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Growth and Production of Synthetic Maize Mutants (M3) at Different Water Availability Levels

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Abstract

Drought causes hampered nutrient absorption and water uptake in plants hence abnormal growth and lower yield. One of alternative solutions is the development of maize varieties tolerant to drought stress through plant breeding. This study aims to find superior M3 mutant genotypes of maize that can adapt to dry land with high productivity. The study was conducted in green house of the Faculty of Agriculture, Universitas Hasanuddin using Split Plot Design. Water availability level was set as main plot consisted of 60%, 80% and 100% and maize mutant was set as subplot consisted of four M3 mutants, namely Bisma 100 gy, Bisma 200 gy, Lamuru 100 gy, and Lamuru 200 gy. In addition two varieties were used as controls ie. Bisma, and Lamuru resulted in total of 6 genotypes tested in the study. Mutant genotype of Bisma 200 gy showed tolerance to all water availability levels and significantly differed to other mutants including its parents indicated with higher yield. 100% water availability condition resulted in higher production than the other two water availability conditions with the average yield produced was 44.1 g per plant

Key Words: drought, mutant, synthetic maize, tolerance, water availabity

A. Introduction

Maize is one of the important food crops in Indonesia. In the last five years, corn production in Indonesia has increased from about 19.4 million tons in 2012 to 23.6 million tons in 2016 or 21.6%. Similarly there was a 10.6% increase in the productivity since 2012 (4.80 t / ha) until 2016 (5.31 t / ha). The increase in production and productivity from 2012 to 2016 has a positive implication in the decrease of national corn import volume in the last three years from about 3.3 million tons in 2015 to only about 508 thousand tons by 2017. This shows a significant decrease in corn imports in 2017 since 2015 amounted to 84.43%. Expansion of harvested area cultivated with maize for the last 5 years became one of reasons for the production increase. Data recorded shows the area of land cultivating corn in 2012 was 3.9 million ha and increased to 4.4 million ha by 2016 (Pusat Data dan Sistem Informasi Pertanian, 2017).

Despite this success, the productivity increase of 5.3 t / ha is still lower than the genetic potential of the superior varieties in Indonesia that can obtain 7 - 8 ton per hectare (Aqil M., Rapar, C., & Zubachtirodin, 2012). Efforts in aggregating maize production through expansion of plantation areas are constrained on several issues, including the fact that about 70% of maize crops developed on low productivity land due to environmental stress. According to Efendi R., Sunarti, S., Musa, Y., Farid, M., Rahim, M.D., & Azrai, M. (2015), different agro-ecological conditions such as climate, fertility and pathogenesis present in the particular cultivation environment affect productivity of maize.

The expansion of planting areas to new areas that are generally aimed at the less productive lands due to environmental stresses become the factor inhibiting plant growth. Among the various types of environmental stress, drought stress is the most common stress, both in Indonesia and around the world. This causes corn crops are at greater risk of drought stress.

The drought stress is one of the largest stresses affecting growth and production in agriculture lands around 26% (Kalefetoglu & Ekmekci, 2005) with decreasing yield of maize in the tropics around 17-60% (Monneveux, P., Sa¡nchez, C., Beck, D., & Edmeades, G. O., 2005). Drought stress due to limited and uncertain availability of water on agricultural land due to climate anomalies (global warming), limited water supply to agricultural land as it competes with the water demand in industrial and residential sectors. In Indonesia, the cultivation of maize is mostly done in dry land and rainfed rice fields of about 79% and 10% respectively (Kasryno, 2002). Common obstacles encountered on these lands are limited water availability and less fertile land (Monneveux *et al.,* 2008). At certain moments the corn crops are even experiencing drought stress that causes the greater decrease in yield. The results of the study Banzinger M., Edmeades, G.O., Beck, D., & Bellon, M. (2008), stated that drought stress resulted in 80 - 100% yield decline.

Drought stress causes hindered nutrient absorption and water uptake causing abnormal growth and decreasing yield. Corn is very sensitive to drought stress especially in the period of 1 week before up to 2 weeks after flowering. Drought during this period will cause the plant to experience an increase in ASI (Anthesis Silking Interval) so that pollination is not synchronized (Edmeades, G.O., Bolanos, J., & Lafitte, H.R., 1992 ; Banziger *et al.,* 2008), hence the formation of seeds that are not optimal even none of the seeds are formed due to the presence reduction in photosynthesis results (Zinselmeier C., Westgate, M. E., & Jones, R.J., 1995; Schussler & Westgate, 1995).

