

Selection Strategy of Drought Tolerance on Red Rice Mutant Lines

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ABSTRACT

Most of Indonesian red rice is not grown in dry land. New varieties could be bred through mutation breeding. This research objective was to evaluate the selection strategy of drought tolerant on red rice. The study was conducted on Ultisol soil in Bangka Belitung Province, Indonesia, in February 2012-February 2015. It consisted of three activities, selection M5, evaluation of selected M5 and evaluation of drought critical period on selected M6. Mutant of M5 was selected and evaluated with polyethylene glycol (PEG) -0.5 MPa and less of soil moisture. Selected M6 was evaluated to observe a critical period of drought stress. Evaluation with PEG produces five superior mutants that more vigor than the elders. Selection on low humidity shows that M₅-G_{R150}-1-9 produces higher filled grain and weight filled grain than other mutants and elders. The selected red rice line M₅-G_{R150}-1-9-13, has a better tolerance towards drought than its control. It could be obtained such mutant lines which have a high yield, early maturing and drought tolerant from the result of six generations gamma irradiation mutant selection.

Keywords: drought; gamma; mutant; red rice; selection

INTRODUCTION

The main obstacles in rice cultivation are drought stress and limited irrigated land. The limited amount of irrigated land is becoming an issue in the development of red rice varieties. Red rice varieties are currently classified as types of paddy as Aek Sibudong, Bahbutong (Aryana, 2009) and Inpari 24 (Utama, 2015). Red rice varieties of upland species released is only Inpago 7 (IAARD, 2012). Drought tolerance trait has to be owned by the red rice mutant to be cultivated on upland.

Drought tolerance of rice is controlled by many genes (polygenic) (Sudharmawan, 2010).

There were about 245 genes with direct impact and 413 genes with no direct impact have been identified as drought tolerance genes (Degenkolbe et al., 2009). The tendency of drought tolerant that is supported by drought resistance genes, had different and supportive roles (Sudharmawan, 2010). The selection strategy should be able to single out mutants which have resistant genes and are still expressed in drought stress conditions.

Pedigree selection is widely used in self-pollinated plants selection. The accuracy level of this selection is higher. The frequency of plants which have a recombinant was increasing (Bos & Caligari, 2008). The genes of superior lines were concentrated in a certain assembly point within the genes, and the pedigree was vividly learnt (Syukur, Sujiprihati, & Yunianti, 2010). Pedigree selection was used in 85 % of rice varieties in America (Guimarães, 2009). The modification of the selection method is performed to determine the differences in plants phenotype in order to accelerate the selection process.

Polyethylen Glykol (PEG) is used for the rice selection that have drought tolerance nature. Drought-tolerant red rice was selected by PEG-0.5MPa (Ballo, Nio, Mantiri, & Pandiangan, 2012). Drought tolerance in rice can be selected by observing less of soil moisture. The selection was carried out to, among other, *aman* rice genotypes at 40 % and 70 % degree of moisture (Zubaer, Chowdhury, Islam, Ahmed, & Hasan, 2007) and upland rice cultivars in moisture level of 10-90 % (Supriyanto, 2013). Asch, Dingkuhn, Sow, & Audebert (2005) proved that a decrease of 14% moisture reduces the biomass of upland rice. The varieties of upland rice that are more tolerant to drought stress were selected by using PEG and moisture content (Effendi, 2008).

Selection of drought-tolerant rice has been done by providing stress at critical periods of the plant. The critical period of rice paddy was experienced at the age of 16 days before blooming

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and 10 days after blooming (Kumar, 2011). The panicle length of the rice had decreased as a result of drought throughout the generative phase (Chutia & Borah, 2012), weight of 1,000 grains (Zain, Ismail, Mahmood, Puteh, & Ibrahim, 2014) and the production up to 94 % (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009). Drought stress at booting phase decreased the amount of fertile panicle, number of filled seeds (Pandey, Kumar, Pandey, & Thongbam, 2014), leaf area and dry weight of rice plants (Zubaer, Chowdhury, Islam, Ahmed, & Hasan, 2007). Drought stress on the grain filling phase lowered the crop production up to 60 % (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009; Sokoto & Muhammad, 2014). Rice plants are unable to withstand drought stress on the generative phase.

