in drought conditions have good tolerance to drought even though the yield is not optimal. However, rice plants are sensitive to water shortages which can cause panicle reduction and high sterility, resulting in a significant decrease in grain yield (Angie et al. 2019).

Root length is positively correlated with root volume because the longer the root length, the larger the root volume. The rice plant can find the water well with long roots and a high root volume. Rice plants that survive water shortage conditions have a large and long rooting system that can penetrate deeper soil layers to maintain water status in plant tissues (Sihombing et al. 2017). The result showed that root volume was positively correlated with filled grain number. Long roots can absorb more water, so the need for water when filling the grains is sufficient. The rice yield components are directly proportional to the root system (Dang 2020). In conclusion, 23A-56-30-25-1, 23A-56-30-25-12, and 23A-56-30-25-13 lines of upland rice have good tolerance to drought based on the character of leaf rolling, leaf drying, crop yield, and plant growth percentage (%).

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