One alternative solution to the problem is to develop corn varieties that are tolerant of drought stress through plant breeding to support the utilization of lands that have limited water availability. Development of varieties that are tolerant to environmental stress can be done through mutation techniques using gamma-ray radiation. This method used to select several mutant genotypes that are expected to arise as a candidate for new superior varieties that is tolerant to drought and able to produce high yield. At the further stage, selection stages should be carried out by testing the mutants at some levels of drought conditions.

B. Methodology

The study was carried out in Greenhouse of Faculty of Agriculture, Universitas Hasanuddin. The experiment was set in pot trial based on Split Plot Design with water availability level set as main plot and maize mutants as sub plots. Three water levels represent the water availability conditions in the field were used and maintained during the study from planting to harvest namely 60% (A1), 80% (A2) and 100% (A3) of water supply. Maize mutants used in the study was obtained from previous trial. Four mutant genotypes selected were Bisma 100 gy (M1), Bisma 200 gy (M2), Lamuru 100 gy (M3), and Lamuru 200 gy (M4). In addition two varieties of each parental mutant genotypes, Bisma (M5), and Lamuru (M6), were used as controls resulted in total of six genotypes used. Each treatment was repeated three times with 3 plant units. Thus there were 3 levels of water availability and 6 maize mutants that replicated 3 times resulted in total of 162 units of experiments.

The experiment used 10 kg of soil media per bucket to be filled with water until a predetermined concentration. Seeds from each mutant who passed the selection during the previous planting season was germinated. Determination of the extent of water availability was conducted using a weighing method based on field capacity.

$$WC_{ad} = \frac{a-b}{b} x100\%$$

$$WC_{fc} = \frac{c-d}{d} x100\%$$

$$SW_{fc} = w\, x \frac{(100 + WC_{fc})}{(100 + WC_{ad})}$$

$$Vol = SW_{fc} - w$$

Notes:
WC_{ad} = air dried water content (%)
WC_{fc} = field capacity water content (%)
SW_{fc} = soil weight at field capacity (g)
Vol = water volume added to reach field capacity (ml)
a = initial air dried soil sample weight (g)
b = air dried soil sample oven weight (g)
c = initial field capacity soil sample weight (g)
d = field capacity soil sample oven weight (g)
w = used air dried soil weight (g)

The observed character data included plant height, number of leaves, number of dried leaves, Anthesis Silking Interval (ASI) and production, were analyzed using the Analysis of Variance (ANOVA) for Split Plot design. The significant effect of treatments was analyzed further using the Least Significance Difference (LSD) test to determine the difference between the tested mutants.

C. Results

1. Plant Height

Analysis of variance results indicate that water availability levels and mutants treatments significantly affect plant height, respectively. Table 1 shows that at 100% water supply (A3) resulted in the best plant height with an average height of 190.09 cm. The mutant genotype that showed the best response was Lamuru 200 gy (M4) with an average value of 185.97 cm although not significantly differed with other genotypes except for genotype Bisma (M5).

Table 1. Mean of maize mutant plant height (cm) on difference levels of water availability.

Genotypes	Water availability levels			Mean	$LSD_{M\,0.05}$
	A1 (60%)	A2 (80%)	A3 (100%)		
M1 (Bisma 100 gy)	149.17	176.00	175.67	166.94^{ab}	28.60
M2 (Bisma 200 gy)	140.83	164.17	172.17	159.06^{ab}	
M3 (Lamuru 100 gy)	143.07	166.70	180.33	163.37^{ab}	
M4 (Lamuru 200 gy)	158.41	177.33	222.17	**185.97^{a}**	
M5 (Bisma)	105.25	163.33	184.67	151.08^{b}	
M6 (Lamuru)	139.50	161.17	195.17	165.28^{ab}	
Mean	137.41^{y}	166.54^{xy}	**190.90^{x}**		
$LSD_{A0.05}$		29.98			

Values followed by same letter in the same column (a, b) and the same row (x, y) are not significantly different (p=0.05).