Various drought-stress screening methods can be used to ensure the tolerance of plants to drought stress. Mechanisms of genotype influenced big changes to the environment of genetic heterogeneity to stress tolerance and recovery after stress (Allard & Bradshaw, 1964). Stable red rice genotypes produce relatively similar results when planted in dry environment or rice fields (Aryana, 2009). Therefore, the existence of mutants which have stable drought tolerance trait and not influenced by environmental circumstance is important to be obtained. The research objective was to find the best method to select drought-tolerant red rice mutant.

MATERIALS AND METHODS

The study was conducted on Ultisol soil on Bangka Belitung Province, Indonesia, in February 2012-February 2015. It was conducted for six planting seasons from mutant 1 (M1) - mutant 6 (M6). The main materials of this study are the upland of Bangka red rice accession (*Balok Mas*, *Celak Madu*, *Mayang Anget*, *Mayang Duku*, *Mayang Nibung*, *Ruten Puren* and *Radix*). The rice accession has been treated with 150, 200 and 250 gray gamma radiation.

The selection of pedigree has been done on M1-M4. The selection aimed to obtain early maturity of red rice mutant with a great deal of yield. Mutants (M1) derived from the accession and three dose of gamma irradiation planted in a single plot. Selection of individuals was started from M1 plants for early maturity characters performed on each population. M3 was obtained from M2 seed selection that was planted in the

plot. M4 obtained from individual selection is done on M3 (Picture 2). The M4 selected lines used as material selection to obtain drought-tolerant mutants.

The first research was conducted selection on M5 with germination test in a solution of polyethylene glycol (PEG) 8000 -0.5 MPa. The research material was 36 mutant lines selected on M4 and three accessions. The research used completely randomized design (CRD) with three replications. PEG was used for seed damping in laboratory for 14 days. Traits observed were plumula length, root length, and germination.

The second research was to test the soil moisture by decreasing its level. The research materials were 5 lines of M5 which were selected from the first research. The experiment was conducted in a CRD split plot design. The subplots were M₅-G_{R150}-1-4, M₅-G_{R150}-1-9, M₅-G_{R150}-2-2, M₅-G_{R150}-2-3, M₅-G_{R200}-1-2 along with the *Radix* accession. The main plot was the soil moisture of 50 %, 75 % and 100 % (control). The mutant rice is planted in polybags and each plot consists of six plants. The less soil moisture treatment began 1 week after planting up to harvesting. Traits observed included plant height (cm), root weight (g), root length (cm), number of filled grain per panicle (grains), percentage of filled grain, and weight of filled grain (g).

The third research was to test the tolerance to drought in critical periods. The research materials were five selected lines of M6 from the second research. The experiment was conducted in a CRD split plot design. The sub plots were M₆-G_{R150}-1-4-14, M₆-G_{R150}-1-9-13, M₆-G_{R200}-1-2-18, M₆-G_{R150}-2-2-2, M₆-G_{R150}-2-3-13 along with the *Radix* accession. The main plot was drought stress (non-irrigation) in the phase of tillering, booting-flowering, seed filling and control. Stress treatment was given by covering plant using the transparent plastic for 14 days. Traits observed were plant height (cm), number of tillers (stem), number of filled grain (grain), percentage of filled grain, and weight of filled grain (gram). The data was analyzed by Fisher's exact test and the Least Significant Difference (LSD) with 5 % significance level.

RESULTS AND DISCUSSION

Pedigree selection which was committed against M1-M4 red rice mutant managed to produce mutants which have the early maturity of less than 115 days after planting (dap). Selected mutants

which were able to maintain early maturity came from *Ruten Puren* accession to the treatment gamma irradiation rays with dose of 200 gray, *Radix* accession with dose of 150 and 200 gray. The other five accession varieties, namely, *Balok Mas*, *Celak Madu*, *Mayang Anget*, *Mayang Duku*, and *Mayang Nibung* were unable to maintain early maturity.

