The improvement of early maturity red rice mutant trait for drought tolerance

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Abstract

Accession of red rice does not have the traits of early maturity, drought tolerance and high yield. Mutation is needed to obtain the desired genetic resources. Gamma-ray irradiation is the right method because it was proved capable of producing hundreds of new varieties, which are better than the previous. This study aimed to identify the improvement of early maturity and drought tolerance of red rice mutant to gamma ray irradiation results. The research materials are M4, M5 and M6 strain, which are derived from the Bangka’s accession local red rice (Celak Madu, Ruten Puren and radix). The three accession seed have been treated with gamma-ray irradiation doses of 150, 200 and 250 Gray. The results showed gamma ray irradiation dose of 150 Gray and 200 Gray to red rice accession can form a mutant with the character of harvesting time less than 115 days after planting and was more drought tolerant than the previous. M6-GR150-1-9-13 strain was selected as a candidate for early maturity, drought tolerant and high yield mutant.

Introduction

Red rice paddy has nutritional value and anthocyanin higher than white rice. The anthocyanin content of local Indonesian red rice paddy is 0.03 to 210.50 mg/100 g and Thai rice is 109 mg/100 g. The amino acid alanine in red rice paddy turns off the function of free radicals. Antioxidant activity of several red rice cultivar reached 54.2% at concentration of 0.25 mg/mL.

Red rice paddy accession has longer time to harvest (121-138 days) with a flowering time of 91-107 days. Red rice accession is not classified as drought tolerant so it has lower yield (an average of 2 tons/ha). Currently, there is only one variety of red rice classified as upland types, which is Inpago 7 (4.6 tons/ha). The properties of early maturity and drought tolerance were controlled by quantitative genes (Hdl to Hdl4 based on QTL analysis) which influenced by environment. There were 413 and 245 genes found to be significantly induced and suppressed by applying gradual, long-term drought stress to four rice cultivars with differing drought resistance.

Genetic improvement of plants could be done through physical mutation using gamma irradiation. Gamma ray irradiation dose of 200 Gray has been proved to reduce harvesting age of rice up to 65-69 days compared to the previous. Selection of gamma irradiation results are mutant strain which is more drought tolerant, has early maturity and yielding of more than 5 tons/ha. Gamma ray irradiation on rice seed is capable to form permanent red rice varieties and increase the genetic diversity. Stable genotypes showed positive response if it is grown in different environments. Drought tolerance test in rice has been done with a solution of polyethylene glycol (PEG) by reducing soil moisture and stress in rice critical phase. The research was aims to determine the role of gamma ray irradiation for the improvement of early maturity and drought tolerant traits of red rice mutants.

Materials and Methods

The research was conducted in ultisols (Balunijuk, Bangka Belitung, Indonesia) and it took place from November 2013 to February 2015. The plant material used was 16 of fourth mutant (M4) of red rice origin from Celak Madu accession with irradiation dose of 150 Gray (M4-GC150-1, M4-GC150-2 and M4-GC150-3) and 250 Gray (M4-GC250-1, M4-GC250-2 and M4-GC250-3); Ruten Puren accession with irradiation dose of 200 Gray (M4-GP200-1, M4-GP200-2, M4-GP200-3 and M4-GP200-4) and 250 Gray (M4-GP250-1); radix accession with irradiation dose of 150 Gray (M4-GC150-1, M4-GC150-2 and M4-GC150-3) and 200 Gray (M4-GC200-1 and M4-GC200-2). Those M4 were selected because of their early maturity at the third mutants (M3).

The first research was the selection of 1920 individual pedigree of M4 to get early maturity and high grain yield traits. Harvesting age (dap), number of productive tillers, panicle length, number of filled seeds, filled seed weight and weight of 1000 seeds were observed.

