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Performance of selected maize inbred lines to drought stress

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Abstract

Drought stress limits maize production. We studied the drought stress tolerance level of maize inbred lines to select potential parental lines for hybrid production. Glasshouse scale pot experiment was arranged in Completely Randomized Design with eight inbred lines as the treatment. Each inbred line was placed in two conditions; normal and drought stress with three replications. Drought stress condition was achieved by suspending water supply when 50% of the plants had approached V11 phase where maize has grown 11 open leaves, until one plant in the same inbred lines shown heavy withered response. Results showed that line of Gg44 consistently revealed the lowest increase of leaf rolling score (3.8%), the lowest reduction of plant height (3.9%) and plant top dry weight (2.3%) on drought stress condition. The line of Gg44 also produced the lowest stress sensitivity indices (SSI) and the highest stress tolerance indices (STI) for these three traits indicating high tolerance of the line to drought stress. Hence, the line has the potential use in producing maize hybrids that are able to alleviate the negative impacts of drought and high temperature on its growth.

Keywords: Inbred lines, Selection criteria, Drought stress, Tolerant

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Introduction

Maize is one of the major cereal crops in the world that has increased rapidly in area, production, and productivity during the last decade (FAOSTAT, 2016). Maize is regarded as an important commodity in Indonesian economy and food security because of the high demand for foods, feeds and raw materials for industrial use (Indonesia Investment, 2015). Integrated efforts to increase national maize production are inevitable to achieve national food security and food sovereignty. The government has made significant advances in hybrid maize research and development. Approximately more than 35 hybrids are planted on more than 85% of the total maize area (Andayani et al., 2018). But, the maize yield is poorly affected by drought stress (Tai et al, 2011) which is one of the main constraints of maize production in Indonesia (ICERI, 2004). Recently, hybrid maize varieties were tolerant of environmental stress, especially tolerate drought stress, have not been widely released.

Drought stress refers to the influence of environmental factors which cause water to be less available for plants. Drought can be defined as the level of dryness compared to the normal amount of water or the average rainfall at some places, thus it affect maize production (Bänziger et al, 2000). Planting of drought-tolerant maize on certain areas which often experience drought stress can overcome the problem of harvest failure. Drought stress blocks water uptake and nutrient absorption, resulting in abnormal growth and loss of yield. When occurs during germination, drought stress causes a decreasing of rate and percentage of germination (Willanborb et al., 2004). On the stage of flowering, drought may cause enlargement of ASI (Anthesis-Silking Interval) due to the delayed of silk growth. Drought also causes no seeds formed due to the reduction of photosynthesis products (and kernel and ear abortions (Bolaños and Edmeades, 1996).

Plants show certain symptoms as a direct response to drought. This symptom is an important trait to determine the drought tolerance level of a genotype. The availability of inbred lines that are tolerant to drought is prerequisite in production of drought-stress tolerance hybrids. This research was conducted to observe the level of tolerance of eight maize inbred lines to drought stress. Selection of parental inbred lines and determining traits used as selection criteria on obtaining inbred lines that are drought-stress tolerant is an important key for the success of drought tolerance hybrids breeding.

Material and Methods

Experimental design

The study was conducted in the glasshouse of the Faculty of Agriculture, Universitas Andalas, Padang, from August to December 2014. Temperature daily of the glasshouse was 38.3±3.8^{o}C, ranged from 28 – 44^{o}C, while relative humidity was 54.0±6.8. Two series of pot experiments, normal and drought-stress were conducted in a Completely Randomized Design (CRD) with three replications for both stress and normal condition. Each replication contained two pots per genotype. For normal moisture condition experiment, water was administered to reach 75% field capacity. The drought stress condition was achieved by discontinuing water supply when 50% of plants have approached V11 phase. V11 was the vegetative phase where maize has grown 11 open leaves and the most susceptible phase to drought stress (Ransom, 2013). Watering was stopped until one of plants in the same genotypes showed heavy-withered response. After the drought-stress phase, water supply was reverted to normal.

