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# Morphological and molecular characterization of maize lines tolerance to drought stress

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**Abstract.** *Amzeri A, Badami K, Santoso SB, Sukma KPW. 2022. Morphological and molecular characterization of maize lines tolerance to drought stress. Biodiversitas 23: 5844-5853.* The early step in assembling varieties with early maturity, high productivity, and resistance to drought stress is to carry out morphological and molecular characterization among lines resistant to drought stress. The research aimed to characterize the morphology and molecular characteristics of maize lines resistant to drought stress to develop breeding and cultivation programs. The research used a randomized complete block design with twenty genotypes (maize lines) as treatment and three replicates. A total of 57 morphological characters and seven RAPD primers were used to assess the relationship based on morphological characters and RAPD markers. The results showed that the similarity coefficient based on the morphological characters was 0.69-0.97, while the similarity coefficient based on the RAPD marker was 0.64-0.95. The phenotypic diversity coefficient (PDC) was greater than the Genotypic Diversity Coefficient (GDC) in all observed quantitative characters. The broad sense heritability values of the tested maize lines ranged from 0.83 to 0.99. Production per hectare was significantly positively correlated with eight tested quantitative characters. G1 and G15 were the genotypes that can be used as parents to assemble hybrid maize varieties, while G10, G11, G16, and G17 could be used as maize for cultivation programs.

**Keywords:** Drought stress, genetic parameters, maize lines, molecular characterization, morphological characterization

## INTRODUCTION

Suboptimal land use is a strategic step in increasing maize production in Indonesia. The suboptimal land area in Indonesia is ±80% (144.5 million hectares), which consists of 133.70 million hectares of dry land with wet climate and 10.80 million hectares of dry land with dry climate (Mulyani et al. 2016). Madura Island is an area of East Java Province, Indonesia with a planting area for maize of approximately 300,000 ha (30% of maize area in East Java Province, Indonesia). However, productivity at the farmer level is still low on average 2.15 tons per hectare (BPS-Statistics Indonesia 2019). Maize production area in Madura Island is mostly suboptimal land (dry land with dry climate), characterized by insufficient water availability due to low rainfall (less than 2,000 mm/year) and short rainfall period (3-5 months) (Mulyani and Sarwani 2013). The results of research by Suhartono et al. (2020) show that the average annual rainfall in Madura Island was 1346.89 mm/year. Low rainfall is one of the causes of low maize productivity on Madura Island. In addition, the problem of low rainfall also occurs worldwide. Benestad et al. (2022) reported that the global area of daily rainfall decreased from 43 to 41% of the global area between 1950 and 2020.

One strategy for overcoming the problem of low maize productivity on land with low rainfall is to assemble high-yielding varieties resistant to drought stress. This requires many breeding materials equipped with information on the important characteristics of each material. The initial step in assembling high-yielding and drought-tolerant varieties is to carry out morphological and molecular characterization among lines resistant to drought stress. Previous studies showed that four local Madurese maize and five introduced maize were tolerant to drought stress induced by Polyethylene Glycol 6000 during vegetative phase (Suhartono and Amzeri 2021). The research resulted in 20 drought-tolerant lines.

Morphological characterization will provide information on morphological differences among lines and relationships between the tested lines. These information plays an important role in selecting parents to be used to assemble varieties (Li et al. 2020). Crosses between distantly related parents will produce largely segregated offsprings, making it easier to choose the desired variety (Goulet et al. 2017; Marone et al. 2021). Morphological characterization results are often biased by environmental influences (Pandey et al. 2015), therefore molecular characterization is required to complement morphological information (Blazakis et al. 2017; Lutateknekwa et al. 2020).

Molecular characterization is identification at DNA level by conducting indirect selection on the desired character, namely on markers associated with that character, thus can increase the efficiency of parent selection (Hasan et al. 2021). The Random Amplified Polymorphic DNA (RAPD) method can be used to identify individuals at DNA level. The advantage of this method is that it is relatively simple and requires a small quantity of DNA (5-25 ng) in each Polymorphic Chain Reaction

(PCR). It quickly detects polymorphisms at many loci (Lizawati et al. 2019) and is the fastest for collecting polymorphisms in genomic DNA (Matsumoto et al. 2022).

Information on several genetic parameters in maize plants is needed to determine the appropriate breeding method for obtaining maize varieties with early maturity, high productivity, and resistance to drought stress. Calculating the phenotypic and genotypic diversity, heritability, and correlation between quantitative maize characteristics is essential to support the formation of the desired variety (Sravanti et al. 2017; Bartaula et al. 2019). The heritability value gives an overview of the genetic influence on plant appearance (Schmidt et al. 2019), whereas the information of the correlation between characteristics makes it easier to choose the desired plant character (Kumar et al. 2014).

Information on morphological characterization, molecular characterization, and genetic parameters of drought-tolerant maize lines will help maize breeders to choose the effective breeding methods and parent selection as well as predict new varieties characteristics. Therefore, this research aimed to characterize the morphology and molecular characteristics of maize lines resistant to drought stress for further breeding and cultivation programs.

