Selection of maize genotypes for drought tolerance improvement

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Abstract. Climate change alters crop production in tropical and subtropical regions, and drought is a big challenge in maize production nowadays and near future. Breeding drought-tolerant varieties is important to keep production stability and food security. This research was undertaken to evaluate agronomic performance and yield potential of several maize genotypes in a drought stress environment, and to identify potential genotypes for further utilization in a maize breeding program. The experiment was conducted using an augmented design with 370 test genotypes and 6 checks at Sukoharjo Village, Kediri, East Java, from September 2014 to January 2015. The plants suffered from drought stress in the pre-anthesis period. Results of the analysis of variance showed that the effect of genotype is significant on grain yield, ear yield, plant height, and ear height. Linear correlation analysis showed that grain yield is negatively correlated with leaf senescence. Selection for tolerant genotypes was conducted using selection index and cluster analysis. Line 249 and 252 are considered potential for subsequent breeding processes because they have similar traits with a formerly known drought-tolerant variety, namely Bima 19URI.

1. Introduction

Maize is one of the staple crops playing a key role as food, feed, and source of energy. In Indonesia, maize production faces serious challenges regarding water supply due to the increasing demand for fresh water for domestic and industrial use. This situation could be getting worse as it is predicted that climate change will increase the drought level globally [1]. For maize growers, this issue is crucial because drought is the most significant abiotic stress affecting maize cultivation [2]. Although maize as a C4 plant consumes water more efficiently than those of C3, drought stress still contributes to the reduction of maize production up to half of its optimum yield as well as the other vegetative traits such as plant height and ear production [3].

The main approach to mitigate the risk of drought-linked harvest failure in maize is through constructing varieties tolerant to drought. The adoption of drought-tolerant varieties showed an increase in production by 15%. Additionally, the risk of harvest failure dropped by 30% compared to non-drought tolerant varieties [4].

The development of drought-tolerant maize varieties relies on the ability to utilize water effectively so that the kernels are successfully developed within drought stress [5]. However, breeding and genetics studies on this issue are facing big challenges [6]. One of these is that
the heritability of yield and other agronomic traits are likely low under drought stress environments, and therefore the characteristics obtained from such experiments might not be observed in the following generations [7]. One suggested approach is to select the genotypes based on several drought tolerance-related traits simultaneously [8].

A direct selection towards high-yielding maize varieties has shown little progress under drought conditions due to high variability caused by the environment and genotype-environment interaction factors. In this case, the use of secondary traits, besides yield, could enhance the selection process. The selection index could weigh multiple traits at the same time to compensate for the low heritability from drought trials [6].

This research was conducted to observe the agronomic traits and yield of maize genotypes under a drought stress environment. In addition, the index selection was performed to identify potential genotypes suitable for parental line candidates for breeding drought-tolerant varieties.

2. Materials and Method

The observation was done in a farming area of Sukoharjo village, Plemahan sub-district, Kediri regency in East Java from September 2014 to January 2015. This location can be found at the coordinate of 7°43’26.4” S and 112°09’33.6” E with an altitude of approximately 43 meters above sea level.

There were 370 test genotypes, collections of Indonesian Cereals Research Institute, Maros, South Sulawesi, and 6 check genotypes (Lamuru, Bima 19, Bima 20, MR-4, MR-14, and Nei 9008) used during this research. The test genotypes are the third selfing generation of a cross between CML 440 and MR-4. Lamuru is an open-pollinated variety while MR-4, MR-14, and Nei 9008 are inbred lines. The other check genotypes, Bima 20 and Bima 19, are three-way cross hybrid varieties [9]. Bima 19 was used as a drought-tolerant check genotype while MR-4, MR-14, and Nei 9008 were used as drought-prone check genotypes [3].

The research was arranged using a single-factor augmented design as explained by Federer (1994). The check genotypes were replicated five times whereas the test genotypes were randomly placed without any replication. The plot size is a single row of 2.5 m, and there was a total of 400 plots in the experiment.

Seeds were sowed at 75 cm x 25 cm distance with one seed per hole. Prior to this, seeds were treated using fungicide (active materials: dimetomorf 60% and azoksistrobin 10%) where its dose was 5 g L-1. The first fertilization was applied on the first day using organic fertilizer dosed at 800 kg ha-1. The second and last fertilization was applied at 18 and 33 days after planting using NPK dosed at 250 kg ha-1. The plants were irrigated at the same time as fertilization.

The data analysis consisted of F-test, Pearson correlation analysis, index selection, and cluster analysis. The F-test was done using two methods; the first was the least square means to calculate the value of square means while the second was the restricted maximum likelihood to estimate the value of adjusted means. The index selection was done through traits and scaling used by [3]: $I = 5P1+3P2-2P3-2P4-P5$. Those letters stand for the adjusted value of grain weights per plant, ear per plant, leaf senescence score, tassel size, and leaf rolling score respectively. The last analysis, cluster analysis, was performed using the Ward method which was based on the Euclidean distance matrix. The dendrogram was drawn in a circular shape through “ape” package in R i386 3.1.1 [11].
3. Results and Discussions

3.1. General condition

Overall, only 53% of the seeds planted survived. This could be caused by low seeds vigor as many of which had been stored for a considerably long time before being sowed. Until harvesting day, plots consisting of at least three plants were 293. Among this number, only 114 plots made up more than three ears. The data for analysis came from these criteria.