The genotypes evaluated were eight maize inbred lines from different base population of tropical maize hybrids *i.e.* P51, P3, BM40 and BC47, and composite varieties *i.e.* Gg44, Gg53, SgB24, and CL52. The selected inbred lines were those that showed good performance and high yield on field evaluation (Dewi-Hayati and Nazir, 2011). Each line was grown in plastic pots which were containing 10 kg of Inceptisol of soil. The pot size was 20 cm in height, while 20 and 30 cm in beneath and upper diameter, respectively. Into the soil, 150 kg N, 100 kg P_2O_5 and 100 kg K_2O per hectare were added in the form of urea, SP36 and KCl. Other cultivation practices were in accordance with the recommended standards of technical culture.

Data collection

Data collected were days to tasseling, leaf rolling score, proline content, plant height, plant top dry weight and root dry weight. Days to tasselling was observed as the number of days from planting to the day when the tassel shed pollen. Leaf rolling score (1 – 5) was determined in the morning when plant(s) showed severe rolling symptoms due to watering discontinuation. The score of 1 was given when leaf was on turgid condition and no rolling was observed, while severely rolled leaf was given the score of 5 (Bänziger et al., 2000). Plant height was measured from stem base in the soil surface to the base of tassel branch. Plant top and root dry weight were recorded after drying in the oven at 80^{o}C for 48 hours. Measurement of proline content was as described by Bates et al. (1973). Proline content was determined as mg/g of fresh leaf weight as formulae:

$$Proline\ content = \frac{Absorbance\ x\ ml\ Toluene}{Molecular\ weight\ of\ Proline\ x\ leaf\ fresh\ weight}$$

Tolerance indices to drought stress was assessed by comparing performance difference between series of with and without drought-stress using Stress Sensitivity Indices (SSI) based on Fischer and Maurer’s (1978) and Stress Tolerance Indices (STI) based on Fernandez (1992).

$$SSI = \frac{[1 - \frac{Es}{En}]}{[1 - \frac{\overline{Es}}{\overline{En}}]}$$

$$STI = \frac{(En)(Es)}{\overline{En}^2}$$

where En and Es represent performance of inbred line under normal and stress conditions, respectively, while $\bar{E}$n and $\bar{E}$s is mean performance of all inbred lines under normal and stress conditions, respectively.

Statistical analysis

Analysis of variance for each trait within drought stress and non-stress condition was determined using the General Linear Models (Proc GLM). The means of differences for traits were performed using Duncan test at 5% significance level. The data in both series of with and without drought-stress were compared in *t* test at 5% level. Correlation analysis was employed to determine association among the traits. All statistical analysis was computed using SAS software (SAS/STAT®, 2003).

Results and Discussion

Performance of traits among inbred lines

Drought stress is one of major environmental factors that limit plant productivity severely. Maize as a major cereal crops is a very sensitive to drought. Analysis of variance revealed significant differences for days to tasseling, leaf rolling score, plant height, plant top dry weight and root dry weight in response to drought, while in normal condition, significant differences were observed for days to tasseling, plant height, plant top dry weight and root dry weight (Table 1). [Table 1]

Anthesis was delayed on plants in drought stress condition, ranged from 4 – 9 d or 6.2 – 12.8%, while the days to tasseling of two lines *viz*. CL52 and Gg53 became insignificantly shorter for 1 – 3 d (Table 1). Data of days to silking was not able to collect under drought stress condition due to mostly plants in all inbred lines evaluated produced ear without silk protrusion. Traore et al. (2000) reported the delayed of tasselling to 3 d on the Bt maize under the drought stress condition. However, some inbred lines in this study *viz*. CL52 and Gg53 showed a faster emergence of tassel in drought stress condition. The earlier tasseling of CL52 and Gg53 in drought stress condition indicated that somehow this condition initiated anthesis. The genetic makeup of the lines may be responsible for the characteristic.

On drought stress condition, all the evaluated lines could only produce small ear which almost all failed to grow silk. This is in agreement with Bänziger et al. (2000) who reported that silks are more seriously damaged than tassels. Oury et al. (2016) detected that rate of silk growth decreased in drought condition and stopped 2 to 3 d after first silk emergence. With the exception for line CL52 and Gg53, on normal moisture condition plants were still able to produce the silks at 2 to 7 days after anthesis, however, the pollen to pollinate the silks was not adequate. Asynchronous flowering was happened due to drought imposed. Moreover, drought stress and high temperature affect pollen fertility (Saini and Westgate, 2000), pollen abortion (Andersen et al., 2002) and even mortality (Aylor, 2004). Hence, there was no grain kernel produced on both conditions in this study.