## MATERIALS AND METHODS

### Genetic materials and experimental site

The planting material was twenty maize lines tolerant of drought stress, consisting of eight local Madura maize lines and twelve introduced maize lines (Table 1). The research was conducted from April to August 2022. The research was conducted in Bangkalan District, Madura, Indonesia (7°09'14.8" S, 112°44'01.6" E, 5 m a.s.l). The average annual rainfall is 1,631 mm with an average of 124 rainy days. The average temperature is around 30ºC with an average humidity of 68%. The soil type is grumusol with pH of 7.1.

**Table 1**. Twenty maize lines resistant to drought stress

| Genotype | Code | Origin |
|---|---|---|
| G1 | TS-5-20 | Madura Island, Indonesia |
| G2 | T2S-5-11 | Madura Island, Indonesia |
| G3 | DuS-5-02 | Madura Island, Indonesia |
| G4 | ES-5-24 | Madura Island, Indonesia |
| G5 | MS-5-06 | Madura Island, Indonesia |
| G6 | CS-5-43 | Madura Island, Indonesia |
| G7 | DS-5-3-1 | Madura Island, Indonesia |
| G8 | GS-4-2-1 | Madura Island, Indonesia |
| G9 | An-S-4-1-5 | ICERI, Indonesia |
| G10 | La-S-4-2-4 | ICERI, Indonesia |
| G11 | Bi-S-4-1-10 | ICERI, Indonesia |
| G12 | Su-S-4-2-4 | ICERI, Indonesia |
| G13 | Su-S-4-1-12 | ICERI, Indonesia |
| G14 | Su-S-4-1-15 | ICERI, Indonesia |
| G15 | Su-S-4-3-16 | ICERI, Indonesia |
| G16 | Lm-S-4-2-12 | ICERI, Indonesia |
| G17 | Lm-S-4-2-2 | ICERI, Indonesia |
| G18 | Ba-S-4-3-1 | Probolinggo, East Java, Indonesia |
| G19 | Ba-S-4-2-2 | Probolinggo, East Java, Indonesia |
| G20 | Pl-S-5-2 | Kediri, East Java, Indonesia |

### Experimental design, management, and data collected

The research used a randomized complete block design with twenty genotypes (maize lines) as treatment and three replicates, so there were 60 experimental units. Each genotype was planted in a 1 m x 5 m plot with plant spacing of 70 cm × 20 cm. Each plot contained 50 plants. Fertilization is given to plants three times. The first fertilization was applied when the plants were seven days after planting (DAP) using 100 kg $ha^{-1}$ urea, 200 kg $ha^{-1}$ SP-36, and 50 kg $ha^{-1}$ KCl. The second fertilization was applied when the plants were 25 DAP using 100 kg $ha^{-1}$ urea and 50 kg $ha^{-1}$ KCl. The third fertilization was applied when the plants were 40 DAP using 100 kg $ha^{-1}$ urea. Plant pests and diseases were controlled according to plant conditions.

The observed morphological characters were quantitative and qualitative characters. In total, there were 58 characters based on the classification of plant habitus, leave, stem, panicle, ear and agronomy.

### DNA isolation and PCR amplification

The DNA isolation procedure followed the Plant Genomic DNA Kit protocol (Tiangen). RAPD primers used in this research as follows: OPC-15 (GACGGATCAG), OPA-02 (TGCCGAGCTG), OPA-01 (TGGCGACCTG), OPA-10 (GTGATCGCAG), OPB-12 (CCTTGACGCA), OPA-15 (CCAGTACTCC), OPC-07 (GTCCCGACGA). PCR mixture and cycles condition followed the procedures described by Te-Chato et al. (2005).

The PCR mixture consisted of 20 µL Kit Mega Mix Blue, 2.5 µL primer, and 2.5 µL primer "DNA template" of 1/50 concentration of DNA isolate. The PCR was programmed to include pre-denaturation at 94ºC for 1 minute, followed by 45 cycles of denaturation at 94ºC for 1 minute, annealing at 36ºC for 1 minute and extention at 72ºC for 1 minute. The final cycle was allowed an additional 5 minutes period of extension at 72ºC. The amplification products were separated by electrophoresis on 2% agarose gel. A total of 5 µL of each sample was placed in a gel well. Electrophoresis was carried out at 100 volts for 30 to 45 minutes. The electrophoresis process can be terminated, when the sample reaches the fourth line from the end pole. The visualization of DNA bands was carried out using an Geldoc UV-transilluminator and documented.

### Data analysis

A cluster analysis were analyzed using Numerical Taxonomy and Multivariate System (NTSYS) program version 2.1. A cluster analysis applied SAHN (Sequential Agglomerative Hierarchical and Nested) approach was conducted with an Unweighted Pair Group Method Arithmetic (UPGMA) procedure in order to group the genotype based on morphological characters and RAPD markers. Quantitative character data were analyzed using the STAR software version 2.0.1. Estimation of environmental variance, genetic variance, phenotypic variance, and broad heritability ($h^2_{bs}$) were calculated based on the formula of Hallauer et al. (2010). The genotypic diversity coefficient (GDC) and phenotypic diversity coefficient (PDC) were calculated based on the formula of Singh and

Chaudhary (2004). Pearson correlation coefficient analysis was calculated based on the formula of Walpole (1982).

