Numerical Classification of Western Balkan Drought Tolerant Maize (Zea mays L.) Landraces

V. Babić^{1*}, J. Vančetović¹, S. Prodanović², N. Kravić¹, M. Babić¹, and V. Anđelković¹

ABSTRACT

Global warming and predictions of climatic changes additionally put breeding for drought tolerance in the focus of breeding programmes for maize. Extensive studies on the existing gene bank collection of the Maize Research Institute "Zemun Polje" have been performed with the aim to identify and form initial sources for the development of maize inbreds more tolerant to drought. All accessions (about 6,000) were exposed to controlled drought stress in Egypt. Out of this number, approximately 8% of the tested genotypes were selected. In this study attention was given to 321 selected Western Balkan maize landraces, adapted to temperate climate growing conditions and the day length. Data derived from morphological characterization according to CIMMYT/IBPGR descriptors for maize, along with the application of numerical classification methods, were used to define homogeneous landraces groups based on morphological similarities. Results obtained from hierarchical and non-hierarchical analyses revealed the formation of 11 divergent groups. According to the obtained grain yield and visually scored stalk lodging and stay green, approximately 15% of the accessions from each of 11 groups were selected. Further investigations are towards defining their heterotic patterns and their possible utilization in developing and improving synthetic populations.

Keywords: Correspondence analysis, Discriminant analysis, Homogenous groups.

INTRODUCTION

Water deficit and extremely high temperatures during flowering, pollination, and grain filling are harmful to most crops, including maize. Both changes in total seasonal precipitation or in its pattern as well as soil water retention capacity are important to agriculture. Potential future adaptations of maize yield to climate changes would require either increased tolerance to maximum summer temperatures in existing maize varieties or a change in maize varieties grown (Southworth et al., 2000; Khodarahmpour et al., 2011).

Although germplasm collections maintained within gene banks are valuable sources for genetic studies and breeding purposes, they represent a raw material loaded with unfavourable agronomic traits, thus, requiring long-term breeding progress. A concept of pre-breeding, allowing the formation of a core collection, is an effective tool to extensively characterize, explore, and use genetic resources stored within a large collection (Brown, 1989; Agrama et al., 2009). The examples are development of a core subset of 900 medium- and latematuring maize local populations from temperate regions of the former Yugoslavia (Radovic and Jelovac, 1994), regional core collections of Brazilian and Uruguayan maize landraces (Abadie et al., 1999; Malosetti and Abadie, 2001). Within the pre-breeding, successful results have been achieved by creating small synthetic

¹ Maize Research Institute, Zemun Polje, Slobodana Bajića 1, 11185 Belgrade, Serbia.

² Department of Crop Science, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia.

^{*}Corresponding author; e-mail: vbabic@mrizp.rs

populations of certain agronomic traits of importance (Nass and Paterniani, 2000). According to Berthaud (1997), there are three ways to use genetic resources: (1) linear model based on *ex situ* conservation in which a desirable accession to be used is directly drawn from a gene bank; (2) triangle model in which a broad genetic basis is progressively reduced by selection to the stage in which the elite material is adequate for use, and (3) circular model based on *in situ* conservation in which use, propagation, and selection are integrated.

There are many examples of the application of the triangle model of use of maize genetic resources, such as Latin American Maize Project (LAMP) (Sevilla et al., 1994); Germplasm Enhancement of (GEM) (Pollak, 2003), Maize **INRA/PROMAIS** project (Gallais and Monod, 1998). Hierarchical Open-ended Population Enrichment (HOPE) (Kannenberg, 2001). The triangle model seems to be the most adequate for the of European improvement maize populations, mainly due to their lower yields and poorer resistance to lodging compared to the elite material. The ideal proportion of non-breeding material for the incorporation into commercial programmes is still undefined. According to Bridges and Gardner (1987), this depends on the aim of breeding (short and long-term programmes), as well as on traits of non-breeding and elite material per se.

Western Balkan landraces, being well adapted to local conditions, could have a significant role as sources of favourable traits in the process of increasing genetic variability of cultivated maize hybrid varieties (Leng, 1962; Reif *et al.*, 2005; Le Clerc *et al.*, 2005; Hadi, 2005; Babic *et al.*, 2012b). Heterosis is a key factor for the development of hybrids, as well as the information on genetic diversity of their potential parental components. Due to the great scope of the work, it is sometimes necessary to classify a large number of accessions into homogenous groups and, then, on the basis of certain criteria, to select a subset of accessions for further work (Smith *et al.*, 1990).