The second research was the selection of drought tolerant trait in M5 strain that has 36 mutant lines, which were selected on M4. Research activities was started with the selection of seeds in a solution of PEG 8000 -0.5 MPa for 14 days and gave 5 strain of M5 which has the highest germination. The treatments were arranged in a split-plot design such as M5-GR150-1-4, M5-GR150-1-9, M5-GR150-2-2, M5-GR150-2-3, M5-GR150-1-2 and radix accession. The main plot was the soil moisture of 25, 50, 75 and 100% (control). Traits observed were symptoms of rolling, drying of leaves, plant height, number of leaves, the number of tillers, root length, weight of roots, weight of canopy, the number of filled seeds, weight of 1000 seeds, weight of filled seeds, fertility of the grain and the index of drought tolerance trait that calculated by the formula: S=(1-Y/Yp)/(1-X/Yp). The third research was drought tolerant trait selection of M6. There were 5 best strain of M6 from the second research. The research was arranged in a split-plot design such as M6-GX150-1-4, M6-GX150-1-9-13, M6-GX250-2-2-2, M6-GX250-2-3-13, M6-GX250-1-2-18 and radix accession. The main plot was the seeding phase of tillering, booting-flowering, seed filling and control. Drought stress was given for 14 days by covering the mutant using plastic. The main traits observed were filled seeds weight. The data of M5 and M6 were analyzed by using Fisher’s exact test and least significant difference (LSD) test at the 0.05 level of significance.

Key words: Drought tolerant; early maturity; gamma; mutant; red rice paddy.

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Results and Discussion

The selection of 1920 lines of M4 red rice paddy with selection intensity of 1.87% secured 36 of M5 strain. The selected mutant strains derived from Ruten Puren accession with gamma ray irradiation dose of 200 gray, radix irradiation dose of 150 and 200 gray. Mutants derived from the Celak Madu accession of the gamma ray irradiation dose 150 and 250 gray, and Ruten Puren accession dose of 250 gray was unselected of having early ripening traits since it had a harvesting age more than 120 days after planting.

$M_GP200-1$ mutant derived from Ruten Puren irradiation gamma ray dose of 200 gray has the lowest harvesting age and highest number of tillers. All mutants have had a similar harvest age (Table 1). These mutants reduced harvest time by 22.81 days earlier than others. Gamma-ray irradiation doses of 150 and 200 gray are effective to gain early maturity mutants. Mutants derived from radix accession reduced harvest time by 17-19 days after irradiation with doses of 150 and 200 gray. Each rice accession has different sensitivity levels.

The M4 results of gamma irradiation have a different character than the previous as shown

| N. | Mutant name | Age of harvest (dap) | Weight of filled seeds (gram) | Number of filled seeds (grain) | Weight of 1000 seeds (gram) | Number of tillers (stems) | Panicle length (cm) |
|----|-------------|----------------------|--------------------------------|--------------------------------|-----------------------------|--------------------------|-------------------|
| 1  | $M_GG_200-1$-1 | 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 10 | $M_GG_200-1$-10 | 110                  | 24.24                          | 1024                           | 23.83                       | 12                       | 24.0              |
| 11 | $M_GG_200-1$-1 | 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 12 | $M_GG_200-1$-2 | 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 13 | $M_GG_200-1$-3 | 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 14 | $M_GG_200-1$-4 | 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 15 | $M_GG_200-1$-5 | 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 16 | $M_GG_200-1$-6 | 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 17 | $M_GG_200-1$-7 | 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 18 | $M_GG_200-1$-8 | 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 19 | $M_GG_200-1$-9 | 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 20 | $M_GG_200-1$-10| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 21 | $M_GG_200-1$-11| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 22 | $M_GG_200-1$-12| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 23 | $M_GG_200-1$-13| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 24 | $M_GG_200-1$-14| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 25 | $M_GG_200-1$-15| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 26 | $M_GG_200-1$-16| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 27 | $M_GG_200-1$-17| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 28 | $M_GG_200-1$-18| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 29 | $M_GG_200-1$-19| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 30 | $M_GG_200-1$-20| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 31 | $M_GG_200-1$-21| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 32 | $M_GG_200-1$-22| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 33 | $M_GG_200-1$-23| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 34 | $M_GG_200-1$-24| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 35 | $M_GG_200-1$-25| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| 36 | $M_GG_200-1$-26| 110                 | 24.28                          | 1025                           | 23.83                       | 12                       | 24.0              |
| Ruten Puren accession | 134.0 | 18.56                          | 1051.2                          | 22.00                         | 14                       | 24.0              |
| Radix accession | 130.0 | 12.29                          | 649.7                           | 22.50                         | 5                        | 19.5              |

The criteria selection of harvesting age is more than 115 dap, and the number of extra tillers was 4 stems.