## RESULTS AND DISCUSSION

### Morphological characterization

Grouping based on morphological characters produces a dendrogram with similarity coefficients ranging from 0.69-0.97 or a morphological diversity of 0.03-0.31 (Figure 1). The large similarity between maize lines indicates that these maize lines have a close relationship (Tucker et al. 2018). At 0.69 similarity, there are two main groups. Group 1 consists of G1, G2, G3, G4, G5, G6, G7, G8, G18, G19, and G20, while group 2 consists of G9, G10, G11, G12, G13, G14, G15, G16, and G17. Group 1 was united by the similarity of stem diameter, ear stalk length, plant biomass, and production per hectare, while group 2 was united by similarity of character for leaf width, leaf length, ear length, ear shape, number of kernel rows on the ear, and number of kernels per ear, kernel length, and kernel width (Tables 2 and 3)

Group 1 formed two subgroups, i.e., group A consisting of G1, G2, G3, G4, G5, G6, G8, G18, G19, and G20 while G7 separated from group A to form group B due to differences in leaf sheath anthocyanin color, stem color, The color of anthocyanins on the basis of maize husk, kernel surface color, and cob color. Group 2 formed two subgroups, i.e., group C consisting of G9, G10, G11, G12, G13, G14, and G15. Group D consists of G16 and G17. Group C and group D were separated due to differences in anthocyanin color in the first leaf sheat, angle between leaf and stem, leaf sheath anthocyanin color, number of panicle main side branches, ear hair anthocyanin color intensity. Variations in the characters of maize hair, maize panicle, ear, and maize kernels are shown in Figure 2.

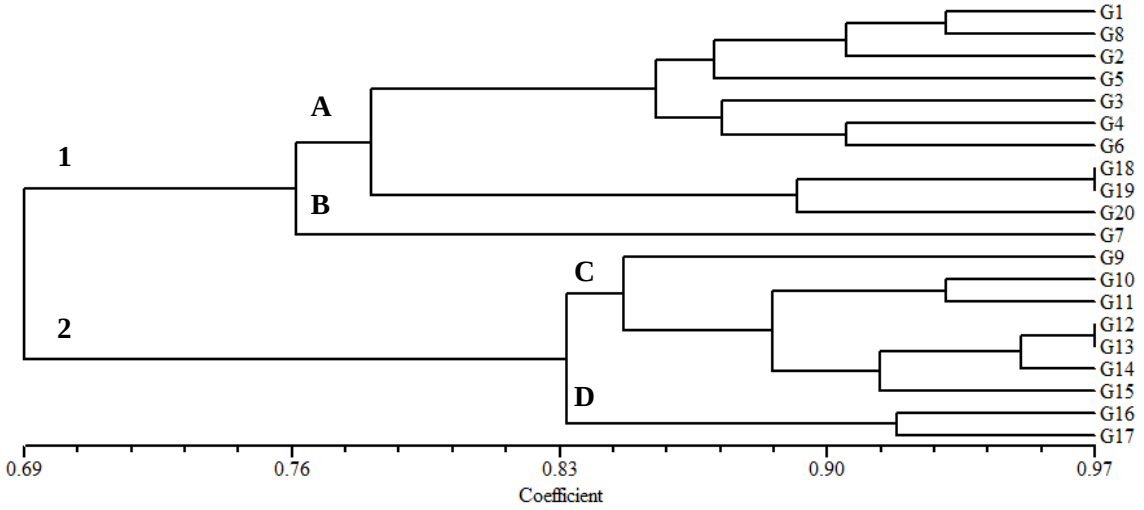


**Figure 1.** Dendrogram of drought-tolerant maize lines based on morphological characters

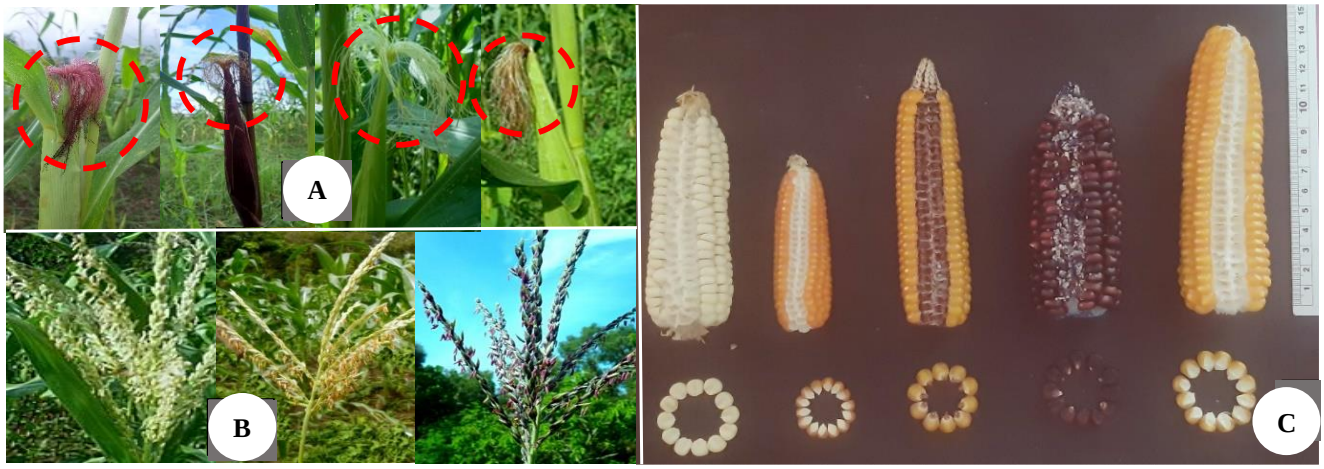


**Figure 2**. Maize morphological characters. A. Maize hair variations, B. Maize panicles variations, C. Variations of maize ear and kernel