2. Number of Leaves

Analysis of variance results indicates no significant effects of all treatments on the number of leaves of the maize genotypes. Figure 1 shows that the highest leaf numbers showed by the genotypes of Bisma 200 gy (M2) mutant and Lamuru (M6) at 60% of water availability with an average value of 15.67 and 15.33 leaves, respectively. While the lowest number of leaves is shown by Bisma 100 gy mutant (M1) on water availability of 80% and 100% with an average value of 13.67 leaves.

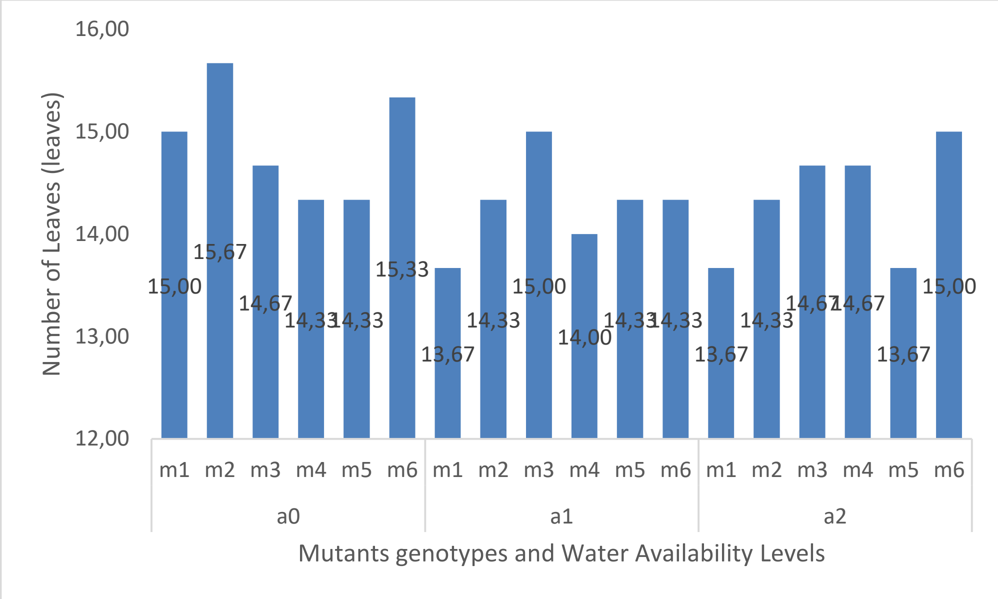

Figure 1. Number of leaves of Maize mutant genotypes at difference levels of water availability (M1: Bisma 100 gy mutant; M2: Bisma 200 gy mutant; M3: Lamuru 100 gy mutant; M4: Lamuru 200 gy mutant; M5: Bisma and M6: Lamuru; A0: 60%, A1: 80% and A3: 100% water availability)

3. *Number of dry leaves*

Water availability and mutant treatments had a very significant effect on the number of dry leaves of maize plants, whereas no significant interaction between mutant and water availability level treatments. Table 2 shows that at 60% water supply (A1) resulted in the lowest number of dry leaves with an average dry leaf number of 5.67 leaves which is not significantly different with dry leaves number at 80% water availability condition (5.87 leaves). The mutant treatment that showed the best response was M2 with an average value of 4.78 leaves and significantly different with the other mutant treatment.

Table 2. Mean of number of dry leaves (leaves) of the maize mutant on difference levels of water availability.

Genotypes	Water availability levels			Mean	$LSD_{M\ 0.05}$
	A1 (60%)	A2 (80%)	A3 (100%)		
M1 (Bisma 100 gy)	6.33	6.00	6.00	6.11^{b}	1.03
M2 (Bisma 200 gy)	4.33	4.67	5.33	**4.78^{a}**	
M3 (Lamuru 100 gy)	6.67	7.33	7.00	7.00^{b}	
M4 (Lamuru 200 gy)	5.33	5.67	8.33	6.44^{b}	
M5 (Bisma)	6.67	6.00	7.00	6.56^{b}	
M6 (Lamuru)	5.33	5.67	7.67	6.22^{b}	
Mean	**5.67^{x}**	**5.87^{x}**	7.07^{y}		
$LSD_{A\ 0.05}$		0.454			

Values followed by same letter in the same column (a, b) and the same row (x, y) are not significantly different (p=0.05).