One of the possible ways of grouping the accessions into homogenous groups is the application of the multivariate analysis (Crossa et al., 1995). In genetic studies, a frequently used analysis is the hierarchical cluster analysis. However, application of some non-hierarchical cluster analyses, such as the k-means or the two-step cluster analyses, could be of help (Babic et al., 2012a). One of the principal dilemmas for interpreting the cluster analysis refers to the decision on the number of clusters that is the most adequate for the undertaken studies. In the cases when there is insufficient information about the observed units or when the subjective estimation should be avoided, the assumption on the number of included clusters can be checked by another analysis, i.e. discriminant analysis (Kovačić, 1998; Grillo et al., 2011; Smykalova et al., 2013).

Data derived from morphological characterisation of selected Western Balkan accessions according to CIMMYT/IBPGR descriptors for maize, were used to define homogeneous groups based on morphological similarities. The aim of this study was to select from a larger pool, on the basis of classification results and agronomic traits of importance, the smaller number of landraces for further work related to their heterotic pattern. Finaly, the obtained information will help to develop synthetic populations with increased tolerance to drought, as a source of new inbreds within commercial maize breeding.

MATERIALS AND METHODS

Plant Materials and Field Trials

Project for drought tolerance was established by MRIZP, and one of the first steps was to identify potential sources of drought tolerance within the existing gene bank collection. The study was performed with the collection of 6,382 maize genotypes: 2,217 landraces, collected from all agro-ecological sites of the former Yugoslavia (Western Balkans) and 4165 introduced genotypes (2,830 inbred lines 1,335 populations). The and field experiment was set up at the location of Sids, in the Nile valley, 120 km south of Cairo (Egypt). Based on MRIZP data for characterization and evaluation, the genotypes were classified into the five maturity groups: I: Extra-early, II: Early, III: Medium, IV: Medium-late and V: Late. A Randomized Complete Block Design (RCBD) with two replications was used in the experiment. White dent synthetic population Giza 2, developed in Egypt from CYMMIT DTP-1, over one cycles of S1 recurrent selection for drought, was used as the check (Shaboon, 2004).

The annual precipitation at the location of Sids was nill, and available moisture was controlled by furrow irrigation. Water was applied until the soil was well soaked up to the full saturation with dry patches around plant itself (about 10 cm, Figure 1). Irrigation was applied not later than 15 days prior to flowering within each maturity group. The occurrence of approximately 10% of tassels per a certain maturity group was a signal to terminate irrigation. After that, one Supplemental irrigation was done 25-30 days after pollination, depending on the maturity group. The intensity of this irrigation was much smaller and amounted to about 101 m⁻². Afterwards, irrigation was not applied. The average air temperature during the growing season (August-November) was 28.5° C. The soil of the experimental site at Sids was clayey.

The material was evaluated twice: at vegetative and harvest stages. Under conditions described above, most of the accessions did not survive until the harvest. However, 785 genotypes with complete data have been analysed by the two-step cluster analysis (Everitt, 1980) (SPSS Windows Version 15.0). The results obtained from this part of the experiment were previously reported (Babic et al., 2012a). Accordingly, genotypes of the second and the third cluster, containing 558 accessions (321 local landraces, 98 introduced inbred lines and 139 introduced landraces) were selected. Introduced inbred lines were directly included into the testing process and a part of results on combining abilities, molecularand physiological-biochemical genetic studies were reported (Babic et al., 2011; Assenov et al., 2013; Kravic et al., 2013).

A total of 321 local landraces were the objective of the present investigation. The majority of the landraces originated from Montenegro (29%) and Bosnia and Herzegovina (21%), while 25% of



Figure 1. Field trials at Sids, Egypt.



accessions originated from Serbia, mostly from the southern parts of the country (province Kosovo and southern Serbia). A smaller number of accessions originated from a coastal part of Croatia (8%), Macedonia (9%), and Slovenia (6%). Selected landraces were mainly early and medium-early maturing landraces. predominantly collected in hilly-mountainous regions of the Western Balkans, which are under certain influence of the Mediterranean climate (Babic et al., 2012a).