**Table 2.** Morphological (quantitative and qualitative) character scoring on twenty maize lines

| | Genotype | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **G1** | **G2** | **G3** | **G4** | **G5** | **G6** | **G7** | **G8** | **G9** | **G10** | **G11** | **G12** | **G13** | **G14** | **G15** | **G16** | **G17** | **G18** | **G19** | **G20** |
| H1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| H2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| H3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| L1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 1 |
| L2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 |
| L3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| L4 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |
| L5 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| L6 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 |
| L7 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 3 | 3 | 2 |
| L8 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 |
| L9 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 2 | 1 | 1 | 1 |
| L10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| L11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| L12 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 2 | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| S1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| S2 | 3 | 3 | 3 | 2 | 3 | 3 | 4 | 3 | 3 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 4 |
| S3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| S4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| S5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 |
| P1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 5 | 4 | 6 | 6 | 6 | 6 | 7 | 4 | 3 | 3 | 3 | 4 |
| P2 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 1 | 1 | 4 |
| P3 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 3 |
| P4 | 3 | 0 | 2 | 1 | 1 | 1 | 3 | 3 | 3 | 2 | 2 | 0 | 1 | 2 | 3 | 2 | 2 | 2 | 2 | 3 |
| P5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 1 | 1 | 1 |
| P6 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 1 | 1 | 1 |
| P7 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 3 | 1 | 1 | 1 | 1 | 1 |
| P8 | 3 | 3 | 2 | 2 | 0 | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 |
| P9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 1 | 1 | 1 |

Note: H1: plant height; H2: the ratio of the length of the top ear to the length of the plant; H3: plant biomass; L1: anthocyanin color in the first leaf sheath; L2: the tip shape of the first leaf; L3: number of leaves: L4: angle between leaf and stem; L5: leaf pattern; L6: leaf width; L7: leaf sheath anthocyanin color; L8: leaf length; L9: leaf surface total rating; L10: leaf sheath hair; L11: number of leaves on the cob; L12: leaf color; S1: stem zig-zag degree; S2: the color of anthocyanins in the root; S3: stem color; S4: fall stem; S5: stem diameter; P1: days to 50% tasselling; P2: the color of anthocyanins on the basis of maize husk; P3: the anthocyanin color does not include the base of the petals; P4: anthocyanin color in fresh anthers; P5: anther grain density; P6: the angle between the main axis and the panicle side branches; P7: place the panicle side branch; P8: number of panicle main side branches; P9: main shaft length above the panicle lowest side branch

**Table 3.** Morphological (quantitative and qualitative) character scoring on twenty maize lines

| | Genotype | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **G1** | **G2** | **G3** | **G4** | **G5** | **G6** | **G7** | **G8** | **G9** | **G10** | **G11** | **G12** | **G13** | **G14** | **G15** | **G16** | **G17** | **G18** | **G19** | **G20** |
| P10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 1 | 1 |
| P11 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 1 | 1 | 1 |
| P12 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| E1 | 2 | 2 | 2 | 0 | 1 | 0 | 2 | 2 | 6 | 5 | 7 | 7 | 7 | 7 | 7 | 5 | 4 | 3 | 3 | 5 |
| E2 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| E3 | 0 | 1 | 3 | 1 | 3 | 2 | 0 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 3 | 4 | 4 | 3 | 3 | 3 |
| E4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| E5 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 |
| E6 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 |
| E7 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| E8 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 |
| E9 | 2 | 2 | 0 | 2 | 2 | 2 | 2 | 2 | 3 | 0 | 1 | 1 | 1 | 0 | 2 | 0 | 1 | 0 | 1 | 1 |
| E10 | 4 | 4 | 5 | 4 | 4 | 3 | 6 | 4 | 0 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 3 | 2 | 3 | 3 |
| E11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E12 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| E15 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| E16 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| E17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E18 | 2 | 2 | 0 | 2 | 1 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| E19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| E20 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| E21 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| E22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| E23 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |

Note: P10: main shaft length above the upper side branch on a panicle; P11: panicle side branch length; P11: panicle type; E1: Days to 50% silking; E2: anthocyanin color in ear hair; E3: ear hair anthocyanin color intensity; E4: ear stalk length; E5: ear length; E6: ear diameter; E7: ear shape; E8: number of kernel rows on the ear; E9: kernel type; E10: kernel surface color; E11: kernel base side color; E12: anthocyanins in cob petals; E13: anthocyanin color intensity on cob petals; E14: the age of the maize husks dries up; E15: ear height; E16: maize husk closure; E17: ear damage; E18: kernel row arrangement; E19: number of kernels per ear; E20: kernel length; E21: kernel width; E22: kernel thickness; E23: cob color; E24: endosperm color; E25: 1000-kernel weight; E26: production per hectare

