Growth and production of sixth generation of brown rice mutants in a high altitude location

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Abstract. This paper presents the result of an experiment which aimed to determine the growth and production of some 6th generation Sinjai brown rice mutants (M6) in the highlands. The experiment was conducted in an irrigated paddy field with a height of 750 meters above sea level. The arrangement was a randomized block design (RBD). The treatments consisted of 8 brown rice mutant lines (g1, g2, g3, g4, g5, g6, g7, g8) and Sinjai brown rice parent as control (g9). The results showed that the mutant lines g2 and g3 produced the highest grain production of 3.10 tons ha\(^{-1}\) and 3.13 tons ha\(^{-1}\) respectively. Mutant strains g2 and g3 were short in plant height with a high number of productive tillers, flowering age and a faster harvest time, as well as highest grain length. g2 and g3 strains are the most potential lines to be developed in the highlands. The growth character and production components that have a real positive correlation to production per hectare are production per clump and presentation of filled grain per panicle.

1. Introduction
The utilization of rice varieties with certain superior characteristics is the key to the success of increasing rice production in Indonesia. Local rice is a high-yielding variety and can adapt well in the local area. Local rice is resistant to local pests and endemic diseases. For this reason, the use of local varieties in breeding programs is recommended. The purpose of local plant breeding is to broaden the genetic background of the superior varieties to be produced [1].

Brown rice is one of the local rice varieties which has a much higher nutritional content compared to white rice varieties. Local brown rice is a source of protein and minerals such as selenium which can increase endurance, and a source of vitamin B which can nourish nerve cells and the digestive system. Brown rice also has a high fiber content so that it can prevent constipation. However, as with local rice in general, brown rice has several weaknesses, namely longevity with a low average yield [2].

In order to overcome this problem, one of the modern methods used is by mutation of plants [3]. Gamma rays have often been used to induce plants to mutate. These rays have the ability to penetrate deep into plant tissue. Mutation induction is directed to change one or several important characters that benefit plants while maintaining most of the original characters [4]. The availability of brown rice lines from the 5th generation mutation (M5), can be developed into the 6th generation which can later be used as new varieties of rice after multilocation testing [5].
2. The M6 mutant hierarchy
Mutation is one technique to increase genetic diversity by using gamma ray irradiation, so that plants are obtained with the expected traits/characters. In this experiment, local sinjai rice seeds used as a comparison (without mutations) had weaknesses complained of by the wider community such as the relatively long harvesting time and low production per unit area. In overcoming the weaknesses of local rice, through mutation breeding can produce local strains with a faster harvest time and production per unit area high.

Interaction between varieties and height causes different effects on the growth of rice. The high altitude ecosystem (> 700 meters above sea level), has a special characteristic that is low temperature. According to Lee [6] low temperature stress extends the vegetative phase, causes pollen sterility, and inhibits seed filling so that plant life is longer and the percentage of empty grains per panicle is higher.

Shaleh’s research in 2011 produced brown brown mutant seed originating from first Sinjai generation (M1) from the parent seed that was irrigated at PATIR-3 BATAN (Center for Application of Radioactive Isotopes and Technology - National Nuclear Energy Agency). The M1 planting carried out in the Village Karattuang of Bantaeng Regency obtained a second generation of brown rice mutants (M2). The third generation (M3) was obtained after planting the second generation of mutants in the village of Kera-Kera, Makassar. The M3 was then planted at the Hasanuddin University experimental farm, Makassar to obtain the fourth generation brown rice (M4). Furthermore, M4 planting was carried out in Watu Village, Marioriwawo Subdistrict of Soppeng District, which produced the fifth generation of brown rice (M5).

3. Methodology
This experiment employed a randomized block design (RBD), with 9 treatments and 3 replications. The treatments consisted of 8 brown rice mutant lines (G1, G2, G3, G4, G5, G6, G7, G8) and sinjai brown rice parent as control (G9).

3.1. Field and seeds preparation
The nursery land was perfectly ploughed, leveled and divided into 9 plots with 200 x 100 cm plot size and 30 cm distance between plots.

Rice seeds were soaked for 24 hours. Immersion aimed to uniform seed germination. Then the seeds are drained and stored in plastic bags for 2 x 24 hours. After the storing, the seeds were sown in each nursery plot. After 20 days old, they are ready to be transplanted to the planting area.