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Table 2. Mean, standard deviation and variance of the Selected M4.

| Selected mutant (M4) | Number of mutant | Mean of harvesting (hst) | $\sigma^2$ | Mean of tillers (stem) | $\sigma^2$ | Mean of filled seeds (gram) | $\sigma^2$ |
|----------------------|------------------|--------------------------|----------|------------------------|----------|-----------------------------|----------|
| M4-GR58-1            | 10               | 110.00±0.0               | 0.00     | 8.70±1.64              | 2.68     | 18.75±1.56                  | 26.58    |
| M4-GR58-2            | 2                | 113.00±0.0               | 0.00     | 6.50±2.12              | 4.50     | 16.17±2.87                  | 8.24     |
| M4-GR58-4            | 6                | 113.00±0.0               | 0.00     | 7.33±1.51              | 2.27     | 17.64±3.66                  | 38.73    |
| M4-GR58-1            | 11               | 113.09±1.3               | 1.69     | 6.73±1.49              | 2.22     | 21.98±2.22                  | 38.73    |
| M4-GR58-2            | 3                | 113.00±0.0               | 0.00     | 6.33±0.58              | 0.33     | 13.86±1.70                  | 2.88     |
| M4-GR58-3            | 1                | 112.00±0.0               | 0.00     | 6.00±0.00              | 0.00     | 16.96±0.00                  | 0.00     |

Table 3. Scrolling, drying and mutants death (M5) percentage to a few test of soil moisture (%).

| Mutant     | Moisture % | Tillering phase | Elongation phase | Booting phase | Flowering phase | Filling seed phase | Mutant death |
|------------|------------|-----------------|------------------|--------------|----------------|-------------------|--------------|
| M5-GR69-1 | 100        | 11.11 ±0.0      | 5.56 ±0.0        | 0.00 f       | 0.00 f         | 5.56 ±0.0         | 10.00 f      |
| M5-GR69-2 | 75         | 22.22 ±0.0      | 5.56 ±0.0        | 5.56 ±0.0    | 0.00 f         | 0.00 f            | 5.56 ±0.0    |
| M5-GR69-3 | 50         | 66.67 ±0.0      | 8.33 ±0.0        | 8.33 ±0.0    | 0.00 f         | 0.00 f            | 5.56 ±0.0    |
| M5-GR69-4 | 25         | 66.67 ±0.0      | 8.33 ±0.0        | 8.33 ±0.0    | 0.00 f         | 0.00 f            | 5.56 ±0.0    |

Note: All data are mean ± standard error. Significant differences determined by LSD test at $p<0.05$. Treatment means followed by the same letter within a column are not significantly different. All experiments were done in triplicate.

1. Soil Moisture, G: Symptoms of Scrolling Leaves, K: Symptoms of Drying Leaves, M: Death Plant. Based on field observations, the red rice mutant enter the phase of tillering at the age of 28-42 day (day of planting/bts (hart setelah tanam)), elongation phase at 45-70 day, the phase of panicle formation and gestation at the age 70-77 day, flowering phase of 77-91 day, while the phase of seed filling >91 hot/day. The same notation means no significance, different notation means significant.
using the characters of scrolling leaves.\textsuperscript{13} Leaf rolling and leaf drying were correlated negatively to the rice yields.\textsuperscript{19} However, in mutants, it was correlated to the number of empty seed, resulting in lower yield. Symptoms of drought stress is the rolled leaves, dried leaves, delayed booting and imperfect seed filling.\textsuperscript{20} Rice, which is sensitive to drought, has higher leaf rolling score.\textsuperscript{21} Leaf rolling is used to determine drought tolerance of rice. Mutants with the lowest percentage of leaf rolling are more drought-tolerant.

Scrolling leaves of mutants led to leaf drying in flowering phase. The highest numbers of drying leaves were on M5-GR150-1-4 and M6-GR150-2-2. M5-GR150-2-3 was the most drought tolerance mutants (Table 3). Critical period of mutants in the vegetative phase is tillering stage. Generative phase occurs with the increasing number of mutants, which experienced leaf drying and death. Mutants on 25% soil moisture have experienced death in inmination and flowering phases. Mutants on 100% soil moisture were dead due to low soil aeration and high amount of water, which tied the clay fraction. Planting on ultisols medium has low porosity due to clay content which reached 40.20%.