Due to a large number of selected landraces (321), their assignment into homogeneous groups was undertaken. Twoyear two-replicate trials were set up according to the randomized block design at the location of Zemun Polje, using standard cropping practices. Landraces were sown in two rows with 20 plants per row. A total of 30 plants per landrace were analyzed according to CIMMYT/IBPGR descriptors for maize (1991). In addition to these traits, stalk quality, stay green, and grain yield were estimated. Collection site data (Longitudes: LO, Latitudes: LA and Altitudes: AL), from which the landraces were sampled, were taken from maize database passport data. The aim was to check whether the grouping of landraces, based on the morphological description, was in agreement with their geographical distribution.

Statistical Analyses

Although CIMMYT/IBPGR descriptors for maize contain over 40 different morphological traits, only twenty-seven with scale measures were used for the statistical analyses. Due to different units of measurement, standardization of these 27 traits was undertaken, in order to provide equal contribution of traits to create homogenous groups of accessions. The average values for number of days to anthesis (NDA) were presented, although not used in statistical analyses.

Non-hierarchical (Two-Step Cluster: TSC analysis) and hierarchical (hierarchical cluster analysis) procedures were used (http://www.norusis.com/pdf/SPC_v13.pdf). As data magnitude exceeded both the processing capacities of the available equipment and software for the application of hierarchical cluster analysis, the two-step analysis was applied in the first stage. In this way, 310 analyzed Western Balkan landraces were divided into three large groups (TSC1, TSC2 and TSC3), and further analyzed by the hierarchical cluster analysis. As a measure of the distance, the squared Euclidean distance was calculated using 27 chosen phenotypic traits. Ward's method of clustering was applied (Ward, 1963). According to the obtained dendrograms, a division into 17 divergent groups was assumed. To emphasize the complexity of interrelations between the studied maize landraces, according to morphological similarities, the correspondence analysis was (Greenacre, 1988; Blasius done and Greenacre, 1998). Its advantage is in the fact that it does not hypothesize the assignment of units into certain groups, presenting continued variability more precisely, especially in the cases of significant genetic exchange between geographically close populations (Cavalli-Sforoza, 1994).

For checking the division into 17 groups obtained by the cluster analysis, the discriminant analysis was used, thus minimizing the probability of a wrong classification between groups (Milligan and Cooper, 1985). This analysis also provided the graphical display of cluster centres and their distribution on the discriminant territorial map.

All statistical analyses were performed using the SPSS programme Windows Version 15.0.

RESULTS AND DISCUSSION

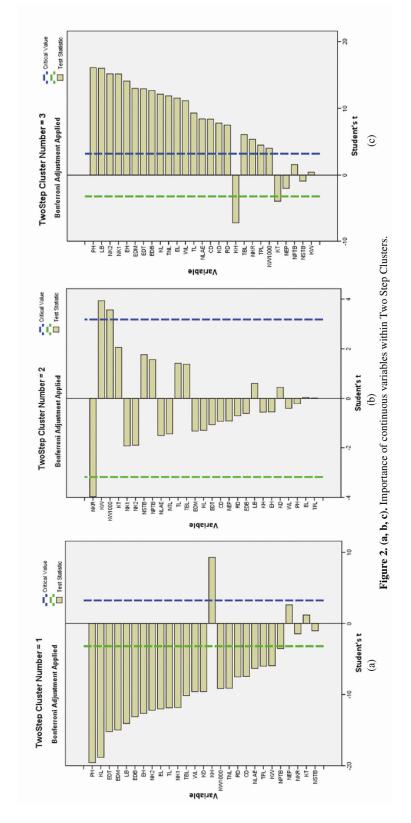
Out of 321 selected and analyzed local landraces, description of 11 landraces could not be done because of a poor emergence rate, high susceptibility to herbicides, stalks susceptibility to lodging and breaking, and ear barrenness. Based on values for 27 analyzed phenotypic traits, selected maize landraces (310) were classified by the twostep cluster analysis into three large groups. The first group (TSC1) encompassed 81 landraces with values for kernel hardness (KH) significantly above the grand mean (P > 95%). Average values for plant height (PH), kernel length (KL), ear diameter at the tip (EDT), ear diameter in the middle (EDM), length of blade (LB), ear diameter at the base (EDB), ear height (EH), number of kernels per row 2 (NK2), ear length (EL), tassel length (TL), number of kernels per row 1 (NK1), length of tassel branches (TBL), width of blade (WL), kernel (KD), 1000-kernel weight dentiness (KW1000), total number of leaves (TNL), rachis diameter (RD), cob diameter (CD), number of leaves above the ear (NLAE), length of a tassel peduncle (TPL), kernel width (KW) and number of primary tassel branches (NPTB) were significantly below the grand mean (P> 95%) (Figure 2-a). The group (TSC2) included second 136 for landraces with values observed parameters between values for TSC1 and TSC3. Average values for the number of kernel rows (NKR) were significantly below, while the values for KW and KW1000 were significantly above, the grand mean (P> 95%) (Figure 2-b). The third group (TSC3) comprised 93 landraces with values for KH and kernel thickness (KT), significantly below the average. Values for PH, LB, NK2, NK1, EH, EDM, EDT, EDB, KL, TNL, EL, WL, TL, NLAE, CD, KD, RD, TBL, NKR, TPL, KW1000 were significantly above the grand mean of the observed maize landraces (Figure 2-c).

