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Variability for Agro-Morphological Traits of Maize (*Zea mays* L.) Inbred Lines Differing in Drought Tolerance

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ABSTRACT

Drought tolerant genotypes have high yield in optimal conditions and lower, but stable yield in dry environments. Gene bank collection (6,000 accessions) of Maize Research Institute was tested under controlled drought in Egypt, and in temperate climate. The mini-core collection of 15 inbreds and 26 populations was created. Inbreds together with lines B73, A632, Mo17 and few commercial inbreds with different tolerance to drought, were evaluated for agro-morphological traits (plant and ear height, total number of leaves, number of leaves above ear, ear leaf length and width), grain yield, number of rows per ear and number of kernels per row, under optimal and increased density in the field in 2014. Since optimal precipitation for maize growing in Serbia is 425 mm, total precipitations of 873.2 mm along with the average temperature of 18.8˚C were exceptionally good for maize production. According to Principal Component Analysis, traits that contributed to the differentiation and were in common for both densities were: number of kernels per row, grain yield and leaf width. Obtained results indicated that inbreds T4 and T8 performed the highest stability, together with commercial T1 and T2 lines, in both experimental conditions. Cluster analysis based on grain yield and morphological traits, grouped them together with the other drought tolerant lines, apart of B73 and lines that showed sensitivity to drought in previous studies. Higher density conditions, simulating mild stress, contributed to more accurate separation of lines from mini-core collection, which could be used as a source for drought tolerance in breeding programs.

Keywords: drought, inbred lines, gene bank, maize, grain yield.

Introduction

The major implications for global food supply are addressed to drought, due to the expected gradual climate change effects over the next century, and the variation in climatic extremes in the short term that it is expected to bring. Increased temperature is a more predictable outcome than changes in rainfall patterns accompanying climate change (Edmeades, 2013). Moreover, as a general notion, major maize producing areas will be subjected to an evolving array of maize diseases and pests that are new to those areas.

In the regions relying on in-season rainfall, there is considerable inter-annual variation in rainfall total and distribution (Löffler *et al.,* 2005). In some years, yield can be significantly reduced by transient water limitations of varying timing, duration, and severity. Many of these water limitations have minor to moderate impact on yield. However, widespread and prolonged drought that substantially reduces grain yield over a wide area can occur in some years, as in 2012 being the most recent occurrence (Boyer *et al.,* 2013).

Maize (*Zea mays* L.) is one of the most important cereal crops with the largest annual global production (Golbashy *et al.,* 2010). However, most of the 160 M ha of production area is highly affected by drought. The EU countries produce around 12% of global maize, and high air temperatures and water deficit in 2012 reduced their yields by an average of 12.5% (MARS, 2012). In Serbia, maize is the most important crop grown mainly without irrigation, which seriously affected genetic potential for yield (Videnovic *et al.,* 2013), and its yield in dry 2012 has been reduced by an average of 48%.

Breeding progress relies on considerable genetic variability for the trait of interest (e.g. grain yield under drought stress), high selection intensity through screening a large number of genotypes and high broad-sense heritability for the trait of interest (Grzesiak, 2001). Thus, the ability to develop high yielding and stable cultivars is an ultimate goal in most breeding programs. The consistent performance of a genotype, both with high or low yield across different environments is considered as yield stability (Epinat-Le Signor *et al.,* 2001). An ideal maize genotype should have a high mean yield combined with a low degree of fluctuation under different environments (Annicchiarico, 2002). Since drought stress influences the reduction of growth, development and production of plants (Mohammadai *et al.,* 2012), one of the most important goals for maize breeders has been to enhance the stability of performance of maize when exposed to stresses (Campos *et al.,* 2006).

Maize Research Institute "Zemun Polje" gene bank maintains 5,806 accessions and is among the ten largest in the world (FAOSTAT, 2010), thus offering the great opportunities for different breeding purposes. After two-year of screening for drought tolerance in Egypt under managed stress environment (MSE) conditions and further testing in the temperate climate regions of Macedonia and Serbia, a mini-core collection of 41 accessions (15 maize inbred lines, 13 local and 13 introduced landraces) was established (Vancetovic *et al.,* 2010; Babic *et al.,* 2011).