Maize leaves will roll if the rate of water loss through transpiration is higher than water absorption from the root (Bänziger et al., 2000). Plants roll their leaves as a way to survive the limited water condition. In normal conditions, the leaf rolling score did not show significant difference. The leaf rolling score ranged from 1 – 2. Score 1 indicated that plant leaves were on turgid condition, while score 2 indicated that plants were in a light stress which would be recover by watering. Score 2 was possibly also as a response of plants to high temperature at glass house.

Leaf rolling responses were observed increased in all lines which imposed to drought. Drought stress by discontinuing of water supply on V11 phase caused leaf rolling with the score ranged from 2 – 3, *viz*. the level of leaves-rolling increased by 3.3 – 24.8% (Table 1). Line Gg44 and BC47 showed the lowest increase of leaf rolling score (3.8 and 3.3%, respectively) compared to the other lines, indicated that the two lines were more tolerant to drought.

Proline content is an indicator of plant stress which increases proportionately in water stressed condition. Both in normal and drought stress conditions, the proline content produced by plant did not show significant difference, indicating that the inbred lines evaluated produced similar content of proline. Several lines *viz*. BC47, CL52 and SgB24 on the contrary produced insignificantly lower proline content in drought stress condition compared to that in normal moisture condition.

High temperature of experimental house (38.3ºC, with range 28 – 44ºC) possibly had effect to high proline content on plants in normal moisture condition. Tandzi et al. (2019) imposed maize at high temperature regime (40ºC/25ºC) for 3 days/nights to screen maize inbred lines for tolerance to combined stresses *i.e.* drought and heat stress. Hence, the inbred lines may be imposed to combined stresses in this study.

Table-1: Days to tasseling, leaf rolling score, proline content, plant height, plant top dry weight and root dry weight of maize inbred lines in drought and normal conditions

Inbred lines	Drought stress condition	Normal condition	*t* test	Drought stress condition	Normal condition	*t* test	Drought stress condition	Normal condition	*t* test
	-------Days to tasseling -------			---- Leaf rolling score# -----			--- Proline content (mg.g^{-1}) ---		
Gg44	68.7±1.2 b	62.7±1.15 e	*	1.83±0.29 c	1.67±0.3 a	ns	0.322±0.25 a	0.250±0.08 a	ns
BC47	74.0±1.0 a	69.7±0.58 cd	*	2.0±0.50 bc	1.83±0.3 a	ns	0.254±0.07 a	0.311±0.10 a	ns
P51	76.0±2.6 a	70.0±2.0 c	ns	2.2±0.28 bc	1.50±0.5 a	ns	0.190±0.09 a	0.196±0.03 a	ns
P3	73.7±1.5 a	67.3±0.58 d	*	2.8±0.28 c	1.67±0.6 a	*	0.233±0.02 a	0.220±0.10 a	ns
Cl52	74.3±3.1 a	77.3±1.53 a	ns	2.8±0.28 c	2.0±0.0 a	*	0.199±0.05 a	0.169±0.07 a	ns
BM40	76.0±2.7 a	69.7±1.15 cd	*	2.8±0.28 c	2.0±0.0 a	*	0.462±0.26 a	0.238±0.05 a	ns
Gg53	74.3±3.1 a	75.0±1.73 b	ns	2.0±0.0 bc	1.67±0.6 a	ns	0.439±0.00 a	0.333±0.09 a	ns
SgB24	76.3±2.3 a	67.7±0.58 cd	*	2.5±0.0 ab	2.0±0.0 a	ns	0.253±0.01 a	0.296±0.06 a	ns
	------- Plant height ------			---- Plant top dry weight -----			--------- Root dry weight -------		
Gg44	145.0±9.6 a	149.8±3.1 b	ns	15.6±0.20 a	15.9±0.24 b	ns	2.5±0.28 bcd	3.49±0.39 bc	ns
BC47	131.5±6.3 ab	135.1±3.7 d	ns	7.3±0.46 c	10.3±1.09 d	*	1.4±0.32 d	1.87±0.16 e	ns
P51	128.7±12.6 ab	142.7±3.8 bc	ns	13.0±0.70 ab	17.2±1.22 ab	ns	2.0±0.72 cd	4.02±0.49 ab	*
P3	115.2±15.5 b	124.2±1.5 e	ns	7.2±0.56 c	12.0±0.87 c	*	1.6±0.33 cd	4.59±0.17 a	*
Cl52	92.4±8.2 c	100.1±2.6 f	ns	7.0±1.45 c	9.9±0.07 d	ns	1.7±0.49 cd	1.23±0.08 e	ns
BM40	128.2±2.4 ab	138.6±5.4 cd	ns	12.0±2.37 b	17.5±0.58 a	ns	3.4±0.72 ab	2.66±0.15 d	ns
Gg53	140.4±3.6 a	147.1±4.1 b	ns	13.7±1.89 ab	15.8±1.19 b	ns	2.6±0.33 abc	2.80±0.25 cd	ns
SgB24	147.2±15.2 a	158.6±1.8 a	ns	14.2±2.47 ab	16.2±0.41 ab	ns	3.5±0.95 a	3.10±0.81 cd	ns