Drying leaves is a sign of mutant experience high drought stress. Drought stress on inmination and seed filling phase causes a higher decrease in yield. Drought caused reduction in the distribution and allocation of dry matter, the reduction of photosynthetic capacity as a result of closing of stomata, inhibition of metabolism and damage to the chloroplast.\textsuperscript{22} A previous study shows that lack of water in the reproductive phase decreases the amount of panicles and increased sterility.\textsuperscript{23} Drought stress at 3 weeks before harvest degrades chlorophyll so that it lowers the rate of photosynthesis. M5-GR150-1-9 and M6-GR150-2-3 experience the lowest drying leaves stress in grain filling phase. Both of M5 strains were more drought-tolerant to the other mutant.

Low symptoms of drying leaf were on M5-GR150-2-3 and M5-GR150-1-9. Low symptoms of leaf drying M5-GR150-2-3 and M5-GR150-1-9 correlated with a lower index value tolerance (IST) (Table 4). Both mutants were more drought-tolerant than other mutants and previous. Genotypes with low IST indicated drought tolerance.\textsuperscript{19} Upland rice mutant superiority has the higher number of grains per panicle, an early ripening age and harvesting, larger seed size and form shape red purplish rice (forming anthocyanin).\textsuperscript{24}

The increase of drought-tolerant traits of the mutant compared with radix accession aside from indication by the value of IST is also demonstrated by filled seed weight/clump. M5 and M6’s filled seeds weighs are higher than Radix accession. The highest average of filled seed weight is M5-GR150-1-9. The mutant is more tolerant to drought than other mutants and radix accession to the decrease in 75% of soil moisture. M5-GR150-1-9-13, which is a descendant of the M5-GR150-1-9, also had a high average weight of filled seeds. M5-GR150-1-9-13 strain has the highest weight of filled seeds in drought stress in tillering stage (Figure 1). The number of filled seeds per panicle is positively correlated with yield.\textsuperscript{25} M5 and M6 strains, which have higher weight of filled seeds also have a high yield. The results show that the improvement of early maturity traits through gamma ray irradiation may form drought-tolerant trait of mutant red rice and increase crop yields.

Table 4. M5 tolerance and radix accession to the decline of 75% soil moisture.

| Plant traits | M5-GR150-1-4 | M5-GR150-1-9 | M5-GR150-2-2 | M5-GR150-2-3 | Radix accessions |
|--------------|--------------|--------------|--------------|--------------|-----------------|
| Plant height | 1.00 p       | 1.01 p       | 1.04 p       | 0.95 m       | 1.00 p          | 0.97 m          |
| Number of leaves | 0.98 m      | 0.96 m      | 0.95 m      | 0.93 m      | 1.08 p          |
| Number of tillers | 0.9 m       | 1.21 p     | 0.77 m        | 0.78 m       | 1.33 p          |
| Root length | 0.9 m        | 1.02 p       | 0.88 m       | 1.08 p       | 1.09 p          |
| Root weight | 0.89 m       | 1.07 p       | 0.77 m       | 0.62 m           | 2.64 p          |
| Crown weight | 0.94 m     | 1.15 p       | 0.93 m       | 0.86 m       | 1.2 p           |
| Number of filled seed | 0.97 m | 1.02 p | 0.92 m | 0.97 m | |
| Weight of 1000 seeds | 1.17 p | 1.5 p | 1.51 p | 1.53 p | 0.44 t |
| Weight of filled seeds | 1.05 p | 1.14 p | 0.67 m | 1.13 p | 1.12 p |
| Filled seed grain | 1.03 p | 0.91 m | 1.02 p | 1.09 p | 1.05 p |
| Index tolerance: t, tolerant <1; m, medium 0.5-1; p, sensitive >1.\textsuperscript{25} |

Figure 1. The mean weight of M5 filled seeds (A), M6 filled seeds (B) and radix accession to the treatment of several drought tolerance soil moisture test and drought stress in the critical period of the plant.
Conclusions

Gamma-ray irradiation dose of 150 and 200 gray on Bangka local red rice paddy accession can form mutant, which has the characteristics of early maturity, drought tolerant and higher yield than the previous. M6-Gr150-1-9-13 strain was selected as a candidate for early maturity, drought tolerant and high-yielding mutant.

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