Understanding the environmental and agronomic responses of maize varieties is fundamental to improve the efficiency of maize production. Accordingly, we evaluated a set of 15 maize inbred lines from drought tolerant mini-core collection, together with lines B73, A632, Mo17 and few commercial inbreds with different tolerance to drought, under optimal and increased density in the field. By measuring agro-morphological traits of importance, the aim of this study was to distinguish genotypes from drought tolerant mini-*core* collection with a high mean yield combined with a low degree of fluctuation under different environmental conditions applied.

Materials and Methods

Public maize inbred lines B73, A632, Mo17, three drought susceptible (S1, S2 and S3) and two drought tolerant (T1 and T2) commercial inbreds, along with a set of 15 maize inbred lines from Maize Research Institute "Zemun Polje" drought tolerant mini-core collection (from T3 to T17) were evaluated in the present study.

The experiment was carried out in 2014 in Zemun Polje, Serbia (44°52´N, 20°19´E, 81 m asl), in two plant densities. The soil was slightly calcareous chernozem with 47% clay and received the usual compound of mineral fertilizer. Chosen inbreds were tested in two-replicate trial, set up according to Randomised Complete Block Design. Plants of each genotype were sown in a single row plot per replica, with 10 hills per row and spaced 0.75 m apart. Spacing between hills were 20 cm (i.e. D-20) and 40 cm (i.e. D-40), respectively. Plots were overplanted and thinned to two plants per hill after seedling establishment. Morphological traits, such as - plant height (PH), total number of leaves (TNL), number of leaves above upper most ear (LAE), ear height (EH), leaf length (LL), leaf width (LW) and grain yield (Y) were recorded for each entry in both replicates, on ten representative plants per maize inbred. Grain yield was calculated per plant, after manual harvesting and drying to 14% of moisture content. Yield components: number of kernel rows per ear (NKR) and number of kernels per row (NKR) were recorded on ten randomly chosen ears.

Data matrix was constructed according to mean values for seven agro-morphological traits observed and their standard deviations (SD). Cluster analysis was conducted using square Euclidean distance and complete linkage method. Principal Component Analysis (PCA) was performed based on the phenotypic correlation matrix of the adjusted means of the inbred lines for the nine agro-morphological traits using SPSS 16.0 (http://spss-for-windows-evaluation-version.software.informer.com/). The matrix of distances between maize inbreds was calculated upon the standardized principal components with eigenvalue higher than one. Traits with a correlation > 0.7 were considered as significant for that component. Correlation analysis between the traits observed was performed using Pearson's correlation coefficient.

Results and Discussion

Grain maize producers in Europe experienced an excellent season in 2014, that led to record yields for the EU countries as a whole (MARS, 2014). In Serbia, maize grain yield was around 25% above the five-year average. Rainfall accumulation of 863.2 mm, exceeded by far the optimal sum of precipitation (e.g. 425 mm), estimated for maize vegetative period (Vasic and Kerecki, 1988). Near optimal ammount of rainfall during June, laid the foundation for a good season. Moreover, the rains of July (150 mm above the optimum level) were especially beneficial to the growth of maize, reflected in remarkably vigorous canopy expansion. Ample soil moisture levels sustained the flowering phase and the subsequent early grain-filling period with a very positive effect on yield formation. Fewer hot days (Tmax > 30°C) were recorded in June, July and August (7, 8 and 7, respectively). Consequently, no drought or extraordinary hot spells compromised the pollination of maize. In August, water supply was at near optimal levels during the grainfilling stages. However, abundant rainfall and overly wet soil conditions in September (around 80 mm above the optimum level), hampered and caused significant delays to the harvest, which mainly increased the drying costs, but did not cause considerable yield losses. Thus, extremely high rainfall accumulation in 2014, allowed us to evaluate yield stability among maize inbred lines previously chosen as drought tolerant.

Yield and yield stability across diverse environments and multiple years (i.e. weather conditions) are some of the most important selection targets for plant breeding (Moose and Mumm, 2008). Genotypes with yield stability tend to have higher stress tolerance (Tollenaar and Lee, 2002) and demonstrate greater resource use efficiency (Ipsilandis and Vafias, 2005), allowing them to reach more of their total yield potential as the maximum yield achieved under stress-free growing conditions and nonlimiting resources (Fasoula and Fasoula, 2002).