4. *ASI (Anthesis Silking Interval)*

Analysis of variance results indicate that treatment of water availability levels and mutants had a significant effect on the ASI of the maize mutants. Table 3 shows that earlier ASI was obtained at 100% water supply (A3) with an average of 4.56 days and was significantly different from other genotypes. The mutant treatment that showed the best response on ASI parameter was Bisma 200 gy (M2) with an average value of 4.11 days and was not significantly different with Lamuru 100 gy (M3) and Lamuru 200 gy (M4) mutants.

Table 3. Mean of ASI (*Anthesis Silking Interval*) (days) of maize mutant on difference levels of water availability.

Genotypes	Water Availability Levels			Mean	$LSD_{M\ 0.05}$
	A_1 (60%)	A_2 (80%)	A_3 (100%)		
M1 (Bisma 100 gy)	6.00	6.00	5.00	5.67^{b}	1.46
M2 (Bisma 200 gy)	3.33	5.00	4.00	**4.11^{a}**	
M3 (Lamuru 100 gy)	7.00	5.67	4.00	5.56^{ab}	
M4 (Lamuru 200 gy)	6.33	6.33	2.67	5.11^{ab}	
M5 (Bisma)	7.00	7.00	5.67	6.56^{b}	
M6 (Lamuru)	6.67	6.67	6.00	6.44^{b}	
Mean	6.06^{y}	6.11^{y}	**4.56^{x}**		
$LSD_{A\ 0.05}$		1.05			

Values followed by same letter in the same column (a, b) and the same row (x, y) are not significantly different (p=0.05).

5. Production

Water availability level and mutants interacted significantly on affecting the production of maize genotypes tested in the recent trial. Table 4 shows that at 100% water availability condition, the genotype of Lamuru (M6) gave the best crop production with an average of 50.96 g per plant and is not significantly different from the production of Bisma 200 gy and Lamuru 200 gy mutants at the same water availability level. At lower water availability level, highest production was shown by Lamuru 100 gy (M3) and Bisma 200 gy (M2) mutants obtained when water condition was at 80% and 60% water supply, respectively. At 80% water availability level, production of Lamuru 100 gy mutants (M3) was not significantly differed with the production of Bisma 200 gy (M2), while at 60% of water availability, Bisma 200 gy mutant (M2) produced higher grain compared to other mutants even when compared to its parent (M5).

Table 4. Mean of plant production ($g.plant^{-1}$) of maize mutants on difference levels of water availability.

Genotypes	Water Availability Levels			$LSD_{M\ 0.05}$
	A_1 (60%)	A_2 (80%)	A_3 (100%)	
M1 (Bisma 100 gy)	23.17$\frac{y}{b}$	6.50$\frac{z}{d}$	36.69$\frac{x}{b}$	7.23
M2 (Bisma 200 gy)	**32.65**$\frac{z}{a}$	41.00$\frac{y}{a}$	**55.93**$\frac{x}{a}$	
M3 (Lamuru 100 gy)	10.80$\frac{y}{c}$	**42.63**$\frac{x}{a}$	37.88$\frac{x}{b}$	
M4 (Lamuru 200 gy)	6.52$\frac{y}{c}$	4.02$\frac{y}{d}$	27.63$\frac{x}{c}$	
M5 (Bisma)	23.22$\frac{y}{b}$	14.33$\frac{z}{c}$	**51.36**$\frac{x}{a}$	
M6 (Lamuru)	21.10$\frac{y}{b}$	23.17$\frac{y}{b}$	**56.97**$\frac{x}{a}$	
$LSD_{A\ 0.05}$		7.26		

Values followed by same letter in the same column (a, b) and the same row (x, y) are not significantly different (p=0.05).

D. Discussion

Maize mutants differed significantly on plant height, number of dry leaves, ASI (Anthesis Silking interval) and average production while character number of leaves shown no difference. The best results in plant production showed by irradiated maize mutant Bisma 200 gy, is supported by growth characteristic components such as leaf number, ASI (Anthesis Silking Interval) and number of dried leaves.