M5 drought tolerance test showed that in the germination phase, there were differences in the traits of higher germination, root length and

plumula length. Low germination of mutants' seeds was believed to be caused by the inability of the seeds to absorb water. Seed absorbed up to 30 % water content before germination for activation (Lestari & Mariska, 2006). The use of PEG decreased the amount of germination seed and growth of rice seed (Kaydan & Yagmur, 2008). In this study, the seeds were unable to absorb water for germination due to the increase in concentration caused by the addition of PEG.

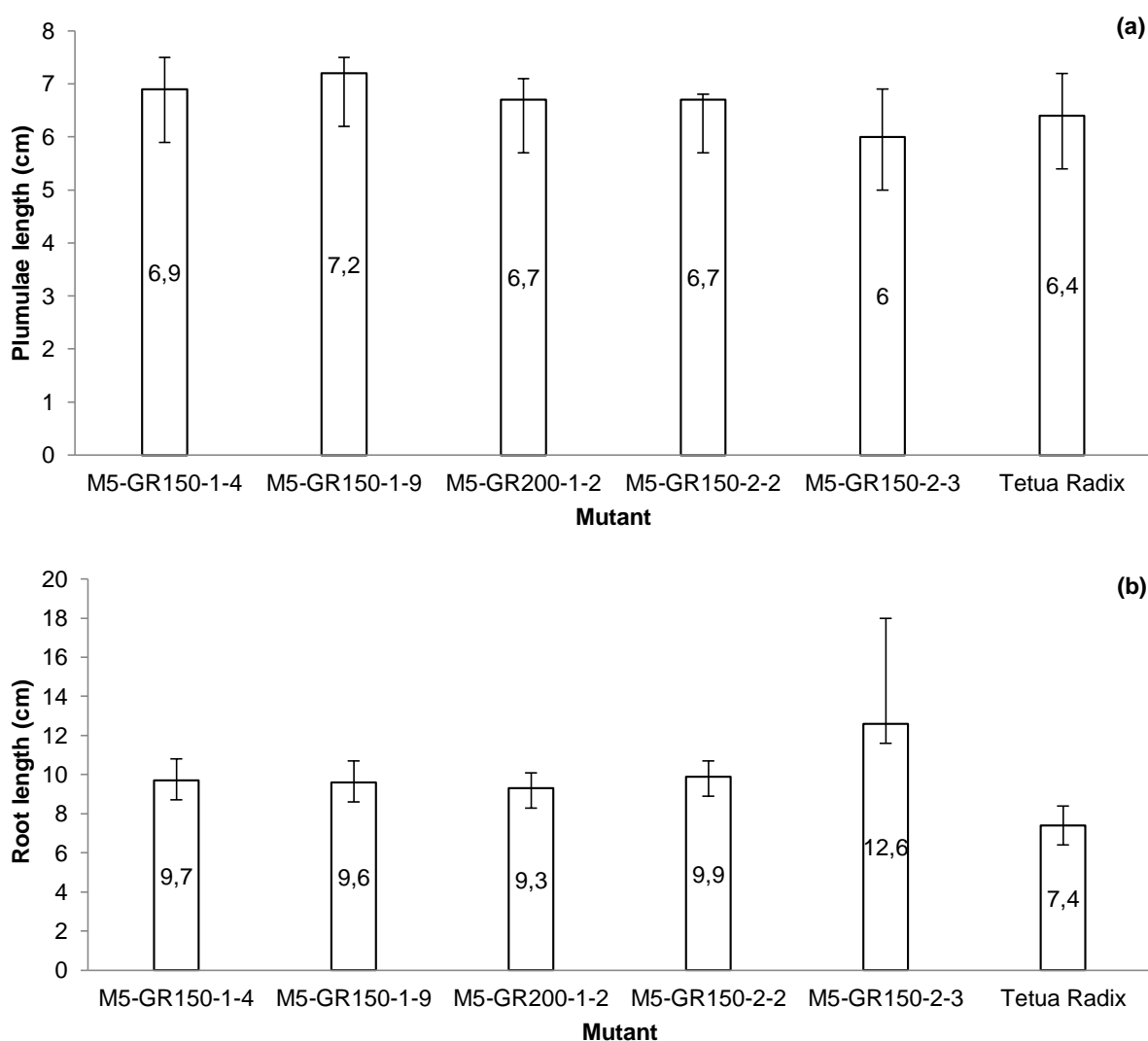


Figure 1. Mean plumula length (a) and root length (b) M5 to a solution of PEG 8000 -0.5 MPa