Plants grown in the same field compete for basic requirements for growth (i.e. sunlight, moisture, and nutrients from the soil), thus, increase of plant population would, to a certain extent, induce stress on the genotypes. Considering that, two plant densities were applied in our experiment: D-40, simulating stress-free growing condition and nonlimiting resources, and D-20, simulating mild stress-induced conditions. Average values for agro-morphological traits observed were presented in Table 1 and Table 2.

Effect of D-20 applied resulted in the reduction of all examined traits. Reduction for morphological traits ranged from 10.2% for TNL to 12.4% for EH. Compared to D-40 growing conditions, average yield reduction was 30.3%, being the lowest in inbreds from drought tolerant mini-*core* collection (i.e. 29.4% reduction), which was in accordance with the results of Andjelkovic *et al.,* (2014). There is substantial genetic variation for plant density tolerance in maize (Sarlangue *et al.,* 2007). Some genotypes yield more as plant density is increased, as was the case for drought tolerant inbred T3, exhibiting 1.3% of yield increase under D-20 (Hashemi *et al.,* 2005; Grassini *et al.,* 2011). Reduction for yield components were 3.7 % for NRE and 11.5% for NKR, respectively.

Dendrogram based on morphological traits and grain yield obtained under D-20 (Figure 1), can be divided into three main clusters (A, B and C). Two inbreds (T15 and T17) from drought tolerant mini-core collection were assigned to cluster A, characterized with the highest values of morphological traits observed. Also, the inbred T15 obtained relatively high grain yield under D-20 conditions. The inbreds T8 and T11, both assigned to cluster B, achieved the highest grain yield compared to all the genotypes from drought tolerant mini-core collection (45.6 g/plant and 49.1 g/plant, respectively), even in compare to public lines B73 and Mo17. The lowest values for the traits observed characterized inbred lines from the cluster C.

Under D-40 plant density, similar distribution of genotypes was observed (Figure 2). This dendrogram can be divided into two main clusters (A and B). Although there was grain yield reduction for drought tolerant inbreds T4, T8 and T11 under D-20 (i.e. 25.5%, 12.7% and 11.2%, respectively), it is important to notice that those inbreds ranged even better compare to grain yield of the rest of inbreds, achieved under higher plant density.

Breeding programs are based on selection for several traits simultaneously and, therefore, knowledge on the genetic association between them is necessary. Correlations detect the strength of relationships between grain yield and the other examined traits. In this experiment, correlation analyses between grain yield obtained under D-20 conditions and morphological traits (Table 3) have shown significant and positive correlations ($P \leq 0.05$ for EH, $P \leq 0.01$ for LW, and $P \leq 0.001$ for PH and LL, respectively). Similar ternd was observed under D-40.

This was in line with study of Rahman *et al.,* (2015). Also, grain yield was in highly significant and positive correlations with ear traits (e.g. NRE and NKR), which is usual association under favorable conditions (Menkir *et al.,* 2009).

In our experiment, PCA was performed for morphological traits, grain yield and yield components in both densities. Under D-20 conditions, PCA revealed that PH, LW, grain yield and NKR contributed to the first axis (PCA1), which explained 59.707% of the total variability. The second axis (PCA2) which explained 16.091% of the variation was defined with TNL, LAE and NRE (Figure 3). Under D-40 conditions, PCA revealed that the majority of the traits observed contributed to the first axis (PCA1), which explained 58.648% of the total variability among the evaluated maize inbred lines. The second axis (PCA2) which explained 15.558% of the variation was defined only with NRE (Figure 4). PCA helps to identify the traits with the highest variability as well as those ones that characterize the distinctness among selected genotypes. According to PCA, traits that contributed to the differentiation and were in common for both densities were: LW, NKR and grain yield.

Conclusion

Ideal genotypes could be considered those that have a large PCA1 score (high yielding ability) and small or absolute PCA2 score (high stability). Higher density conditions, simulating mild stress, contributed to more accurate separation of evaluated maize inbred lines differing in drought tolerance. Since yield stability reflects to higher stress tolerance and greater resource use efficiency, it can be observed that the inbred line T8 from drought tolerant mini-*core* collection was the closest to the ideal genotype, followed by inbred T4, also from drought tolerant mini-core collection together with commercial T1 and T2 lines. These inbreds could be used as a source for drought tolerance in breeding programs.