Tillage for planting land was carried out with hand tractors. The process included plowing which intended to get muddy soil with a depth of 20 cm. After plowing, the land is leveled and then the rice fields are left in muddy and saturated condition.

This experiment contained 9 treatments that were repeated 3 times, with a total plot of 27 units. Plots’ dimension were 5 m x 3 m (15 m²) with 50 cm spacing between plots within the same group and 100 cm spacing between plots in different groups. Each group was randomized to determine the plots to be planted with tested genotypes, and labeled as genotype treatment codes.

3.2. Planting and cultivating
Planting was performed by moving the seedlings from the nursery to the plots that have been prepared. The planting system employed a spacing of 25 cm x 25 cm. One seedling was planted in each hole, make a total of 240 plants in one plot.

Fertilizers were inorganic ones (Urea, KCL, and SP-36). Initial fertilization was carried out at 7 days with a dose of 133.33 kg/ha (200 g/plot) for Urea; 100 kg/ha (150 g/plot) for KCl; and 200 kg/ha (300 g plot) for SP-36. Fertilizer application continued at 37 DAT with a dose of 166.67 kg/ha (250 g plot) of Urea.

Irrigation was given by flowing water ± 10 cm off the ground level. Watering was done 21 days after transplantation because fertilization was carried out at 7 days after transplantation where the required conditions of rice fields when applying fertilizers are in a state of muddy and saturated (not
inundated). Three days after the first irrigation, the water was removed and allowed to return to the condition of muddy and saturated in order to provide an opportunity for the release of productive tillers. In the primordial phase, water was given again to suppress new tillers that are no longer productive. Then 7 days before harvest, the water was removed again and allowed to dry up so that the maturing process of the grain were uniformed.

4. Results and discussion

4.1. Vegetative growth

Results showed that strain did not have significant effect on plant all vegetative growth parameters. Figure 1, 2 and 3 shows the performance of each brown rice mutants on these parameters.

![Figure 1. diagram of plant height (cm) at harvest time](image)

Figure 1 shows that the g9 strain produced the highest average plant height of 102.47 cm, while the g6 mutant strain tended to produce the lowest average plant height of 84.22 cm.

The average plant height shows that the mutant lines g1, g2, g3, g4, g5, g6, g7 and g8 (80-90 cm) have shorter plant height compared to g9 (102.47 cm). This is because g1, g2, g3, g4, g5, g6, g7 and g8 are genes resulting from gene mutations while g9 is the parent strain. Expected lines are lines that have superior properties such as having short and strong stems to avoid being overburdened and can support high production. According to Manurung and Ismunadji [7], the desired trait in the development of superior varieties in rice is short and stiff stems because plants that have these characteristics will resist lodging, respond to fertilization, besides that the ratio between grain and straw is more balanced.

The results of the correlation analysis (see table 6) showed that plant height was significantly negatively correlated to production per hectare, namely $r^2 = -0.73$ *. Increased plant height will have an impact on production decline. Thus the strain that is considered potential and gives the best results to plant height is the g3 mutant strain which has the shortest average.
Figure 2 shows that the g8 mutant strain produced the highest average number of tillers i.e. 17.60, while the g9 strain produced the lowest average number of tillers which was 13.87 tillers.

Figure 3 shows that the g6 mutant strain produced the highest percentage of productive tillers at 78.30%, while the g8 strain produced the lowest number of productive tillers 63.74%.

According to Makarim and Suhartatik [8], productive tillers are one of the yield components that directly influence the level of grain yield. The average number of productive tillers (Figure 3) shows that the mutated lines (g1, g2, g3, g4, g5, g6, g7 and g8) have better growth compared to the comparative lines (g9). The g6 mutant strain with the highest number of productive tillers (13.04 stems) and g3 mutant strains (12.27 stems) differed from the parent strain or comparative strains (g9) which produced the lowest average number of productive tillers of 9.62 stems.

Increasing the number of productive tillers will have an impact on increasing production. Agustamar [9] states that the number of productive tillers is the main actor with a contribution to the
yield of 48.9%, so almost half of the results of rice is determined by the number of productive tillers. This is indicated by the resulting production of g6, g3 higher than g1, g2, g4, g5, g7, and g8. Likewise, g9 which has low productive tillers, also produces low production.

4.2. Generative variables

The results showed that the strains significantly affected flowering age and harvesting time. The following table 1 dan 2 presents these two parameters.