Means with the same letter in each column are not significant (P≥0.05) based on Duncan's Multiple Range Test
ns and * = non significant and significant at P<0.5 based on *t* test
#data transformed by√(x+0.5)

The tolerance inbred line to drought may also be tolerant to high temperature.

Proline accumulation on leaves is a response to drought stress condition and one of the resistance mechanisms to drought stress. Proline level increases with the length of stress as an indication of tolerance to drought stress because it serves as a repository of N compounds and an osmo-regulator which functions as certain enzyme protectors in stress condition. Proline also helps to increase water holding capacity on drought stress condition (Yoshiba et al., 1997). Similar result with the increased of proline content is reported by Sanchez et al. (1998) in pea, spinach and tomato (Umbebese et al., 2009) and maize (Heidari and Moaveni, 2009). However Hanson (1980) stated that the accumulation of proline is not an adaptation to the stress but indicated the accumulation of proline which is only a symptom of stress.

Plant height of each inbred lines varied on each soil moisture condition. Ranking of genotypes within normal moisture and drought stress conditions was relatively similar. The *t*-test showed that the height between drought stress lines and the normal ones were non-significantly different, indicating drought stress did not significantly affect reduction of plant height. Height reduction among the inbred lines was only range from 2.6 – 9.8% (Table 1). The highest height reduction was observed on P51 with 9.8%, while two lines *viz.* BC47 and Gg44 showed the lowest and similar height reduction (2.6%), indicating that these two lines were more tolerant to drought compared to other inbred lines.

Growth and development of vegetative parts of maize are very affected by the decreasing of cell division and proliferation on drought stress condition (Aslam et al, 2015). Traore et al. (2000) found that drought stress condition reduced the height of Bt maize by 15% and leaf area up to 33%. Similarly, Hajibabaee et al. (2012) detected the significant height reduction to forage hybrid maize. Chen et al. (2012) identified that the tolerant inbred lines revealed greater ability to maintain vegetative growth and alleviate damage reproductive tissues under drought condition significantly.

The highest inbred lines in normal condition showed tendency to be the highest in drought stress condition. Similar tendency were also shown by plant top dry weight. However, plant top dry weight reduction varied highly among the inbred lines from 2.3 – 40.1% (Table 1). Line BC47 and P3 showed significantly top dry weight reduction in drought stress condition. The lowest top dry weight reduction was found on Gg44 with only 2.3%, indicating that this line was more tolerant to drought.

The inbred lines showed significant effect on root dry weight within drought and normal condition, however, ranking of genotypes within each moisture condition varied. There are two inbred lines *viz.* P51 and P3 that showed significantly high reduction of root dry weight in drought stress condition. In contrast, line CL52, BM40 and SgB24 showed insignificantly higher root dry weight than that in normal condition. This did not necessarily indicate that these lines tolerate the drought stress, but merely implied that these roots elongate and enlarge better than the other lines.