PEG applications on the planting medium decreased the root length, root distribution and volume of roots of rice seedlings (Adachi *et al.*, 2014). Drought-tolerant rice had higher sprouts of fresh weight and dry weight (Ahadiyat, Hidayat, & Susanto, 2012). The selected mutants by using PEG was more efficient because it shortened the selection time. M₅-G_{R150}-1-4, M₅-G_{R150}-1-9, M₅-G_{R150}-2-2, M₅-G_{R200}-1-2, M₅-G_{R150}-2-3 mutants were selected as the most tolerant to drought stress by their germination traits (Table 1). The fifth mutant has better character than the Radix accession. The mutants which have more drought tolerance are the mutants with 150 and 200 gray gamma radiation exposure.

Table 1. Mean of germination rate (%) in a solution of PEG 8000 M₅ -0.5 MPa

Mutant	Germination (%)
M ₅ -G _{R150} -1-4	100,0 S+
M ₅ -G _{R150} -1-9	100,0 S+
M ₅ -G _{R200} -1-2	100,0 S+
M ₅ -G _{R150} -2-2	100,0 S+
M ₅ -G _{R150} -2-3	98,7 S+
Tetua Radix	41,3

Remarks: S+ = significantly higher than the controls based on the test of least significant difference (LSD) α 5 %

Positive correlation is shown from the results of M₅ drought tolerance test with PEG and a decreased soil moisture. The length of M₅ plumula and root has a higher value than the Radix accession on the PEG test (Figure 1). The average M₅ height and root length after the test with a decreased soil moisture is higher than elders (Table 2).

M₅ has shown some indicators of drought stress under the treatment with 75 % soil moisture which decrease its production. Upland rice experienced a decrease in weight up to 100 seed sand dry grain per tillers on soil moisture up to 90% (Supriyanto, 2013). M₅-G_{R150}-1-4, M₅-G_{R150}-1-9 and M₅-G_{R150}-2-3 mutants have higher filled seed grain and seed weight than Radix accession in the less soil moisture of 75 % (Table 2). According to Bos & Caligari (2008), the arrangement of genes and the complexity of environment affected the interaction between genotype and the environment. The character of M₅ was affected with the drought stress. The traits used for the selection of dried-tolerant rice were root volume, thickness of root,

root weight (Nassir & Adewusi, 2012), and the weight of dry grain (Effendi, 2008). Traits such as number of filled grain and weight of filled seed are important to dried-tolerant mutant selection because of the high correlation with the results.

Lack of water causes the decrease in the number of filled grain and grain weight. Total of crop yield will be low. The decline of filled grain and grain weight that was caused by a lack of water would result in a decline in the rice yields (Pandey, Kumar, Pandey, & Thongbam, 2014). Tolerant rice varieties would perform a tolerance mechanism to accumulate proline and sugar that play a role in the process of biosynthesis of the substrate, hydrolysis, and energy producing (Maisura, Chozin, Lubis, Junaedi, & Ehara, 2014). Sugar and total chlorophyll content in grain played a role in maintaining rice yields in dry stress conditions (Zain, Ismail, Mahmood, Puteh, & Ibrahim, 2014). M₅-G_{R150}-1-9 filled grain has the highest weight in moisture 100 % and 75 % compared to the other mutants, so it is considered to have the potential for high yield and 75 % tolerance to soil moisture.

The results show equivalency between traits of M₅ and M₆, where M₆ also has better plant height, number of filled grain, percentage of filled grain and weight of filled grain than Radix Accession (Table 3). M₆-G_{R150}-1-9-13 has several traits such as higher number of filled grain, percentage of filled grain, and weight of filled grain in the stress treatment of tiller forming compared to other mutants and controls. The number of grain decreased and the rice yields increased due to the lack of water in the vegetative phase of red rice paddy. Rice crop critical period occurred during the formation of flowering and grain filling (Kumar, 2011). The number of rice fertile panicles and number of filled grain decreased due to the lack of water in the blossoming phase. The senescence was accelerated and the rice grain quality was decreasing as a result of drought stress on the grain filling phase (Pandey, Kumar, Pandey, & Thongbam, 2014). The characters declining of almost half of the red rice mutant is due to drought in the fertilizing-flowering phase and grain filling phase.