Table 1. The average flowering time (DAT) of various local Sinjai brown rice mutant lines

| Strain | Average  |
|--------|----------|
| g1     | 103.33a  |
| g2     | 102.00a  |
| g3     | 102.00a  |
| g4     | 102.33a  |
| g5     | 104.33ab |
| g6     | 102.33a  |
| g7     | 106.00b  |
| g8     | 105.33b  |
| g9     | 128.33c  |

LSD 0.05 2.67

Notes: The numbers followed by the letters (a, b, c) which are not the same in the same row and the same column means significantly different in the LSD α test = 0.05

Table 1 shows that the g2 mutant strain had the fastest flowering time (102.00 DAT) and was not significantly different from the g3 mutant strain (102.00 DAT), but significantly different from g9 strain which had the longest average flowering time (128.33 DAT).

Table 2. Average harvest time (DAT) of various local Sinjai brown rice mutant lines

| Strain | Average  |
|--------|----------|
| g1     | 142.67ab |
| g2     | 135.67a  |
| g3     | 135.33a  |
| g4     | 138.33a  |
| g5     | 147.33b  |
| g6     | 139.00a  |
| g7     | 149.00a  |
| g8     | 148.67a  |
| g9     | 180.67c  |

LSD 0.05 8.61

Notes: The numbers followed by letters are not the same on the same line, meaning that they are significantly different in the LSD α test = 0.05

Table 2 shows that the g3 mutant strain had the fastest average harvest time (135.33 DAT) and was not significantly different from the g2 mutant line (138.33 DAT), but it was significantly different from the g9 strain which had the longest average harvest age (180.67 DAT).

The g2 and g3 mutant strains (102.00 DAT) produce the fastest flowering time compared to other strains (Table 1). According to Rohal [10] the faster flowering the faster the plants can be harvested. According to Pramudyawardani, Suprihatno, & Mejaya [11], flowering age is controlled by the action
of dominant additive genes, so that the more dominant genes that are in one individual the longer the flowering age and correlates with the longer harvest time.

Age of flowering has a very significant positive correlation to the age of harvest (r² = 0.97 **) but a negative correlation is very significant to production per hectare that is r² = -0.93 ** (please refer to table 6). It means that an increase in flowering age is in line with an increase in harvesting time. The longer the flowering is the longer the harvest time which causes a decrease in production. Manurung and Ismunadji [7] stated plants that flower faster have a faster generative phase. This is supported by the average harvest time, mutant lines g2 (135.67 DAT) and g3 (135.33 DAT) have more harvest age fast compared to other mutant lines (g1, g4, g5, g6, g7, g8) and comparison lines (g9) which have a longer harvest time (180.67 DAT). Taslim, Partoharjo, & Djunainah [12] added the cause of differences in plant production time is due to the different vegetative phases. This was also stated by Damayanti & Utami [13], that flowering age determines yields. If the flowering is fast, the harvest time is fast and vice versa.

4.3. Grains and panicles
The analysis of variance showed that the strain did not significantly affect panicle length, number of grains per panicle, grain density and weight of 1000 grains. The following figure 4, 5, 6 and 7 depict these variables.

Figure 4. Diagram of average panicle length of each strain

Figure 4 shows that the g1 mutant strain had the shortest panicle of 20.77 cm, while the g9 strain had the longest panicle of 23.14 cm.

Panicles are generative part of the rice. The panicle length is determined by the nature of the parent of the variety and the state of the environment. Panicles vary in length, short (20 cm), medium (20-30 cm), and long (more than 30 cm). The panicle length of a variety, the number of branches per panicle, and the number of grains per branch, depends on the variety and method of farming [8]. The g1 mutant strain produced shorter panicle compared to other mutant lines and g9 comparison lines.
Figure 5 shows that the g6 mutant strain produced the highest average number of grain per panicle at 130.79, while the g5 mutant strain produced the lowest average number of grains per panicle at 115.56.

According to (Makarim & Suhartatik [8], the longer the panicle length is, the more rice is produced. g1 mutant lines with the shortest panicle length produced the highest percentage of empty grains per panicle i.e. 56.02% (table 4). Whereas g7 that have long panicles produced a lower percentage of empty grain per panicle (52.78%) yet not much different from g1 mutant lines. Lestari [14] stated that panicles that are too long can cause the ripening time between the initial grain to the final grain to appear too far so that it produces a lot of empty grain.

