Response of M5 wheat (*Triticum aestivum* L.) mutant to low altitude growing condition

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Abstract. This study aims to study the adaptability of wheat mutant lines in the lowlands and determine the growth and production of the mutant lines. The research was conducted from September to December 2017 at Teaching Farm, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi Province. The study was carried out in the form of experiments arranged based on Randomized Block Design and further tested using orthogonal contrast. The treatments given were five M5 mutant genotypes and two convergent breeding genotypes. Three wheat varieties Dewata, Munal, and Selayar were used as comparisons. The results showed that the convergent breeding line (CB 145) showed good genetic traits on plant height parameters and the number of empty floret. Dewata, Munal, and Selayar varieties showed more average number of tillers (6.00 tillers) and 100 heavier grain weight (4.07 g) compared to the mutant genotypes. Dewata variety showed good genetic characteristics on panicle length parameter (6.50 cm) and grain weight per panicle (0.34 g). CB 169 genotype showed good genetic characteristics on the parameter number of grain per panicle which only produced the number of seeds per panicle (8.33 seeds).

1. Introduction

Wheat is usually used to produce flour, animal feed, or fermented to produce alcohol. Wheat includes cereals that contain carbohydrates and starch-based food. Flour from cereals including wheat has a special character compared to starch from starchy plants such as various tuberous plants. Flour from cereals is not hygroscopic so it has a long shelf life, both in the form of seeds and flour [1].

Food diversification, encouraging the need for flour until now has shown significant development, marked by the development of the food processing industry made from wheat flour as a processed product from wheat seeds as a food raw material. The biggest consumption is 35% for household consumption in the form of wet noodles or dry noodles, 25% for the bread industry, 20% for the instant noodle industry, 15% for the biscuit industry, and the remaining 5% is for fried food. The types of food are very popular by the community ranging from children to parents, both from the lower classes to the top level. The diversity of wheat-based processed products causes wheat production and wheat demand to increase in proportion to the level of public consumption related to income levels and the rate of population growth which is always increasing [2].

Indonesia is one of the countries with high population. The increasing population growth will cause the need for food consumption increase, especially carbohydrate consumption. The Indonesian population consumes large amounts of wheat, which is worth 21 kg per capita, the second largest after rice consumption. In developing countries the increase in demand for wheat is estimated to reach around 2% per year. To meet these needs, we need to increase wheat production twice the current world wheat production. The current rate of increase in wheat production is still too low to meet future wheat needs.
One effort to reduce the value of wheat imports is to develop wheat cultivation in Indonesia. Study conducted by Hakim [3] shows that some farmers have a positive perception of wheat cultivation. This indicates that the potential for wheat to be cultivated in Indonesia. Therefore, wheat varieties are needed that are able to adapt in Indonesia tropical climate. Despite this, wheat cultivation in Indonesia is constrained by climate factors because wheat originally is a subtropical crop. Wheat requires the environment to grow with a temperature range of 8 °C – 10 °C and rainfall of 350 mm - 1250 mm per year. Based on the plant growing condition requirement, wheat cultivation in Indonesia can only be done in the highlands or above 1000 m above sea level (asl). Wheat cultivation should be directed to medium to low elevation area to avoid competition with horticultural crops. Therefore, the wheat breeding program in Indonesia is aimed at assembling superior tropical varieties that are able to adapt in the lowlands growing condition. One of the criteria for the success of a wheat breeding program in Indonesia is the ability to assemble improved varieties that are adaptive at locations with heights <400 m asl. The results of research in Pasuruan and Kuningan with a location height of 600 m asl show that wheat can produce well because the flowering phase of wheat gets cold wind in July-August [4].