Different result was reported by Nejad et al. (2010) that root density, volume and number of roots were reduced under severe drought stress. However, according to Dubrovsky and Gomez-Lomeli (2003), the formation of longer and deeper root is one of the drought stress tolerance mechanisms. The increase of dry root weight in the three lines on drought stress condition might be caused by the expansion of root volume, the extension of root length, or by the increasing of root branches. In all of these schemes more water would be absorbed by the root. Plants are equipped with the ability to expand their roots to the deeper soil layer with higher moisture than soil surface, therefore the roots have bigger chance to absorb more water.

Correlation among traits under drought and normal conditions

Correlation analysis was used to determine the association among traits within drought stress and normal conditions, and traits between drought and normal conditions based on Welcker et al. (2005) to identify tolerant genotypes under stress condition based on their performance on normal condition (Table 2).

Plant height showed significant and positive correlations with plant top dry weight (r=0.62) and root dry weight (r=0.48) on drought stress condition, indicating that taller plants tended to have heavier biomass. Hence, significant and positive correlation was found between plant top dry weight and root dry weight (r=0.58). A bigger root system is important to absorb water and nutrients from the soil, then are transported to the leaves for the photosynthesis process to produce assimilates for plant growth viz. plant height and top dry weight. Similar association between plant height and plant top dry weight (r=0.73) and root dry weight (r=0.41) was also found on normal moisture condition.

Plant height was negatively correlated with leaf rolling score (r=-0.44) on drought stress condition, indicating that taller plants are faster to roll. Rolling is a mechanism of plants to reduce transpiration. Leaves rolling are a way of plant to survive drought stress imposed. Rolling reduce the ability of the leaves to generate assimilates for plant growth because of the increasing evapo-transpiration which decrease the flow of CO_2 into the leaves. Olson et al. (2018) reported that taller plants were more vulnerable to drought stress than the shorter ones.

Table-2: Correlation coefficients between traits evaluated in drought and normal conditions. Correlation coefficients on drought stress (above diagonal), normal conditions (below diagonal), and between similar traits on drought stress and normal conditions (bold, diagonal).

Trait	Plant height	Days to tasseling	Top Dry weight	Root Dry Weight	Leaf Rolling Score	Proline Content
Plant height	**0.83****	-0.16	0.62*	0.48*	-0.44*	0.17
Days to tasseling	-0.53**	**0.32**	-0.13	0.23	0.34	-0.09
Top Dry weight	0.73**	-0.30	**0.77****	0.58**	-0.33	0.39
Root Dry Weight	0.41*	-0.56**	0.44*	**0.08**	0.04	0.37
Leaf Rolling Score	-0.16	0.17	-0.09	-0.37	**0.44***	-0.03
Proline Content	0.33	-0.06	0.33	-0.10	-0.03	**0.35**

*, ** significant at P<0.05 and P<0.01, respectively

Plant height and top dry weight in drought stress condition were shown to have significantly correlation with those in normal moisture (r=0.83 and r=0.77, respectively).