The rainy season at the research location started on the 57th day (Figure 1). Rainy days lasted for 28 days with an average of 14.39 mm. The gap between the last irrigation (33rd day) and the first rainy days (57th day) was 24 days. The normal intensity of irrigation is 14 days so that an absence of water during such a gap is considered as a period of drought stress to the crops observed.

![Figure 1. Precipitation, irrigation, and estimation of drought period during this study](image)

3.2. Analysis of variance

The summary of the F-test (Table 1) shows that the block effect was insignificant for all traits. Meanwhile, the genotype effect among check genotypes was significant for all traits except leaf rolling, leaf senescence, and ear per plant. For test genotypes, the effect is only significant on ear and grain weight per plant. A tolerance for confidence interval seems necessary in this research because of drought stress. This circumstance was likely to rise the variance within test genotypes. Hence, it is difficult to identify the difference among test genotypes.

| Traits              | Group | Genotype | C vs T^a | Check genotypes | Test genotypes |
|---------------------|-------|----------|----------|-----------------|----------------|
| Grain weight per plant (gram) | 0.04ns | 0.35**   | 19.4**   | 1.45**          | 0.09**         |
| Ear per plant+ | 0.28  | <0.01    | <0.01    | <0.01           | 0.02           |
| Ear size (cm)     | 395.72ns | 741.15** | 30342.57** | 2747.70**      | 323.07ns       |
| Ear diameter (cm) | 0.15  | 0.01     | <0.01    | <0.01           | 0.17           |
| Plant height (cm) | 6.70ns | 41.52*   | 2024.48** | 147.54**       | 14.78ns        |
| Stem diameter (cm) | 0.56  | 0.02     | <0.01    | <0.01           | 0.17           |
| Tassel size (cm)  | 132.23ns | 718.31*  | 38140.34** | 3175.04**      | 166.02ns       |

*ns: not significant, *: p < 0.05, **: p < 0.01, ***: p < 0.001
### 3.3. Performance of drought specific traits

The results of the analysis of variance showed a significant difference between check genotypes and test genotypes for the leaf rolling score, in which the former showed a lower mean (Table 2). This refers to a better ability to maintain plant turgor during water scarcity through improvement in water absorption or osmotic adjustment [12].

#### Table 2. Adjusted means of each group of maize genotypes under drought stress condition

| Traits                  | Overall means | Test genotypes means | Check genotypes means | CV (%) |
|-------------------------|---------------|----------------------|-----------------------|--------|
| Grain weight per plant  | 2.70          | 2.30                 | 27.77                 | 4.30   | 44.97 |
| (gram)                  |               |                      | 87.87                 |        |       |
| Ear per plant           | 0.78          | 1.13                 | 1.20                  | 1.32   | 1.09  | 34.35 |
| Leaf senescence (score) | 3.66          | 2.05                 | 1.83                  | 1.85   | 1.82  | 29.74 |
| Tassel size (cm)        | 2.64          | 2.60                 | 3.04                  | 3.82   | 2.59  | 26.97 |
| Leaf rolling (score)    | 2.36          | 2.42                 | 1.73                  | 1.84   | 1.63  | 29.97 |
| Plant height (cm)       | 54.38         | 51.95                | 89.47                 | 118.49 | 66.41 | 36.88 |
| Stem diameter (cm)      | 1.47          | 1.47                 | 1.76                  | 1.84   | 1.63  | 28.26 |
| Ear diameter (cm)       | 2.48          | 2.43                 | 3.39                  | 3.91   | 2.63  | 18.11 |
| Ear size (cm)           | 9.43          | 9.24                 | 12.85                 | 15.02  | 9.59  | 22.33 |

CV: coefficient of variation

Leaf senescence is a characteristic that suggests the ability of a maize plant to stay green. Regarding the result of correlation analysis, genotypes with lower leaf senescence scores were likely to yield higher ($r = -0.29; p<0.01$). A regression of yield on the leaf senescence score is shown in Figure 2. Maize genotypes that can sustain their green leaves longer may be able to produce more grains [13].