According to Nur [5] evaluation of 47 wheat lines in the Malino highlands, South Sulawesi (1500 m asl), Bantaeng moderate land, South Sulawesi (800 m asl), and Malang lowlands, East Java (325 m above sea level) showed a decrease in yield from the highlands to the lower plains. The results of the 47 lines evaluated were 5.05 tons ha⁻¹ each in Bantaeng and 0.37 tons ha⁻¹ in Malang. Wahyu et al. [6] has conducted research on 10 wheat introduction lines from India, Turkey, CIMMYT, and national varieties as a comparison conducted in the Seameo Biotrop experimental garden, Tajur, Bogor which has a height of 300 m above sea level). The recent study is one of the studies conducted to evaluate the mutant lines grown at lower altitude.

2. Methodology
The study was conducted from September to December 2017 at the screen house, Teaching Farm, Faculty of Agriculture, Hasanuddin University, Makassar. The location is at an altitude of 25 meters asl. The study was conducted in the form of an experiment using a randomized block design (RBD). consisting of five mutant lines (M5), namely Nias350 3.8.9, N350 3.1.3, N250 4.2.1, N350 3.2.2, and N250 4.6.2, two convergent breeding genotypes, namely CB 145 and CB 169, three comparison varieties, namely Dewata, Munal, and Selayar. Each treatment was repeated 3 times, so that there were 30 experimental units.

2.1. Seed sources
Plant materials used in this study were seeds of 7 wheat mutant genotypes (M5) which are the result of a lowland wheat development program carried out by the Faculty of Agriculture, Hasanuddin University. The mutation treatment is carried out with various levels of gamma ray irradiation in the parents of tropical wheat varieties that have been released by ICRI (Indonesian Cereal Research Institute). In addition, two genotypes from convergent breeding from ICRI were also used. The origin of the genotype is symbolized in the name of the genotype attributed to the name of the parental line and dosage of gamma irradiation used. While genotypes from convergent breeding are symbolized in a different manner (CB). As a comparison, three Indonesian tropical wheat varieties also were used.

2.2. Planting
Planting media used were a mix of soil and manure with a ratio of 2:1. Soil were previously cleaned from large aggregates, roots and rocks that are not desirable and then dried. Subsequently, the soil were placed into a 25 x 35 polybag of 3 kg planting media per polybag. Wheat seeds were sown in the polybags with three wheat seeds. Thinning was carried out after the plants grew leaving two plants per polybag.
Fertilization was given twice at 10 days after planting (DAP) and 30 DAP. Weeding of wheat plants was conducted every time weeds grow on polybags that have the potential to reduce crop productivity. Plant and disease control were carried out by spraying the insecticide Deltamethrin 25g/L into wheat-infested pests and spraying 70% Propineb fungicide on wheat-infested diseases. Harvesting is done 80% of the plants in each polybag has yellowed (the seeds are dry and yellowing), wheat seeds have been quite hard when massaged by hand.
2.3. Data analysis

Data obtained from observations were analyzed using one-way ANOVA according to a randomized block design. If significantly effect of the treatment found, further analysis was performed with the Orthogonal Contrast Test ($\alpha = 0.05$).

3. Results

3.1. Plant height, length of panicle and number of spikelet per panicle

Variance analysis showed that the treatment had very significant effect on plant height, panicle length and number of spikelet per panicle. Orthogonal contrast test on the average values of these parameters are shown in table 1.

Table 1. Orthogonal contrast test on the mean values of plant height, length of panicle and number of spikelet per panicle of M5 wheat mutant and convergent breeding genotypes on low altitude condition.

| Genotype treatments | Mean values | F value | F table 0.05 | F table 0.01 |
|---------------------|-------------|---------|--------------|--------------|
| Plant Height (cm)    |             |         |              |              |
| g8g9g10 vs g1g2g3g4g5g6g7 | 38.78 vs 42.25 | 40.65** |             |              |
| g1 vs g2g3g4g5g6g7  | 40.24 vs 42.59 | 7.54*   |              |              |
| g2 vs g3g4g5g6g7    | 39.69 vs 43.17 | 16.13** | 4.41         | 8.29         |
| g3 vs g4g5g6g7      | 52.06 vs 40.94 | 158.31**|              |              |
| g4 vs g5g6g7        | 39.39 vs 41.46 | 5.18*   |              |              |
| g5 vs g6g7          | 51.02 vs 36.69 | 219.12**|              |              |
| g6 vs g7            | 39.22 vs 34.16 | 20.49** |              |              |
| g8 vs g9g10         | 30.66 vs 42.83 | 158.00**|              |              |
| g9 vs g10           | 42.77 vs 42.90 | 0.01ns  |              |              |