**Table 4**. Characteristics of plant height, days to 50% tasselling, days to 50% silking, harvest age, and ear height of twenty maize lines

| Genotype | Plant height (cm) | Days to 50% tasselling (days) | Days to 50% silking (days) | Harvest age (days) | Ear height (cm) |
|---|---|---|---|---|---|
| G1 | 157.67 f | 41.33 g | 44.00 f | 71.33 h | 84.33 e |
| G2 | 159.33 ef | 39.67 h | 39.67 h | 70.67 h | 83.47 de |
| G3 | 155.33 f | 38.67 i | 41.33 gh | 71.33 h | 87.30 d |
| G4 | 157.67 f | 33.33 j | 35.67 i | 55.67 k | 84.30 e |
| G5 | 144.67 g | 40.33 h | 42.33 fg | 68.67 i | 83.33 e |
| G6 | 120.33 h | 31.67 k | 33.67 j | 60.33 j | 63.37 g |
| G7 | 157.67 f | 40.00 h | 42.67 fg | 70.33 h | 83.47 e |
| G8 | 162.33 e | 39.33 hi | 41.67 g | 71.33 h | 84.67 de |
| G9 | 160.33 ef | 51.67 d | 52.67 c | 101.67 c | 72.33 f |
| G10 | 203.67 a | 48.67 e | 50.33 d | 88.33 fg | 106.67 a |
| G11 | 186.67 d | 57.67 a | 59.33 a | 94.33 e | 89.33 c |
| G12 | 189.67 cd | 55.33 c | 57.33 ab | 103.67 b | 91.33 bc |
| G13 | 192.33 bc | 55.67 bc | 57.67 ab | 104.67 ab | 93.00 b |
| G14 | 194.00 b | 56.00 bc | 58.00 ab | 104.33 b | 89.00 c |
| G15 | 192.33 bc | 56.33 b | 58.67 ab | 105.67 a | 89.67 c |
| G16 | 190.67 bc | 49.33 e | 51.33 cd | 88.33 fg | 84.67 de |
| G17 | 194.00 b | 49.00 e | 50.33 d | 87.67 g | 84.33 de |
| G18 | 187.00 d | 45.33 f | 47.33 e | 89.33 f | 90.33 c |
| G19 | 190.00 cd | 45.00 f | 47.00 e | 88.67 fg | 90.00 c |
| G20 | 192.67 bc | 49.33 e | 51.00 cd | 98.67 d | 83.67 e |

Note: The numbers followed by the same letter in the same column are not significantly different according to the 5% DMRT test

**Table 5**. Characteristics of ear length, ear diameter, kernel weight per plant, 1000-kernel weight, and production per hectare of twenty maize lines

| Genotype | Ear length (cm) | Ear diameter (cm) | Kernel weight per plant (g) | 1000-kernel weight (g) | Production per hectare (kg) |
|---|---|---|---|---|---|
| G1 | 12.60 e | 3.27 gh | 46.52 g | 188.67 efg | 3101.00 i |
| G2 | 12.63 e | 3.41 fg | 52.03 ef | 209.33 bc | 3468.67 g |
| G3 | 8.73h | 2.59 j | 49.05 fg | 116.33 h | 3269.67 h |
| G4 | 9.97 g | 2.72 i | 34.63 i | 206.67 cd | 2308.67 k |
| G5 | 7.80 i | 3.27 h | 26.65 j | 207.33 cd | 1776.33 l |
| G6 | 11.40 f | 3.26 h | 18.19 k | 219.67 b | 1212.67 m |
| G7 | 12.47 e | 3.42 f | 50.08 f | 257.67 a | 3338.33 gh |
| G8 | 11.13 f | 3.23 h | 42.92 h | 195.33 def | 2861.33 j |
| G9 | 14.93 c | 3.97 d | 54.81 e | 184.67 fg | 5009.67 e |
| G10 | 15.93 b | 4.30 c | 58.39 d | 191.00 efg | 5222.67 d |
| G11 | 15.96 b | 4.43 bc | 50.98 f | 181.33 g | 5645.00 c |
| G12 | 16.93 a | 5.40 a | 75.70 ab | 198.33 cde | 6316.67 b |
| G13 | 16.97 a | 5.33 a | 77.95 a | 198.33 cde | 6969.00 a |
| G14 | 16.80 a | 5.50 a | 73.16 b | 198.33 cde | 7040.67 a |
| G15 | 17.07 a | 5.43 a | 73.82 b | 200.33 cde | 7115.33 a |
| G16 | 16.87 a | 4.50 b | 69.96 c | 191.00 efg | 5681.33 c |
| G17 | 17.03 a | 4.57 b | 69.92 c | 192.67 efg | 5803.33 c |
| G18 | 13.73 d | 3.77 e | 59.32 d | 199.33 cde | 3944.67 f |
| G19 | 13.70 d | 3.73 e | 59.71 d | 193.67 efg | 3980.33 f |
| G20 | 13.93 d | 3.97 d | 60.86 d | 188.33 efg | 4057.00 f |

Note: The numbers followed by the same letter in the same column are not significantly different according to the 5% DMRT test