Table 2. Mean of the fifth mutant traits (M5) of red rice and Radix accession on impairment testing of soil moisture

Trait	Soil Moisture	M ₅ -G _{R150} -1-4	M ₅ -G _{R150} -1-9	M ₅ -G _{R200} -1-2	M ₅ -G _{R150} -2-2	M ₅ -G _{R150} -2-3	Radix Accession
Plant Height (cm)	50 %	81.57 ns	84.42 ns	99.32 ns	85.97 ns	91.93 ns	85.24
	75 %	155.80 ns	165.67 s+	166.47s+	155.39 ns	159.09 s+	147.02
	100 %	165.83 ns	174.75 ns	170.25 s+	173.73 ns	169.02 ns	161.53
	Mean	134.40 ns	141.61 s+	142.23 s+	138.37 ns	142.97 s+	131.26
Root Weight (gram)	50 %	1.53 ns	1.32 ns	1.11 ns	1.22 ns	1.12 ns	3.20
	75 %	7.19 s-	6.60 s-	10.97 s-	7.44 s-	7.61 s-	12.78
	100 %	16.18 s+	12.59 s+	8.19 ns	13.47 s+	17.06 s+	7.22
	Mean	8.30 ns	7.28 ns	6.58 ns	7.38 ns	9.44 s+	7.75
Root Leight (gram)	50 %	10.41 ns	10.35 ns	9.94 ns	11.68 ns	11.29 ns	13.46
	75 %	28.46 ns	27.30 ns	30.53 ns	27.86 ns	33.25 ns	30.39
	100 %	26.33 ns	28.74 ns	28.49 ns	30.06 ns	29.48 ns	26.43
	Mean	21.73 ns	22.12 ns	22.30 ns	23.20 ns	25.15 ns	23.43
Number of Filled grain per panicle	50 %	0 ns	30.00 ns	33.00 ns	0 ns	36.67 ns	7.67
	75 %	721.75 ns	717.72 ns	666.94 ns	785.81 ns	569.66 ns	447.26
	100 %	1241.09 ns	1267.53 ns	1120.17 ns	1068.72 ns	1093.58 ns	776.95
	Mean	654.28 ns	671.75 ns	606.70 ns	618.17 ns	566.64 ns	410.62
Percentage of Filled Grain (%)	50 %	0 ns	14.10 ns	20.26 ns	0 ns	9.63 ns	12.78
	75 %	69.62 s+	59.25 ns	59.37 ns	72.48 s+	71.80 s+	41.85
	100 %	70.72 ns	74.67 ns	61.31 ns	65.97 ns	68.55 ns	53.40
	Mean	46.78 ns	49.34 ns	46.97 ns	51.78 ns	49.99 ns	36.01
Weight of Filled Grain (gram)	50 %	2.21 ns	2.31 ns	2.16 ns	1.38 ns	1.08 ns	0.69
	75 %	20.19 s+	22.28 s+	17.99 ns	22.37 s+	18.23 s+	11.89
	100 %	32.75 ns	33.07 ns	30.79 ns	29.68 ns	30.94 ns	20.02
	Mean	18.38 s+	19.22 s+	16.98 s+	17.81 s+	16.75 s+	10.87

Remarks: The numbers were compared to controls on the same line. S+= higher significance than controls, s-= lower significance than controls, and ns = non significance based on the test of least significant difference (LSD) α 5 %

Table 3. Mean traits of the sixth Mutant (M6) of red rice and Radix accession on non irrigation in critical phases of plant