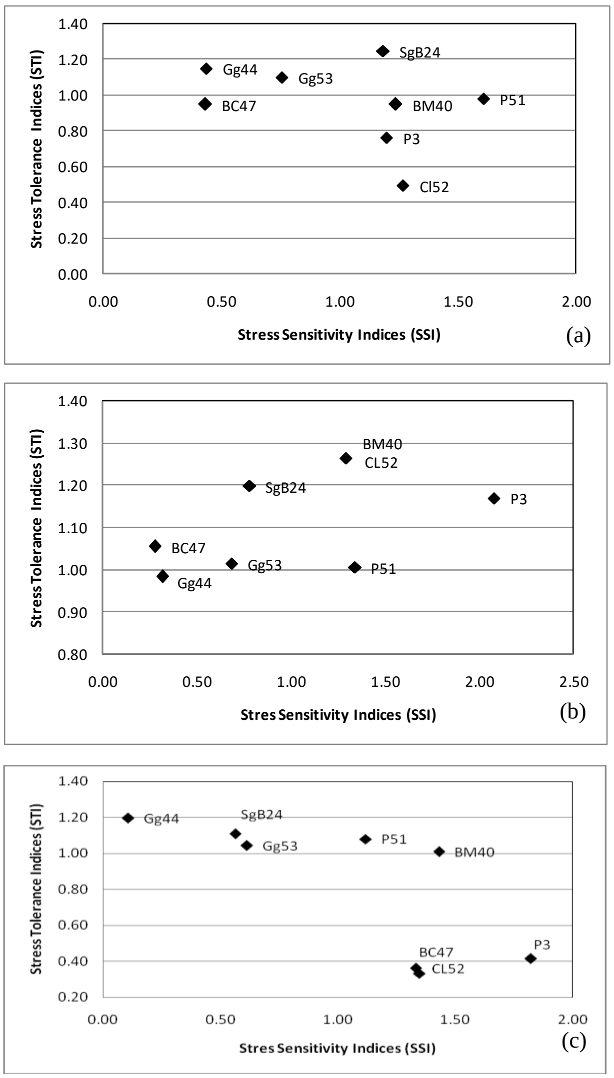

Fig-1: Stress sensitivity indices (SSI) and stress tolerance indices (STI) of maize inbred lines based on: (a) leaf rolling score, (b) plant height, and (c) plant top dry weight

This indicates that the inbred lines which were taller on height and heavier on top dry weight in drought stress condition tended to have similar traits on normal condition and vice versa. Similarly, leaf rolling score in drought stress condition was significantly correlated (r=0.44) with that in normal condition. These results indicate that in general, leaf rolling, plant height and top dry weight could be used as selection criteria to identify tolerant genotypes under their performance on normal condition.

Selection indices

Genotypes with an SSI less than a unit are regarded as stress tolerance, since their yield reduction in stress condition is smaller than the mean yield reduction of all genotypes (Fischer and Maurer, 1978), while genotypes with a higher STI are more tolerant than those with lower STI (Fernandez, 1992). We plotted the two indices as performed in Table 1 to distinguish inbred lines tolerant to drought (Fig. 1). The higher position of inbred lines along the Y-axis and the lower position of inbred lines along the X-axis, the lines will be more tolerant to drought. We used only three traits that showed significantly coefficient correlations between traits in drought and traits in normal condition (Table 2), *viz*. leaf rolling score, plant height and top dry weight.

Based on stress tolerance and stress sensitivity index of leaf rolling score as depicted in the figure (1a), the inbred lines are distributed discretely. Line Gg44 and BC47 showed low SSI and high STI, indicating high tolerance of both two lines to drought stress.

While based on stress tolerance and stress sensitivity index of plant height in the figure (1b), the majority of inbred lines show high SSI, indicating lower tolerant to drought stress condition. However, two lines *viz.* Gg44 and BC47 performed low SSI and high STI, indicating their lower sensitive to drought. Based on plant top dry weight, line P3, CL52 and BC47 have higher SSI and lower STI, indicating high sensitive of these three lines to drought (Fig. 1c), while high stress tolerance index is performed by line Gg44, Gg53 and SgB24. Line Gg44 consistently generated low SSI and high STI of leaf rolling score, plant top dry weight and root dry weight, indicating high tolerance of this line to drought stress condition. Leaf rolling, plant height and top dry weight can be used as criteria of indirect selection of non-stressful moisture condition to obtain a drought tolerant inbred line. It is necessary to confirm the heat stress tolerance of the line due to high temperature condition imposed in this study.

Conclusion

Based on the study, it can be concluded that line of Gg44 consistently revealed the lowest increase of leaf rolling score (3.8%), the lowest reduction of plant height (3.9%) and plant top dry weight (2.3%) on drought stress condition. The line of Gg44 also produced the lowest stress sensitivity indices (SSI) and the highest stress tolerance indices (STI) for these three traits indicating high tolerance of the line to drought stress. Hence, the line has the potential use in producing maize hybrids that are able to alleviate the negative impacts of drought and high temperature on its growth.

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Contribution of Authors

Hayati PKD: Conceived idea, conducted experiment and write up of article
Sutoyo: Designed and helped in experiment

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