The maize variety assembly program is directed at forming superior varieties with early maturity and high production characteristics for maize development on dry land. Maize harvesting age is classified into three, i.e., early maturing variety (90-95 days), intermediate maturing variety (105-110 days), and late maturing variety (115-120 days) (Oluwaranti and Fakorade 2015). The results of statistical analysis on quantitative characters showed that plant height, days to 50% tasselling, days to 50% silking, harvest age, ear height, ear length, ear diameter, kernel weight per plant, 1000-kernel weight, and production per hectare varied between genotypes (Tables 4 and 5). Madura local maize (G1-G8) has an early maturing range from 55.67-71.33 days but low productivity ranging from 1212.67-3468.67 kg per hectare. The research results by Garba and Namo (2013) show that early maturing maize has low production. Furthermore, Bello et al. (2012) showed that intermediate and late maturing varieties gave 17% and 34.29% higher yields than early maturing varieties. G15 had the highest harvesting age and production per hectare characters of 105.67 days and 7115.33 kg per hectare, respectively, compared to other genotypes tested.

**Molecular characterization**

The amplification results from seven primers produced 65 bands from 20 maize lines tested with an average of 9.4 bands. Bands per primer with amplification product sizes ranging from 100-1500 bp on different primers (Table 6). The total number of bands for each primer was different, from 7 bands (OPC-07) to 11 (OPB-12 and OPA-15), while the average polymorphic percentage was 94% (Figure 3). The results of the study by Handi et al. (2013) showed that the test results of 56 maize genotypes using 11 primers produced an average percentage of 97% polymorphic bands. Furthermore, the results of Berhitu et al. (2019) showed that the results of testing Southwest Maluku local maize-Indonesia (var. Kuning Genjah) and hybrid maize (BISI 2 variety) using three primers resulted in an average percentage of 91% polymorphic band.

**Table 6**. Number of polymorphic bands of drought stress resistant maize lines on 7 RAPD primers

| Primer code | Sequence (5'-3') | DNA band | Polymorphic DNA band | Percentage of polymorphisms | Fragment Size (bp) | |
|---|---|---|---|---|---|---|
| | | | | | Lowest | Highest |
| OPC-15 | GACGGATCAG | 10 | 9 | 90 | 100 | 700 |
| OPA-02 | TGCCGAGCTG | 9 | 8 | 89 | 100 | 1000 |
| OPA-01 | TGGCGACCTG | 9 | 8 | 89 | 200 | 1000 |
| OPA-10 | GTGATCGCAG | 8 | 8 | 100 | 150 | 1500 |
| OPB-12 | CCTTGACGCA | 11 | 11 | 100 | 125 | 1300 |
| OPA-15 | CCAGTACTCC | 11 | 10 | 99 | 100 | 1500 |
| OPC-07 | GTCCCGACGA | 7 | 7 | 100 | 200 | 700 |
| Total | | 65 | 61 | 94 | | |