Traits	Stress Phase	M ₆ -G _{R150} -1-4-14	M ₆ -G _{R150} -1-9-13	M ₆ -G _{R200} -1-2-18	M ₆ -G _{R150} -2-2-2	M ₆ -G _{R150} -2-3-13	Radix Accession
Plant Height (cm)	Tillering Forming	131.40 s+	137.97 s+	132.83 s+	130.57 s+	142.67 s+	121.23
	Booting-Flowering	139.93 s+	142.02 s+	144.77 s+	140.30 s+	142.00 s+	118.40
	Seeds Filling	135.20 s+	138.53 s+	139.10 s+	138.33 s+	136.63 s+	111.67
	Control	145.10 s+	144.53 s+	143.80 s+	146.77 s+	144.97 s+	122.20
	Mean	137.91 s+	140.76 s+	140.13 s+	138.99 s+	141.57 s+	118.37
Number of Tillers (stem)	Tillering Forming	11.07 ns	11.70 ns	10.43 ns	12.40 ns	11.23 ns	10.37
	Booting-Flowering	11.80 ns	10.83 ns	13.23 ns	11.60 ns	13.07 ns	10.96
	Seeds Filling	9.80 ns	10.50 s+	11.87 s+	12.57 s+	12.73 ns	8.87
	Control	15.40 ns	12.50 ns	13.50 ns	16.03 ns	14.93 ns	10.40
	Mean	12.02 s+	11.38 ns	12.26 s+	13.15 s+	12.99 s+	10.15
Number of Filled Grain	Tillering Forming	862.00 ns	1256.40 s+	889.53 ns	952.93 ns	975.60 ns	920.00
	Booting-Flowering	742.27 ns	800.10 ns	662.40 ns	750.33 ns	699.60 ns	601.90
	Seeds Filling	720.80 ns	821.60 ns	978.27 ns	881.17 ns	973.53 ns	576.13
	Control	1048.67 ns	1065.33 ns	885.33 ns	957.37 ns	1196.40 ns	693.13
	Mean	843.43 ns	985.86 s+	853.88 ns	885.45 ns	961.28 s+	697.79
Percentage of Filled Grains (%)	Tillering Forming	78.56 s+	81.39 s+	73.58 s+	75.31 s+	76.30 s+	64.15
	Booting-Flowering	63.99 ns	67.73 ns	49.66 ns	57.00 ns	50.62 ns	60.64
	Seeds Filling	66.61 ns	64.88 ns	72.57 ns	61.76 ns	72.18 ns	55.86
	Control	60.80 sn	69.98 s+	69.65 s+	64.82 sn	74.09 s+	53.71
	Mean	67.49 s+	70.99 s+	66.37 ns	64.72 ns	68.30 s+	58.59
Weight of Filled Grains (gram)	Tillering Forming	17.63 ns	26.13 s+	17.51 ns	19.46 ns	18.59 ns	16.72
	Booting-Flowering	11.43 ns	14.83 ns	11.03 ns	10.34 ns	9.95 ns	10.16
	Seeds Filling	12.48 ns	12.22 ns	15.97 ns	12.87 ns	17.17 ns	9.74
	Control	19.78 ns	21.23 ns	17.37 ns	18.19 ns	24.46 ns	16.23
	Mean	15.33 ns	18.60 s+	15.47 ns	15.21 ns	17.54 ns	13.22

Remarks: The numbers were compared to controls on the same line. S+= higher significance than controls, s-= lower significance than controls, and ns = non significance based on the test of least significant difference (LSD) α 5 %