Acknowledgments

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Table 1. Evaluated agro-morphological traits in maize inbred lines differing in drought tolerance.

Inbred line	PH (cm)	EH (cm)	TNL	LAE	LL (cm)	LW (cm)	Y (g/plant)	NRE	NKR
B73	142.5	67.3	17.7	5.8	69.6	8.0	31.1	17.0	21.5
A632	114.9	42.6	16.6	6.1	67.6	6.9	45.0	14.0	21.0
Mo17	121.3	50.6	16.6	4.8	58.6	8.6	30.6	10.0	21.5
S1	144.2	66.3	18.3	6.1	66.6	9.0	54.0	16.0	27.0
S2	124.5	62.5	16.5	5.4	73.7	8.9	43.6	14.0	32.5
S3	127.3	55.8	16.3	5.1	71.6	9.3	55.2	14.0	21.0
T1	119.7	39.3	16.1	5.8	59.1	6.9	38.8	11.0	23.5
T2	138.6	53.3	16.7	5.6	71.7	8.4	70.1	13.0	31.5
T3	91.4	23.8	13.3	5.1	54.6	5.8	30.0	12.0	19.0
T4	110.0	40.8	15.0	5.0	61.5	8.2	39.6	14.0	24.5
T5	102.8	42.6	14.6	4.7	59.2	6.6	23.6	14.0	24.5
T6	85.5	29.3	12.1	3.9	44.8	6.8	18.7	13.0	18.0
T7	74.3	37.8	15.0	4.5	49.4	7.3	16.1	10.0	18.5
T8	111.9	41.0	17.1	5.6	58.0	8.9	45.6	13.0	27.0
T9	103.3	50.2	13.2	4.9	60.2	8.6	42.3	15.0	18.5
T10	92.8	42.4	15.2	5.3	51.7	7.3	19.2	10.0	18.5
T11	110.5	54.0	14.7	5.1	59.6	7.0	49.1	16.0	23.0
T12	102.0	38.3	13.1	5.0	55.7	7.2	37.7	14.0	24.0
T13	98.5	41.8	13.1	4.0	55.9	7.9	40.3	12.0	26.0
T14	91.3	36.8	14.5	5.2	49.3	6.9	35.8	13.0	21.0
T15	147.4	68.3	17.3	6.0	71.3	8.2	49.0	16.0	27.0
T16	97.5	32.5	14.3	4.9	49.8	5.6	29.1	14.0	21.5
T17	136.3	54.8	18.4	6.9	73.9	8.1	31.8	18.0	28.5

The results present mean values of two replications for D-20 plant density applied (20 cm between hills in the row). S - drought susceptible inbred line; T - drought tolerant inbred line.

Table 2. Evaluated agro-morphological traits in maize inbred lines differing in drought tolerance.