Mutant strains with short panicles have a small amount of grain per panicle resulting in high densities per panicle. Whereas the G9 comparison line has a long panicle but the number of grains per panicle is small so that it produces a low density per panicle. g4 mutant strain had the highest average density per panicle (6.36 grains/cm) while g9 had the lowest average density per panicle (3.86). This is supported by Riadi, Sjahril, Kasim, & Djarjo [15] that the large value of panicle density does not
always reflect the high number of grain per panicle and the length of panicle, because the same value of panicle density can be obtained by panicle with less grains but shorter panicle.

Figure 7 shows that the mutant strain g2 produced heaviest weight of 1000 grains which was 23.64 g, while the strain g6 tended to produce the lightest weight of 1000 grains which was 21.93 g.

The strains on the other hand significantly affected the percentage of filled grain per panicle and empty grain per panicle which are presented in the following table 3 and 4.

**Table 3.** The average percentage of filled grain per panicle (%) of various local Sinjai brown rice mutant

| Strain | Average   |
|--------|-----------|
| g1     | 43.98\(^a\) |
| g2     | 53.64\(^c\) |
| g3     | 61.53\(^c\) |
| g4     | 54.95\(^b\) |
| g5     | 48.96\(^a\) |
| g6     | 57.84\(^c\) |
| g7     | 47.22\(^a\) |
| g8     | 48.85\(^c\) |
| g9     | 10.66     |

LSD 0.05 43.98\(^a\)

Notes: The numbers followed by letters are not the same on the same line, meaning that they are significantly different in the LSD \(\alpha\) test = 0.05

Table 4 shows that the g3 mutant strain produced the highest percentage of filled grain per panicle at 61.53% and was not significantly different from the g2 mutant strain with an average of 53.64%, but significantly different from the g1 mutant strain which had lowest percentage of filled grain per panicle of 43.98%.
Table 4. The average percentage of empty grain per panicle (%) of various local Sinjai brown rice mutant

| Strain | Average |
|--------|---------|
| g1     | 56.02c  |
| g2     | 40.36a  |
| g3     | 38.47a  |
| g4     | 45.05ab |
| g5     | 51.04b  |
| g6     | 42.16a  |
| g7     | 52.78bc |
| g8     | 51.15b  |
| g9     | 10.66   |

LSD 0.05 56.02c

Notes: The numbers followed by letters are not the same on the same line, meaning that they are significantly different in the LSD α test = 0.05

Table 5 shows that the g1 mutant strain produced the highest percentage of empty grain per panicle which was 56.02% and was not significantly different from the g7 mutant strain with an average of 52.78%, but significantly different from the g3 mutant strain which had the lowest percentage of empty grain per panicle i.e. 38.47%.

The high number of grains per panicle supports crop productivity, if followed by the large number of filled grains produced. This is supported by the opinion of Siregar & Marzuki [16] which stated that the amount of grains per panicle will determine crop productivity. The high percentage of filled grains per panicle and the low percentage of empty grains per panicle were related to the vegetative phase of each strain. Strains that have a longer vegetative phase can produce more grains, due to the longer time for plants to carry out the process of photosynthesis to produce assimilates which will be used for filling grains as a reproductive gutter. Whereas lines that have a rapid vegetative phase will only produce a small amount of assimilate, possibly causing the grain to become empty.

4.4. Productivity

The variance analysis shows that strain significantly affected production per hectare. The average production per hectare of brown rice mutant lines is shown in table 5.

Table 5. Average production per hectare (ton) with a moisture content of 12% of various Sinjai local brown rice mutant lines

| Strain | Average |
|--------|---------|
| g1     | 1.62b   |
| g2     | 3.10a   |
| g3     | 3.13b   |
| g4     | 2.62ab  |
| g5     | 1.42bc  |
| g6     | 2.93a   |
| g7     | 1.23c   |
| g8     | 1.28c   |
| g9     | 1.27    |

LSD 0.05 1.62b

Notes: The numbers followed by letters are not the same on the same lines, meaning that they are significantly different in the LSD α test = 0.05
Table 7 shows that the g3 mutant strain produced the highest average production per hectare of 3.13 tons and was not significantly different from the g2 mutant strain with an average of 3.10 tons but significantly different from the g7 strain which had the lowest average production per hectare which was 1.23 tons.