| Panicle length (cm) |         |         |              |              |
| g8g9g10 vs g1g2g3g4g5g6g7 | 5.41 vs 4.46  | 18.70** |             |              |
| g1 vs g2g3g4g5g6g7  | 4.65 vs 5.02  | 2.76ns  |              |              |
| g2 vs g3g4g5g6g7    | 4.58 vs 5.10  | 5.39*   |              |              |
| g3 vs g4g5g6g7      | 4.12 vs 5.35  | 28.93** |              |              |
| g4 vs g5g6g7        | 4.40 vs 5.67  | 28.75** | 4.41         | 8.29         |
| g5 vs g6g7          | 6.33 vs 5.33  | 15.85** |              |              |
| g6 vs g7            | 5.32 vs 5.34  | 0.00ns  |              |              |
| g8 vs g9g10         | 6.50 vs 4.86  | 43.01** |              |              |
| g9 vs g10           | 4.98 vs 4.74  | 0.71ns  |              |              |

| Number of spikelet per panicle |         |         |              |              |
| g8g9g10 vs g1g2g3g4g5g6g7    | 9.80 vs 9.00 | 123.18**|              |              |
| g1 vs g2g3g4g5g6g7           | 8.67 vs 9.06 | 0.11ns  |              |              |
| g2 vs g3g4g5g6g7             | 8.06 vs 9.26 | 0.97ns  |              |              |
| g3 vs g4g5g6g7               | 7.04 vs 9.81 | 5.00*   |              |              |
| g4 vs g5g6g7                 | 7.47 vs 10.59| 5.96*   | 4.41         | 8.29         |
| g5 vs g6g7                   | 11.33 vs 10.23| 0.65ns |              |              |
| g6 vs g7                     | 10.41 vs 10.05 | 0.05ns |              |              |
| g8 vs g9g10                  | 11.95 vs 8.73 | 5.62*   |              |              |
| g9 vs g10                    | 9.32 vs 8.13  | 0.53ns  |              |              |

ns: not significantly different; *: significantly different (p<0.05); **: highly significantly different (p<0.01). g1: Nias350 3.8.9; g2: N350 3.1.3; g3: CB 145; g4: N250 4.2.1; g5: CB 169; g6: N350 3.2.2; g7: N250 4.6.2; g8: Dewata; g9: Munal; g10: Selayar.
The contrast test results in table 1 show that for all parameter, the mutant and convergent breeding (CB) genotypes tested or g1, g2, g3, g4, g5, g6, g7, (Nias350 3.8.9; N350 3.1.3; CB 145; N250 4.2.1; CB 169; N350 3.2.2; and N250 4.6.2) were significantly different compared to the average values of plant height, panicle length and number of spikelet per panicle of the comparison lines, namely g8, g9, and g10 (Dewata, Munal and Selayar). Highest average of plant height parameter was shown by the genotype from convergent breeding (CB 145) with an average plant height of 52.06 cm, which significantly different to average plant height of other mutant (g1, g2, g4, g6 and g7) and convergent breeding lines (g5). The average values for parameters of panicle length and the number of spikelet per panicle shown by the comparison genotypes were significantly higher than the mutant and CB lines. Longest panicle and highest number of spikelet per panicle was shown by Dewata variety which also differed significantly to the average values of these parameters in the other two comparison varieties.

3.2. Productive tillers, flowering and harvest ages

The variance analysis showed that the treatment did not significantly affect the number of productive tillers, flowering age and harvest age. The average value of these parameters of the M5 wheat mutant and CB genotypes compared to the comparison varieties as the response of these genotypes to low altitude growing conditions are shown in figure 1.