Inbred line	PH (cm)	EH (cm)	TNL	LAE	LL (cm)	LW (cm)	Y (g/plant)	NRE	NKR
B73	151.1	74.4	18.9	6.5	73.1	8.7	47.7	17.0	25.0
A632	120.5	49.8	17.6	6.9	71.0	7.1	56.7	13.0	24.5
Mo17	141.8	67.8	16.7	5.1	58.9	9.1	21.5	10.0	27.0
S1	148.3	80.0	18.9	6.2	70.3	10.0	92.8	16.0	30.0
S2	131.8	65.8	18.1	5.7	76.2	9.5	67.3	14.0	32.5
S3	135.0	57.5	17.7	6.2	74.9	10.2	77.4	15.5	25.0
T1	132.9	42.0	17.9	6.8	63.4	8.0	69.0	12.0	31.0
T2	145.5	61.0	18.0	6.2	74.7	9.6	88.2	14.0	30.0
T3	100.9	27.5	14.4	5.5	57.5	6.1	29.6	13.0	22.0
T4	114.5	41.6	16.3	6.0	67.3	8.9	53.2	14.0	29.0
T5	110.5	48.8	15.2	5.6	67.8	7.0	37.2	14.0	25.5
T6	89.6	32.3	13.2	4.3	46.6	7.0	33.2	14.0	19.5
T7	81.3	43.3	15.8	5.3	51.9	8.0	29.9	12.0	20.5
T8	119.0	49.3	17.2	6.2	61.5	10.5	52.3	14.0	29.0
T9	116.3	60.5	14.6	5.5	66.2	9.1	61.6	15.0	21.0
T10	103.3	47.0	16.2	5.9	54.3	7.6	26.1	10.0	21.0
T11	115.8	59.8	15.2	5.2	61.1	7.5	55.2	16.0	25.0
T12	116.0	46.6	13.8	5.0	60.3	7.5	57.3	15.0	23.5
T13	107.5	47.0	13.6	5.1	59.8	8.6	49.1	12.0	26.5
T14	93.8	43.8	15.2	5.7	51.5	7.3	52.3	13.0	26.0
T15	156.6	73.3	17.4	6.9	77.2	9.0	76.3	16.0	29.0
T16	110.5	40.3	15.5	6.4	54.7	6.4	35.8	15.0	22.0
T17	148.5	66.3	19.1	7.3	77.8	8.4	52.3	17.0	32.5

The results present mean values of two replications for D-40 plant density applied (40 cm between hills in the row). S - drought susceptible inbred line; T - drought tolerant inbred line.

Table 3. Phenotypic correlations between agronomic and morphological traits in chosen maize inbred lines under different densities.

	NRE	NKR	PH	EH	TNL	LAE	LL	LW
D-20								
Y	0.354^{ns}	0.598^{**}	0.652^{***}	0.514^{*}	0.399^{ns}	0.389^{ns}	0.650^{***}	0.534^{**}
NRE		0.357^{ns}	0.592^{**}	0.576^{**}	0.363^{ns}	0.520^{*}	0.586^{**}	0.217^{ns}
NKR			0.630^{**}	0.516^{*}	0.524^{*}	0.444^{*}	0.655^{***}	0.469^{*}
D-40								
	NRE	**NKR**	**PH**	**EH**	**TNL**	**LAE**	**LL**	**LW**
Y	0.510^{*}	0.588^{**}	0.608^{**}	0.527^{**}	0.505^{*}	0.439^{*}	0.677^{***}	0.597^{**}
NRE		0.222^{ns}	0.456^{*}	0.450^{*}	0.297^{ns}	0.348^{ns}	0.545^{**}	0.191^{ns}
NKR			0.691^{***}	0.512^{*}	0.683^{***}	0.537^{**}	0.672^{***}	0.558^{**}

D-20 and D-40 - 20 cm and 40 cm between hills in the row, respectively; *** - significant at the 0.001 probability level; ** - significant at the 0.01 probability level; * - significant at the 0.05 probability level; ^{ns} - non-significant;

Figure 1. Dendrogram of the 23 maize inbred lines differing in drought tolerance constructed using UPGMA cluster analysis of Euclidean distance values obtained by morphological data and grain yield under D-20 plant density.