Differences in mutant responses to the given treatments primarily on the same cultivar origin but different radiation doses indicated that changes has occurred after mutation. Radiation with gamma rays can cause mutations. The mutations in plants causes a change in gene arrangement, hence causing differences in appearance of each character in plants. According to Wiryosimin (1995), gamma ray irradiation as a mutagen will break the DNA strands. Termination of DNA due to radiation results in changes in the formed amino acids and proteins, which will eventually lead to mutations. The above opinion in accordance with the opinion Sitompul & Guritno (1995), states that the genetic factors of plants is one cause of differences between plants with each other.

The observations on characters correlated significantly with the plant production showed that Bisma 200 gy (M2) genotype tended to show the best results. The mutant treatment showed high characteristic of leaf number, even when water supply decreased to 60% of water available, compared to other mutants and its parent. Therefore, the amount of stomata contained in the Bisma 200 gy mutant leaves were also higher resulting in higher H_2O and CO_2 exchange activities. High transpiration causes a high water loss that affects plant physiological activity resulting in a decrease in production. According to Harjadi & Yahya (1988) and Qing, ZM., Jing, L.G., & Kai, C. R. (2001), the amount of water used by plants is related to photosynthetic activity and dry weight of production. Drought stress affects plant physiology activities including radiation capture, leaf temperature, stomatal conduction, transpiration, transport electron, photosynthesis and respiration that can decrease production (Qing *et al.,* 2001).

Differences in mutant responses to treatments were given primarily on the same cultivar origin but different radiation doses indicated that changes occurred after mutation. Radiation with gamma rays can cause mutations. The occurrence of mutations in plants causes a change in gene arrangement, causing differences in appearance of each character in plants. According to Wiryosimin (1995), gamma ray irradiation as a mutagen will break the DNA strands. Termination of DNA due to radiation causes changes in amino acids and proteins that form, which will eventually lead to mutations. The above opinion in accordance with the opinion Sitompul & Guritno (1995), states that the genetic factors of plants is one cause of differences between plants with each other.

Growth components such as number of leaves show that Bisma 200 gy (M2) that tended to give the best results on water availability of 60% indicate that Bisma 200 gy is more tolerant to drought stress than other genotypes. This is in line with the limits on drought resistance based on breeding aspect and objectives proposed by Murdiyato & Sugiyarto (1992), that crop resistance to drought as the ability of a plant genotype to become more productive to produce more yield than other genotypes.

Corn plant is very sensitive to water availability especially in the period of 1 week before up to 2 weeks after flowering. The best ASI (Anthesis silking interval) value at the 60%, 80% and 100% water supply were Bisma 200 gy (M2). The ASI characteristic shown by the mutant was strongly correlated with the successful of grain formation, hence produce high yield. Genotypes experiencing drought in this period will experience an increase in ASI causing unsynchronized pollination (Edmeades *et al.,* 1992; Banziger *et al.,* 2008) leading to unoptimal even to no grain formation by the reduction of photosynthesis (Zinselmeier *et al.,* 1995; Schussler & Westgate, 1995).

Production is a character that determines the plant tolerance to low water availability. The Bisma 200 gy mutant (M2) produced high yield at 60%, 80% and 100% water supply treatments. High production is strongly influenced by the character of leaf number and ASI (Anthesis Silking Interval), two characters that related to grain formation and filling process mainly use assimilate synthesized in photosynthesis. The ability of the Bisma 200 gy mutant against a wide range of water availability helps the plant in reducing high air circulation in the leaves. According to Earl & Davis (2003), reported that drought stress reduces maize yields caused by three factors, namely (1) decreased canopy absorption in the event of active photosynthetic radiation (PAR), (2) decreased radiation efficiency, while leaves are heavily weightened resulting in prematurely senesence and reduction resulting in the entire canopy obtaining an exchange of CO 2 by absorbing PAR, (3) decreasing the yield index. Short periods of stress that coincide with the critical period will affect the rate of development of plant tissue. Water stress can prevent fertilization due to a decrease in the seed water potential so that the seed growth stops prematurely. Similarly, on soil conditions with high salt content.

E. Conclusion

1. Bisma 200 gy mutant shows tolerance to water availability of 60%, 80% and 100% and were different significantly from other mutants including its parent, which are shown in the production.
2. 100% water availability provides more production than water availability of 60% and 80% with an average of 44.1 g per plant.

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