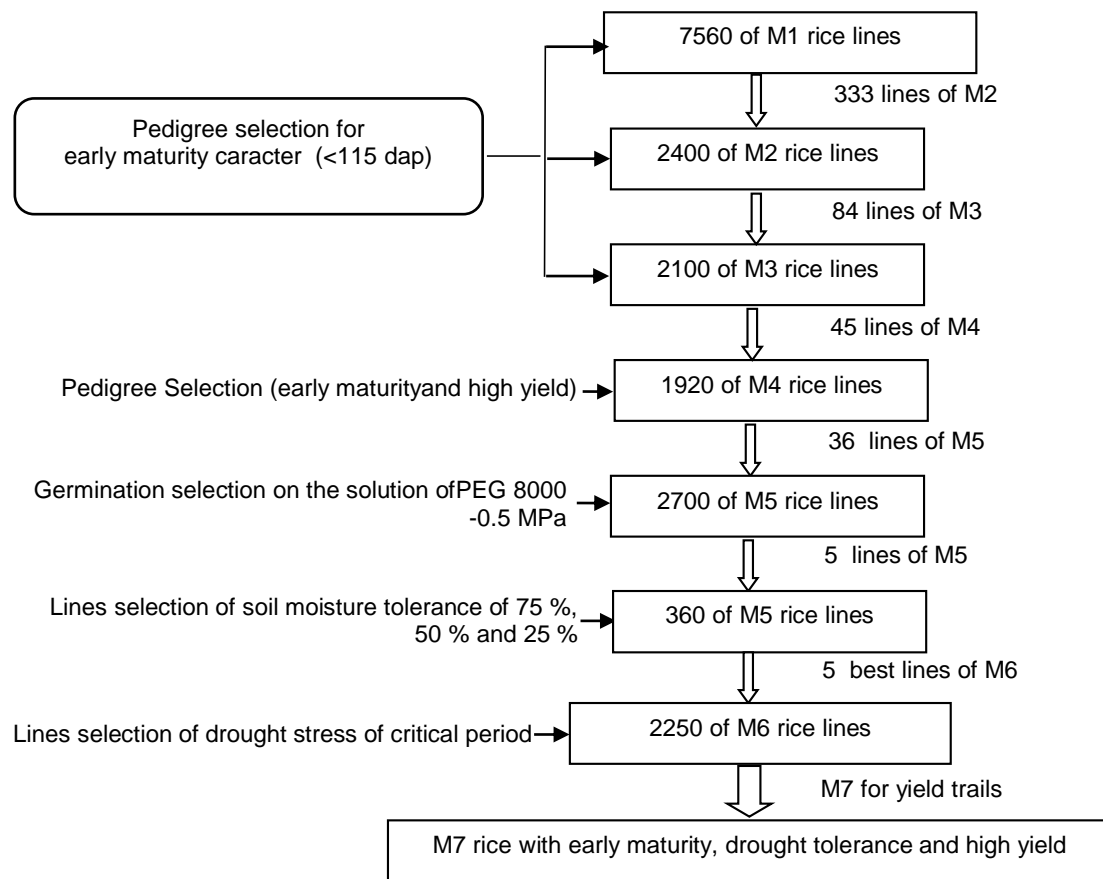


Figure 2. Selection strategy of red rice mutant for superior trait which is tolerant to drought

The character of tolerant mutants was having better results in deficit water conditions. Traits of the number of tillers, number of filled grain, percentage of filled grain, and weight of 100 grains can be used indirectly as selection criteria of red rice grain yield in the drought stress environment (Aryana, Basuki, & Kuswanto, 2011). $M_6\text{-GR}_{150}\text{-1-9-13}$ is drought tolerant mutant because during the selection process, it was able to survive in drought stress on the germination phase with polyethylene glycol (PEG) -0.5 MPa, 75 % in soil moisture and in drought stress on vegetative phase with higher results than the other mutants. Furthermore, the $M_6\text{-GR}_{150}\text{-1-9-13}$ mutants can be recommended as a candidate of drought tolerant mutants with yield from 5.23 to 6.61 t ha⁻¹).

The results showed that pedigree selection was the proper selection used to obtain early maturity and high yield mutants. The pedigree of each mutant strains could be certainly known. PEG solutions could be used to decrease the number of mutant lines that should be selected on the germination phase. To prove the tolerance of plants

to drought stress, impairment moisture test or drought stress test could be carried out because it was proven to be able to select mutant lines that are more tolerant to drought stress.

CONCLUSION

Evaluation with PEG produced five superior mutants that were more vigor than the elders. Selection on low humidity showed that $M_5\text{-GR}_{150}\text{-1-9}$ produced higher filled grain and weight filled grain than other mutants and elders. The selected red rice lines $M_5\text{-GR}_{150}\text{-1-9-13}$ had a better tolerance towards a drought than its control. It could be obtained by such mutant strains which have a high yield, early maturing and drought tolerant from the result of six generations gamma irradiation mutant selection.

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