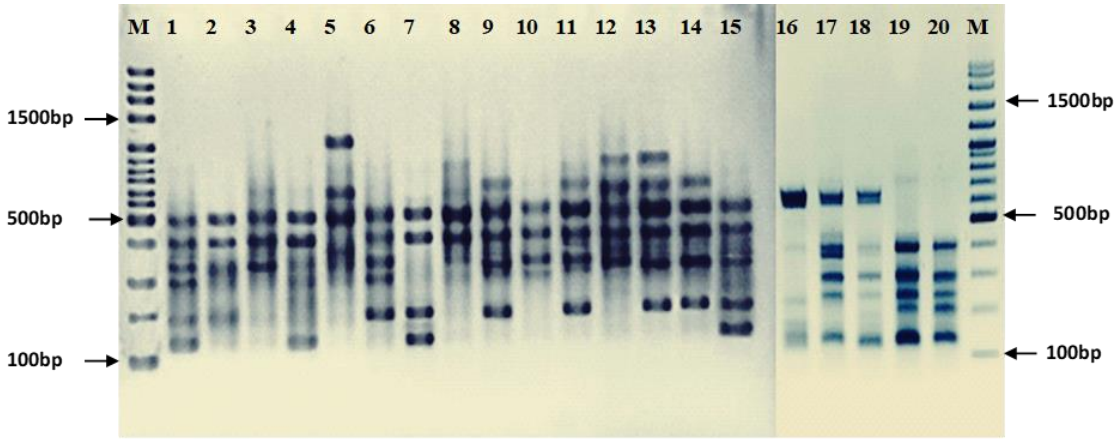


**Figure 3**. RAPD band from 20 maize lines using OPB-12 primer

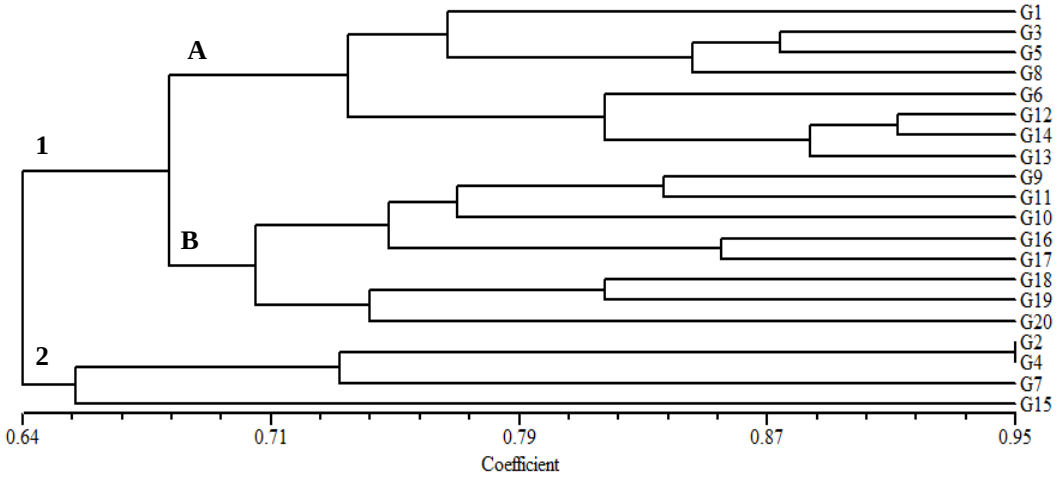


**Figure 4**. Dendrogram of 20 drought stress tolerant maize lines based on RAPD markers

Grouping based on RAPD markers resulted in a dendrogram with a similarity coefficient ranging from 0.64-0.95 or a DNA diversity of 0.05-0.36 (Figure 4). At the 0.64 level of similarity, two main groups can be formed. Group 1 consisted of two subgroups, i.e., Group A and Group B. Group A consists of G1, G3, G5, G6, G8, G12, G13, and G14. Group B consists of G9, G10, G11, G16, G17, G18, G19, and G20. Group 2 consisted of four lines, i.e., G2, G4, G7, and G15. Grouping based on RAPD markers was different from grouping based on morphological characters. This difference is caused by the amplified bands of the RAPD method that are not related to morphological characters (Probojati et al. 2019). Differences in amplified DNA bands, especially the bands' number and size, play a very important role in determining the level of genetic diversity. The number of polymorphic DNA bands can describe the profile of the maize plant genome because it can see the distribution of primer attachment sites on the genome.

**Genetic parameter analysis**

The observed quantitative characters had a Phenotypic Diversity Coefficient (PDC) greater than the Genotypic Diversity Coefficient (GDC) (Table 7). Muladi et al. (2021) stated that the PDC value greater than GDC indicates that the selection can be made based on the appearance of these characters. The PDC value which is almost the same as GDC shows that environmental factors have very little effect on the appearance of plant characters (Magar et al. 2021). Characters with almost the same PDC and GDC values are plant height, harvest age, kernel weight per plant and production per hectare.

Heritability values in the broad sense of the tested maize lines for the evaluated characters ranged from 0.85 - 0.99. Based on the heritability criteria, all the characters tested had high heritability values. High heritability values indicate that genetic factors play a more important role in determining plant characters than environmental factors (Badami et al. 2020; Amzeri et al. 2021). The selection of characters with high heritability values will have a high chance of genetic progress because genetic factors control these characters so that they will be passed on to their offspring (Kartahadimaja et al. 2021). Selection in the early generation can be done on characters with high heritability values (Hakim and Suyamto 2017).

**Table 7**. Values of environmental variance, genetic variance, phenotypic variance, heritability, GDC, and PDC of twenty maize lines

| Character | $\sigma^2_e$ | $\sigma^2_g$ | $\sigma^2_p$ | GDC | PDC | $h^2_{bs}$ |
|---|---|---|---|---|---|---|
| Plant height | 13.49 | 477.05 | 490.54 | 12.52 | 12.70 | 0.97 |
| Days to 50% tasselling | 10.21 | 59.37 | 69.58 | 16.69 | 18.06 | 0.85 |
| Days to 50% silking | 10.3 | 57.88 | 68.18 | 15.82 | 17.17 | 0.85 |
| Harvest age | 10.37 | 252.58 | 262.95 | 18.75 | 19.13 | 0.96 |
| Ear height | 11.91 | 64.92 | 76.83 | 9.38 | 10.20 | 0.84 |
| Ear length | 1.046 | 8.27 | 9.31 | 20.79 | 22.07 | 0.89 |
| Ear diameter | 0.10 | 0.50 | 0.60 | 17.74 | 19.42 | 0.83 |
| Kernel weight per plant | 12.95 | 261.32 | 274.27 | 29.27 | 29.99 | 0.95 |
| 1000-kernel weight | 145.21 | 567.33 | 712.54 | 12.16 | 13.62 | 0.80 |
| Production per hectare | 19332.99 | 3155292.43 | 3174625.42 | 40.31 | 40.44 | 0.99 |

Note: $\sigma^2e$: environmental variance; $\sigma^2g$: genetic variance; $\sigma^2$ p: phenotypic variance; GDC: genotypic diversity coefficient; PDC: phenotypic diversity coefficient; $h^2_{bs}$: heritability in the broad sense. Heritability criteria: high ($h^2_{bs} \geq 0,5$), moderate ($0,2<h^2_{bs}<0.5$), low ($h^2_{bs} \leq 0.2$)