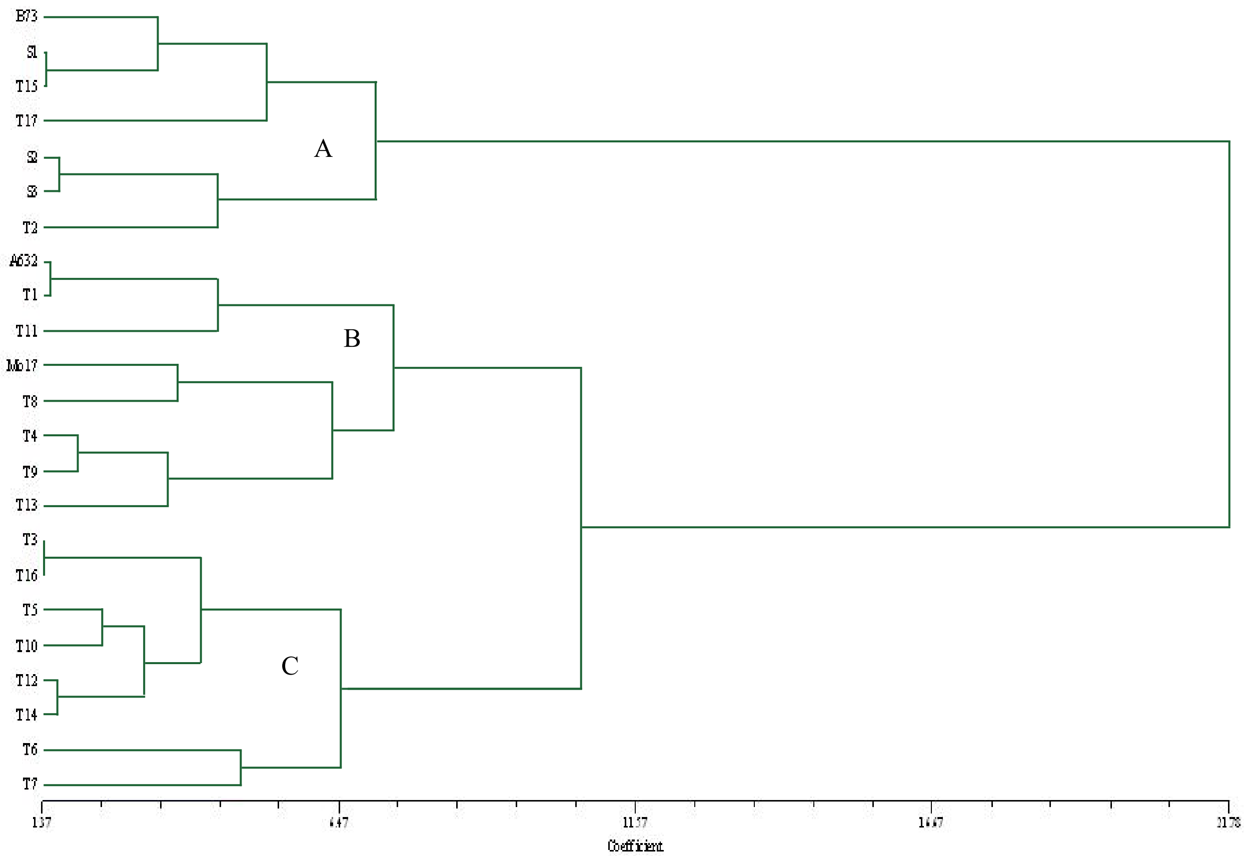
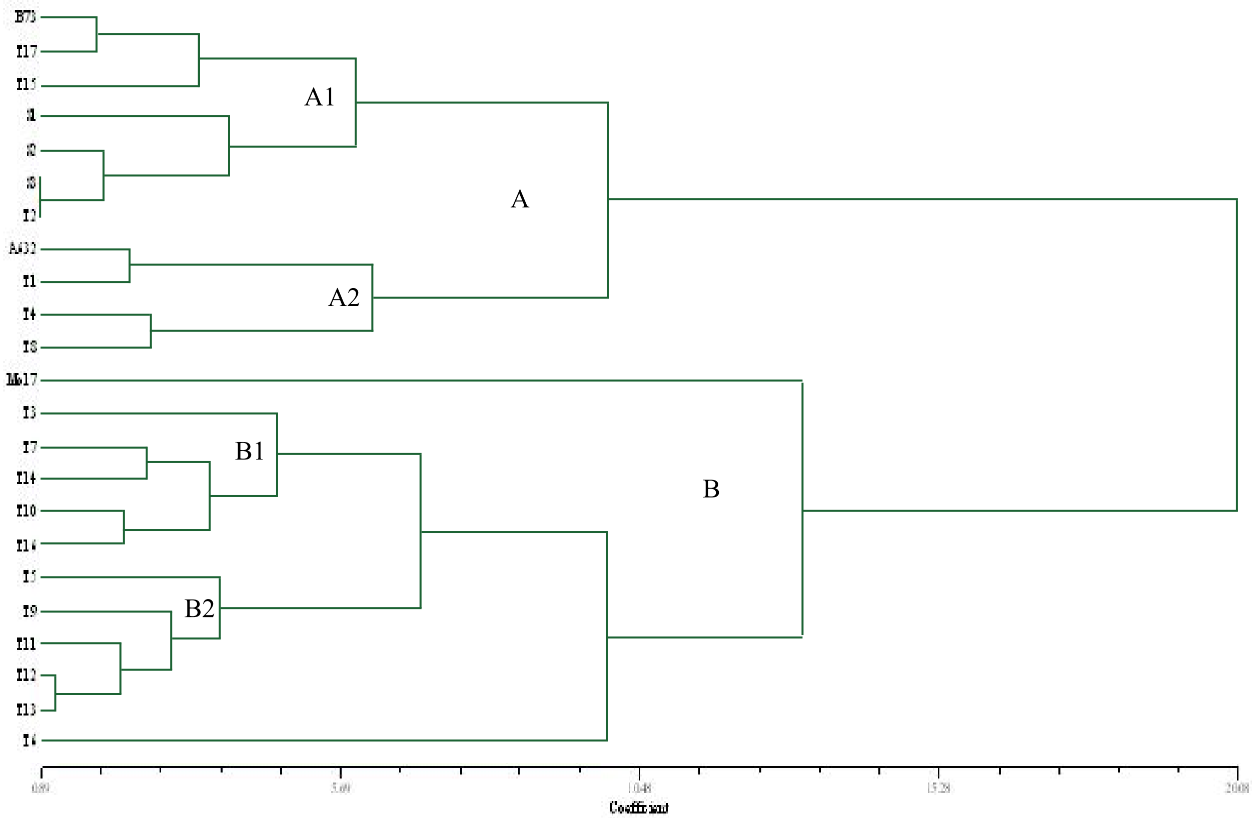