#### Table 3. Linear correlation coefficients among maize traits under drought stress condition

| EW          | EP   | PH   | TS   | LS   | LR   | ES   |
|-------------|------|------|------|------|------|------|
| EW          | 0.47**|      |      |      |      |      |
| EP          |      | 0.53**| 0.00 ns|      |      |      |
| PH          | 0.17 ns| -0.10 ns| 0.45**|      |      |      |
| TS          | -0.29**| -0.22*| -0.21*| 0.21*|      |      |
| LS          | -0.17 ns| -0.05 ns| -0.28**| 0.03 ns| 0.07 ns|      |
| LR          | -0.29**| -0.22*| -0.21*| 0.21*|      |      |
| ES          | 0.63**| 0.26*| 0.39**| 0.06 ns| -0.33**| -0.22*|
| ED          | 0.80**| 0.41**| 0.44**| 0.19 ns| -0.24*| -0.14 ns| 0.65**|

EW: ear weight per plant; EP: ear per plant; PH: plant height; TS: tassel size; LS: leaf senescence; LR: leaf rolling; ES: ear size; ED: ear diameter; **: significant correlation at 5%; ***: significant correlation at 5% and 1%, respectively; ns: not significant; the correlation coefficients were calculated with n=93-97.
Figure 2. Linear regression of grain weight per plant against leaf senescence score (using adjusted means)

3.4. Simultaneous selection
Table 4 lists the result of index selection for the top 10 genotypes. This was performed by calculating the indices for 97 genotypes from the adjusted values of 92 test genotypes and adjusted average values of 5 check genotypes. Genotype 249 topped the list. Meanwhile, the top five genotypes also showed fair performance where the rows in the ears were fully developed in spite of the disturbance of grain development due to drought stress [14].

Table 4. Means and weighted selection indices of the top 10 genotypes

| Genotypes | Drought-tolerant specific traits | Index | PH (g) | ES (cm) | ED (cm) |
|-----------|---------------------------------|-------|--------|---------|---------|
|           | EW (g) | EP  | LS    | TS    | LR     |
| 249       | 61.67  | 1.33| 2.00  | 2.75  | 2.00   | 30.48  | 100.00  | 14.80  | 3.38   |
| Bima 20   | 96.36  | 1.56| 2.65  | 3.50  | 1.80   | 29.95  | 110.49  | 15.40  | 3.55   |
| Bima 19   | 115.00 | 1.10| 3.37  | 4.18  | 1.80   | 25.70  | 77.91   | 12.80  | 3.00   |
| Lamuru    | 67.22  | 0.84| 3.35  | 3.93  | 2.00   | 23.86  | 125.62  | 13.70  | 3.84   |
| 252       | 31.25  | 0.75| 4.25  | 4.00  | 2.00   | 15.70  | 69.00   | 14.00  | 3.43   |
| 109       | 8.57   | 0.86| 2.20  | 2.00  | 2.00   | 15.09  | 68.20   | 10.23  | 2.65   |
| 35        | 11.20  | 1.60| 2.60  | 3.33  | 2.00   | 13.33  | 72.60   | 9.19   | 2.79   |
| 48        | 10.00  | 1.00| 2.20  | 3.00  | 2.00   | 11.95  | 57.80   | 8.60   | 2.99   |
| 40        | 4.00   | 1.00| 1.50  | 2.00  | 3.00   | 11.38  | 50.20   | 11.23  | 2.08   |
| 166       | 8.00   | 1.00| 3.25  | 3.00  | 2.00   | 11.25  | 81.50   | 12.25  | 3.35   |

| General means | EW (g) | EP  | LS    | TS    | LR     | PH (g) | ES (cm) | ED (cm) |
|---------------|--------|-----|-------|-------|--------|--------|---------|---------|
|               | 2.70   | 0.78| 3.66  | 2.64  | 2.36   | -      | 54.38   | 9.43    | 2.48   |
| LSD 5%        | 2.71   | -   | -     | -     | -      | -      | 43.01   | 4.69    | 1.00   |

EW : ear weight per plant; EP : ear per plant; PH : plant height; TS : tassel size; LS : leaf senescence; LR : leaf rolling; ES : ear size; ED : ear diameter; * significantly different according to LSD test at 5%.
3.5. Cluster analysis
There are two main groups according to the result of cluster analysis (Figure 3), namely tolerant genotypes cluster and susceptible genotypes cluster. The coefficient of dissimilarity is 0.19 which indicates that genotypes within groups had a high similarity. Further evaluations are needed to understand whether the clusters represent heterotic groups. It is estimated that genotypes that belong to the same heterotic group have similar genetic traits [15]. Crossing within the same heterotic group should be avoided as it might lead to inbreeding depression. In contrast, one should cross genotypes from different heterotic groups. The potential drought-tolerant lines presented herein could be proceeded to subsequent self-pollinated generations to develop inbred lines. Later on, it can be a good opportunity to develop an improved drought-tolerant hybrid variety by crossing a drought-tolerant line with another potential line from different heterotic groups.

![Figure 3. Groups representing putative tolerant and susceptible maize genotypes](image)

4. Conclusion
There were variations among agronomic characters and yield of maize genotypes under drought stress in terms of grain weight per plant, ear weight per plant, ear size, ear diameter, plant height, and ear height. Correlation analysis revealed that a higher grain weight per plant was associated with a lower leaf senescence score. From the tested genotypes, genotype 249 and genotype 252 were the best candidates for developing inbred lines having putative tolerance to drought stress.

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