Figure 1 shows that the highest number of tillers was 5.17 produced by the Selayar (g10) variety treatment while the lowest number of tillers was produced in the CB 145 (g3) genotype treatment of 3.17 tillers. The earliest flowering age of 52.83 DAP was shown by the N350 3.8.9 genotype (g1) treatment while the latest flowering age of 57.33 DAP was found in the N250 4.6.2 genotype (g7). The earliest harvest age of 48.17 DAP was produced by the Dewata variety treatment (g8) while the N350 3.8.9 and CB 145 genotypes (g1, g3) were found to be the latest of harvesting age.

3.3. Number of empty floret, number of grain per panicle, grain weight per panicle.

Variance analysis showed that the genotype treatment had a very significant effect on the parameters of the number of empty floret, number of grains per panicle and grain weight per panicle. The orthogonal contrast tests of the average values of these parameters are shown in table 2.

Table 2 shows that for parameter of the percentage of empty floret, there was significant difference between the comparison varieties and the mutant and convergent breeding (CB) genotypes. The mutan and CB wheat lines had lower percentage of empty floret compared to the comparison varieties. The lowest average value of the parameter was shown by CB 145 (14.62%). On the contrary, there was no significant difference between the average values of the number of grain and grain weight per panicle shown by the mutant and CB lines compared to the average values of these parameters in the comparison varieties. The CB 169 genotype had the highest number of grain per panicle (8.33 grains), while Dewata variety showed the heaviest grain weight per panicle (0.34 g).
Table 2. Orthogonal contrast test on the mean values of number of empty floret, number of grain per panicle, and grain weight per panicle of M5 wheat mutant and convergent breeding genotypes on low altitude condition.

| Genotype treatments | Mean values | F value | F table |
|---------------------|-------------|---------|---------|
|                     |             |         | 0.05    | 0.01    |
| **Number of empty floret (%)** |             |         |         |         |
| g8g9g10 vs g1g2g3g4g5g6g7 | 21.84 vs 19.53 | 68.90** |         |         |
| g1 vs g2g3g4g5g6g7 | 18.50 vs 19.70 | 4.23ns | 4.41    | 8.29    |
| g2 vs g3g4g5g6g7 | 16.68 vs 20.31 | 37.40** |         |         |
| g3 vs g4g5g6g7 | 14.62 vs 21.73 | 137.90** |         |         |
| g4 vs g5g6g7 | 14.90 vs 24.01 | 1.00ns |         |         |
| g5 vs g6g7 | 25.65 vs 23.19 | 13.78** |         |         |
| g6 vs g7 | 23.22 vs 23.15 | 0.01ns |         |         |
| g8 vs g9g10 | 27.67 vs 18.93 | 174.01** |         |         |
| g9 vs g10 | 20.78 vs 17.07 | 23.56** |         |         |
| **Number of grain per panicle (grain)** |             |         |         |         |
| g8g9g10 vs 1g2g3g4g5g6g7 | 7.56 vs 7.48 | 0.11ns |         |         |
| g1 vs g2g3g4g5g6g7 | 7.50 vs 7.47 | 0.06ns |         |         |
| g2 vs g3g4g5g6g7 | 7.50 vs 7.47 | 0.01ns |         |         |
| g3 vs g4g5g6g7 | 6.50 vs 7.71 | 6.41* | 4.41    | 8.29    |
| g4 vs g5g6g7 | 7.50 vs 7.78 | 0.32ns |         |         |
| g5 vs g6g7 | 8.33 vs 7.50 | 2.54ns |         |         |
| g6 vs g7 | 8.00 vs 7.00 | 2.75ns |         |         |
| g8 vs g9g10 | 8.17 vs 7.25 | 3.08ns |         |         |
| g9 vs g10 | 7.17 vs 7.33 | 0.08ns |         |         |
| **Grain weight per panicle (g)** |             |         |         |         |
| g8g9g10 vs g1g2g3g4g5g6g7 | 0.29 vs 0.29 | 0.86ns |         |         |
| g1 vs g2g3g4g5g6g7 | 0.29 vs 0.29 | 0.02ns |         |         |
| g2 vs g3g4g5g6g7 | 0.28 vs 0.29 | 0.70ns |         |         |
| g3 vs g4g5g6g7 | 0.24 vs 0.30 | 26.39** |         |         |
| g4 vs g5g6g7 | 0.28 vs 0.31 | 5.70* | 4.41    | 8.29    |
| g5 vs g6g7 | 0.33 vs 0.29 | 9.01** |         |         |
| g6 vs g7 | 0.33 vs 0.26 | 18.77** |         |         |
| g8 vs g9g10 | 0.34 vs 0.27 | 25.03** |         |         |
| g9 vs g10 | 0.2 vs 0.27 | 0.19ns |         |         |