The average production per hectare of g2 and g3 mutant lines were 3.10 and 3.13 tons per hectare respectively, which were higher than other lines. The ability of a plant to grow and produce is an important factor in plant development. Mutant strains g2 and g3 which have a short plant height with a high number of productive tillers, flowering time and a faster harvest time, also had the longest panicle and produced the most production per hectare. Thus g2 and g3 are the most potential lines to be developed based on production levels and other parameters. However, when compared to g9, all mutant strains have better growth and yield potential.

4.5. Correlation analysis

According to Sarwono [17] the correlation coefficient shows the strength of the linear relationship and the direction of the relationship between the two random variables. If the correlation coefficient is positive, then the two variables have a direct relationship. Conversely, if the coefficient is negative then the two variables have an inverse relationship. The results of the correlation analysis are presented in table 6.

Table 6. The results of the correlation analysis of some characters of M6 brown rice mutant lines

|       | PH   | NoT  | NoPT | FT   | HT   | PL   | NoGP | GD    | FGP   | EGP   | W1000 | P    |
|-------|------|------|------|------|------|------|------|-------|-------|-------|-------|------|
| PH    |      |      |      |      |      |      |      |       |       |       |       |      |
| NoT   | -0.20** |      |      |      |      |      |      |       |       |       |       |      |
| NoPT  | -0.54** | 0.21* |      |      |      |      |      |       |       |       |       |      |
| FT    | 0.62** | 0.28** | -0.42** |      |      |      |      |       |       |       |       |      |
| HT    | 0.64** | 0.33** | -0.45** | 0.97** |      |      |      |       |       |       |       |      |
| PL    | -0.01 | -0.13 | -0.01 | 0.44 | 0.37 |      |      |       |       |       |       |      |
| NoGP  | -0.53** | -0.11 | 0.80* | -0.29 | -0.25 | 0.21 |      |       |       |       |       |      |
| GD    | 0.21 | -0.36 | 0.47 | 0.00 | 0.14 | -0.21 | 0.31 |      |       |       |       |      |
| FGP   | -0.73* | -0.45 | 0.45 | -0.76 | 0.84** | 0.16 | 0.40 | -0.20 |      |       |       |      |
| EGP   | 0.73* | 0.45 | -0.45 | 0.76 | 0.84** | -0.16 | -0.40 | 0.20 | -1.00 |      |       |      |
| W1000 | 0.18 | -0.10 | -0.23 | -0.17 | -0.33 | -0.10 | -0.31 | -0.69 | 0.17 | -0.17 |      |      |
| P     | -0.73* | -0.52 | 0.32 | 0.93** | 0.97** | -0.16 | 0.41 | 0.12 | 0.93** | -0.93* | 0.22** |      |

Notes: PH=plant height; NoT=number of tillers; NoPT=number of productive tillers; FT=flowering time; HT=harvesting time; PL=panicle length; NoGP=number of grains per panicle; GD=grain density; FGP=Filled grain per panicle; EGP=empty grain per panicle; W1000=weight of 1000 grains; P=productivity

The correlation coefficient shows the relationship between the character of production per hectare with other characters in M6 brown rice mutants. Production per hectare (0.94 **) has a very significant positive correlation with the percentage of number of grains per panicle (0.89 **) but in other characters the correlation is not significantly positive.

The results of the positive correlation were very significant between the character of production per hectare with the percentage of number of grains per panicle (0.93 **) but in other characters the correlation was not significantly positive with the character of production per hectare. The results of the negative correlation are very significant between the character of production per hectare with flowering age (-0.93 **), age of harvest (-0.97 **) and percentage of empty grain (-0.93 **) as well as a negative negative correlation between the character of production per hectare with plant height (-0.97 **).
0.73 *) but the other characters have a negative negative correlation with the character of production per hectare.

The brown rice mutant lines (G1, G2, G3, G4, G5, G6, G7, G8) which have now reached the sixth generation (M6) have produced potential lines with faster harvesting age and production per unit area higher than the comparison mutation line (G9) which is not mutated.

5. Conclusion

Based on the observed parameters, mutant lines g2 and g3 have the highest potential to be developed based on the production as well as other variables. Mutant strains g2 and g3 gave a shorter plant with a high number of productive tillers, faster flowering and harvesting time, produced longest grain and overall produced more per hectare.

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