Figure 2. Dendrogram of the 23 maize inbred lines differing in drought tolerance constructed using UPGMA cluster analysis of Euclidean distance values obtained by morphological data and grain yield under D-40 plant density.



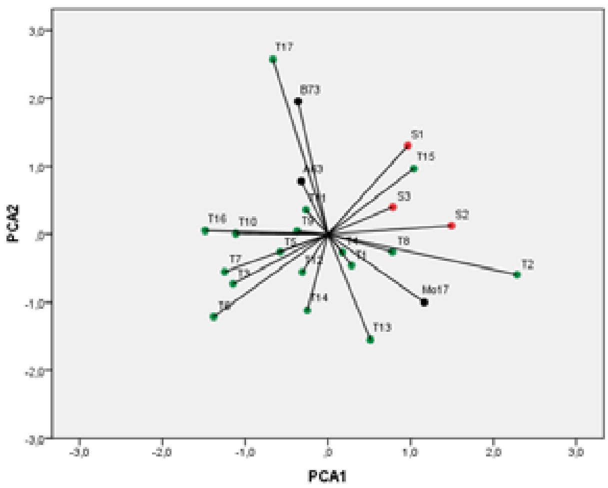


Figure 3. Distribution of the 23 maize inbred lines differing in drought tolerance on the first two principal components PCA1 and PCA2 of the PCA performed for agro-morphological data obtained under D-20 growing conditions.

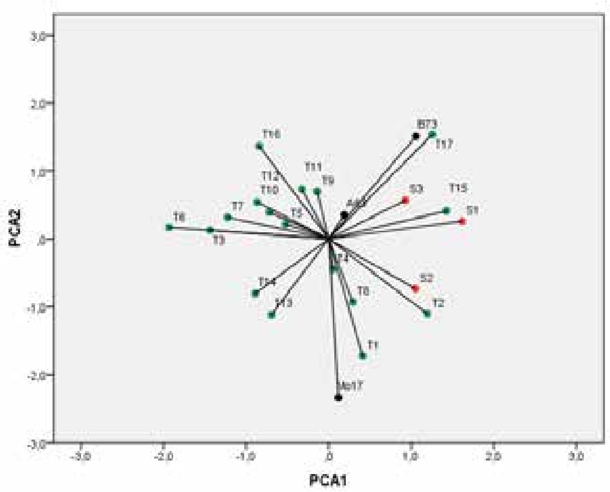


Figure 4. Distribution of the 23 maize inbred lines differing in drought tolerance on the first two principal components PCA1 and PCA2 of the PCA performed for agro-morphological data obtained under D-40 growing conditions.

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