ns: not significantly different; *: significantly different (p≤0.05); **: highly significantly different (p≤0.01). g1: Nias350 3.8.9; g2: N350 3.1.3; g3: CB 145; g4: N250 4.2.1; g5: CB 169; g6: N350 3.2.2; g7: N250 4.6.2; g8: Dewata; g9: Munal; g10: Selayar.
3.4. Weight of 100 grains and productivity

Variance analysis showed that the treatment had a very significant effect on the weight of 100 grains and the production per plant. These parameters were varied between the genotypes (Table 3).

Table 3. Orthogonal contrast test on the mean values of the weight of 100 grains and productivity of M5 wheat mutant and convergent breeding genotypes on low altitude condition.

| Genotype treatments | Mean values | F value  | F table | F table |
|---------------------|-------------|----------|---------|---------|
|                     |             |          | 0.05    | 0.01    |
| Weight of 100 grains (g) |             |          |         |         |
| g8g9g10 vs g1g2g3g4g5g6g7 | 4.07 vs 3.70 | 41.51**  | 4.41    | 8.29    |
| g1 vs g2g3g4g5g6g7 | 3.70 vs 3.64 | 0.09 ns  |         |         |
| g2 vs g3g4g5g6g7 | 3.54 vs 3.66 | 0.38 ns  |         |         |
| g3 vs g4g5g6g7 | 3.68 vs 3.66 | 0.01 ns  |         |         |
| g4 vs g5g6g7 | 3.72 vs 3.63 | 0.19 ns  |         |         |
| g5 vs g6g7 | 3.55 vs 3.67 | 0.31 ns  |         |         |
| g6 vs g7 | 3.64 vs 3.71 | 0.07 ns  |         |         |
| g8 vs g9g10 | 4.60 vs 3.94 | 2.98 ns  |         |         |
| g9 vs g10 | 3.92 vs 3.96 | 0.02 ns  |         |         |
| Production per plant (g per plant) | 1.41 vs 1.07 | 16.80**  | 4.41    | 8.29    |
| g8g9g10 vs g1g2g3g4g5g6g7 | 1.23 vs 1.05 | 0.03 ns  |         |         |
| g1 vs g2g3g4g5g6g7 | 0.96 vs 1.06 | 0.01 ns  |         |         |
| g2 vs g3g4g5g6g7 | 0.74 vs 1.15 | 0.15 ns  |         |         |
| g3 vs g4g5g6g7 | 1.05 vs 1.18 | 1.00 ns  |         |         |
| g4 vs g5g6g7 | 1.22 vs 1.15 | 0.00 ns  |         |         |
| g5 vs g6g7 | 1.25 vs 1.05 | 0.02 ns  |         |         |
| g6 vs g7 | 1.57 vs 1.32 | 0.05 ns  |         |         |
| g7 vs g10 | 1.23 vs 1.41 | 0.02 ns  |         |         |

ns: not significantly different; *: significantly different (p≤0.05); **: highly significantly different (p≤0.01). g1: Nias350 3.8.9; g2: N350 3.1.3; g3: CB 145; g4: N250 4.2.1; g5: CB 169; g6: N350 3.2.2; g7: N250 4.6.2; g8: Dewata; g9: Munal; g10: Selayar.