**Table 8**. The linear correlation coefficient between characters in the maize lines tested

| | PH | DT | DS | HA | EH | EL | ED | KWP | 1000KW | PPH |
|---|---|---|---|---|---|---|---|---|---|---|
| PH | 1.00 | | | | | | | | | |
| DT | 0.79** | 1.00 | | | | | | | | |
| DS | 0.80** | 0.99** | 1.00 | | | | | | | |
| HA | 0.79** | 0.80** | 0.95** | 1.00 | | | | | | |
| EH | 0.75** | 0.46* | 0.46* | 0.39 | 1.00 | | | | | |
| EL | 0.79** | 0.85** | 0.85** | 0.83** | 0.37 | 1.00 | | | | |
| ED | 0.72** | 0.89** | 0.89** | 0.87** | 0.40 | 0.90** | 1.00 | | | |
| KWP | 0.87** | 0.82** | 0.82** | 0.84** | 0.55** | 0.84** | 0.81** | 1.00 | | |
| 1000KW | -0.17 | -0.15 | -0.15 | -0.16 | -0.17 | 0.07 | 0.10 | -0.14 | 1.00 | |
| PPH | 0.82** | 0.93** | 0.93** | 0.89** | 0.51** | 0.90** | 0.922** | 0.92** | -0.13 | 1.00 |

Note: PH: plant height: DT: days to 50% tasselling; DT: Days to 50% silking; HA: harvest age; EH: ear height; EL: ear length; ED: ear diameter; KWP: kernel weight per plant; 1000KW: 1000-kernel weight; PPH: production per hectare. *,**significant at 5% and 1% level of probability, respectively

**Correlation between quantitative characters**

Production per hectare is the main component of maize that has economic value. Indirect selection is often used in plant breeding programs to improve the character of production per hectare to save time, effort, and cost in assembling plant varieties. Assessment of characters significantly correlated with production per hectare is useful for improving the character of production per hectare through indirect selection using characters significantly correlated with production per hectare. Selection will be more effective if there is a correlation between the characters to be selected (Naharudin et al. 2021). The correlation coefficient can be used to measure the closeness of the relationship between the observed characters.

Production per hectare was significantly positively correlated with plant height, male flowering age, female flowering age, harvest age, ear height, ear length, ear diameter, and seed weight per plant. days to 50% tasselling, days to 50% silking, harvest age, ear height, ear length, ear diameter, and kernel weight per plant (Table 8). 1000-kernel weight is not significantly correlated with production per hectare. The strategy to increase production per hectare is to increase the value of characters with a significantly positive correlation with production per hectare.

**Selected maize lines for plant breeding and cultivation programs**

This study establishes maize varieties with early maturity and high production. The results showed that 15 maize lines had early maturity (<95 days), i.e., G1, G2, G3, G4, G5, G6, G7, G8, G10, G11, G16, G17, G18, G19, and G20. Maize lines with high yields (<5000 kg per hectare) were G9, G10, G11, G12, G13, G14, G15, G16, and G17. Determination of the lines to be used as parents in the assembly of varieties with early maturity and high production characteristics is based on the results of the dendrogram of morphological characters, a dendrogram of RAPD markers, analysis of genetic parameters, and correlations between quantitative characters. Dendrograms of morphological character and RAPD markers were used to determine the genetic distance between the tested lines (Rabha et al. 2016; Wang et al. 2022). Genetic parameters are used to determine the role of genes in the characters being tested (Donkor et al. 2022). The correlation between quantitative characters is used to determine the characters that correlate with the production character per hectare to be used as a basis for simultaneous selection (Kinfu et al. 2022).

The results showed that the variety assembly method used in the assembly of maize varieties with early maturity and high yielding characteristics were selection and

hybridization methods. The determination of the variety assembly method was based on the high heritability values for all characters and the high genetic distance between the tested lines. G10, G11, G15, and G16 are lines that can be used for assembling varieties using the selection method because they have early maturity and high production per hectare. In addition, the effectiveness of selection on these lines is very high because it has high heritability values on the character of harvest age and production per hectare. According to Ogunniyan and Olakojo (2014), characters with high heritability values will provide high genetic advance values. The effectiveness of selection in the assembly of varieties must pay attention to the correlation value between the characters being tested. Characters that have a significant positive and negative correlation can be used to make indirect selection in increasing or decreasing the desired plant character (Fellahi et al. 2018). Production per hectare has a significant positive correlation with plant height, so the selection of high plants will increase production per hectare in implementing plant selection.

Assembling varieties between maize lines through hybridization must consider the relationship between the two lines to be crossed. The kinship based on morphological characters and RAPD shows that G1 and G15 have a distant kinship relationship so that it will produce a wide variation of offspring and no depression inbreeding occurs (Voillemot and Pannell 2017). The choice of G1 and G15 lines as parents in the assembly of hybrid varieties with early maturity and high production per hectare was because G1 had early maturity (71.33 days) and G15 had high production characteristics (7115.33 kg per hectare). The combination of the two parents is expected to produce hybrid varieties with early maturity and high production per hectare.

Research results are used directly to use maize lines with early maturity and high production to be applied (planted) in the field. The selection of maize lines that can be developed for cultivation is by selecting lines based on quantitative data with early maturity and high production per hectare. The results showed that G10, G11, G16, and G17 had early maturity and high production per hectare so that they could be used as maize for cultivation programs.

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