Table 3 reveals that production component parameters of the wheat mutant and CB lines were significantly different compared to the comparison varieties. However, when comparing within the mutant and CB lines, there was no significant difference found either for the weight of 100 grains or the production per plant parameters. Highest 100 grains weight and production per plant was shown by the N250 4.2.1 (g4) and N350 3.2.2 (g6), respectively.

4. Discussion

The results of variance show that the genotypes treatment had a significant effect on plant height, panicle length, number of empty floret, number of spikelets, number of grain, and grain weight per panicle, weight of 100 grains and production per plant. The results of orthogonal contrast analysis also show that generally these parameters values were significantly different between the comparison varieties and both the mutant and CB lines. This shows that the selected strains have quite wide diversity and can produce the best strains from several genotypes such as plant height character. Plant height character is a part that is very related to the absorption of light, so that wheat plants that have tall stems get more light so that physiological and biochemical processes take place more leverage than plants with shorter stems.
This is according to the opinion of Sarlikioti [7] which states that the higher the plant, the more light can be absorbed, hence can lead to optimal photosynthesis.

The results showed that the Dewata variety produced the longest panicle length (6.50 cm) and differed significantly with respect to all strains planted, the results also showed that there were very significant differences between varieties with an average panicle length (5.41 cm) and strains that produced average panicle length (4.46 cm). This is because the length of panicle growth produced by each line planted is very diverse. Panicle length is a parameter that can support the high and low productivity of wheat plants. This is consistent with the opinion of Alnopri [8] that panicle length is a parameter that supports high and low productivity. The longer the panicle size, the greater the chance of the amount of grain being formed.

Orthogonal contrast test results on the parameters of the number of empty florets indicate that there were significant differences between the comparison varieties (Dewata, Munal, and Selayar) which produce an average number of empty floret (21.84%) and the mutant/CB lines that produce lesser average number of empty florets (19.53). CB 145 lines produce the lowest number of empty florets (14.62) compared to other lines. This shows that the mutant had a better genetic trait in the number of empty floret parameters. The number of empty florets is an adverse character because it causes a decrease in yield on wheat. The number of empty floret indicates that there is a failure rate of fertilization in wheat plants, causing failure to form seeds. The number of empty florets is caused by the stunting phase of many wheat plants which are sterile due to high temperatures and irradiation of plants is also high. In the lowlands is also characterized by high sun intensity so that the plants fail to be fertilized [9, 10].

Orthogonal contrast test results on the weight of 100 grains parameter showed a very significant difference between the comparison varieties (4.07 g) and genotypes tested that only produced an average weight of 100 grains of 3.70 g. Dewata variety produced the heaviest of 100 grains (4.60 g) and N350 3.1.3 strain produced the lowest weight of 100 grains (3.54 g). on the parameter of production per plant, there was a very significant difference between the comparison varieties (1.41 g) and the tested genotypes which only produce an average production per plant (1.07 g). The difference in the character of seed weight and production of a plant is strongly influenced by genetic characters. In addition, the gene potential of a plant will be maximized if it is supported by optimal environmental conditions. Internal factors that stimulate plant growth are in genetic control, but climate, soil and biological elements such as pests, diseases and weeds and competition between species and outside species also influence it [11].

5. Conclusions
Based on the results of research that has been done, it can be concluded as follows:

- Dewata, Munal, and Selayar varieties show a better average number of tillers (6.00 tillers), an average weight of 100 grains (4.07 g) and production per plant (1.41 g) compared to the mutant and CB genotypes that produce lower average number of tillers, weight of 100 grains, and production per plant (4.52 tillers, 3.70 g, and 1.07 g, respectively).
- CB 169 genotypes shows better genetic characteristics on the parameter number of grains per panicle. Dewata varieties showed better genetic characteristics on panicle length parameter (6.50 cm) and weight of grains per panicle (0.34 g).
- CB 145 strain shows better genetic characteristic on plant height parameter (52.06) and number of empty florets (14.62%).

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