Each of these three groups of Western Balkan landraces, obtained by the two-step cluster analysis, was analyzed separately using the hierarchical cluster analysis. Based on the obtained results, six (1-6), five (7-12) and six (12-17) clusters were assigned to the TSC1, TSC2 and TSC3 group, respectively. According to average values, the first six clusters (TSC1) encompassed extra early to early maturing flinty landraces, with short plants, mainly small ears, rounded and relatively small kernels. Clusters 7-11 (TSC2) consisted of medium early maturing landraces, with flinty and medium sized kernels, plant height ranging from 176 to 186 cm, longer ears with higher diameters related to landraces from TSC1. Moreover, these landraces have higher kernel length and higher 1,000-kernel weight. Clusters 12-17 (TSC3), with the exception of intermediated cluster 14, consisted of dent landraces with higher plants, increased number of kernel rows (with the exception of the cluster 15), bigger ears, longer kernels, and a higher 1,000-kernel weight.

Upadhyaya et al. (2002) used Ward's method to separate core collection accessions into groups of similar accessions according to morphological, agronomic and qualitative traits. In the context of this research goals, experience of the analyst is of crucial importance for interpreting the cluster analysis. Although the cluster analysis is very useful in genetic studies, one of its disadvantages is that it presents the results in the form of discrete groups-clusters, even when continuous variability is present in the data. Numerous grouping methods have been proposed. Cluster analysis has been criticized because of the adequate choice of different options being difficult to verify (Bull and Hogarth, 1990). For a better representation of the core collection, cluster methods should be combined with different sampling strategies (Hu et al., 2000). In order to overcome one of the deficiencies of the cluster analysis, correspondence analysis was applied. The lines in the figures of the correspondence analysis encompass dots of landraces belonging to the same cluster.

In Figure 3, overlapping of the regions of the distribution of clusters 2, 3, and 4, as well as of clusters 5 and 6, indicate a similarity between populations of this clusters. The landraces from the cluster 1 made one clearly separated unit. Figure 4 shows a clear differentiation of the clusters 9, 10, and 11, while the regions of the distribution of landraces from clusters 7 and 8 practically could not be separated.

459



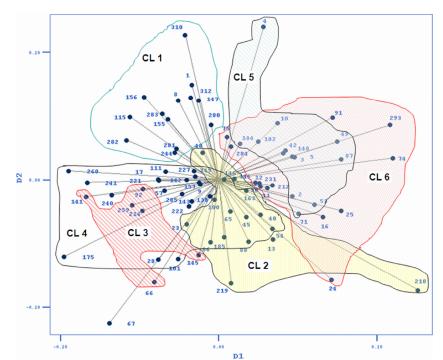


Figure 3. Correspondence analysis of morphological similarities of the TSC1 group of landraces.

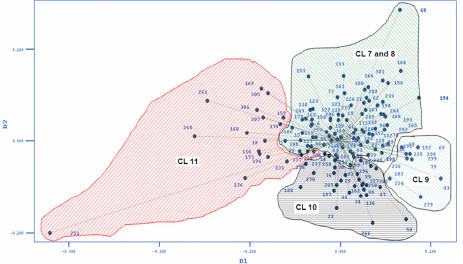


Figure 4. Correspondence analysis of morphological similarities of the TSC2 group of landraces.

Figure 5 represents the overlapping of the regions of clusters 16 and 17, as well as of 12 and 13, while the distribution regions of the clusters 14 and 15 are clearly differentiated. Such a distribution of landraces (i.e. dots) pointed out the assumption that the classification into smaller number of groups (11) would be more appropriate.

One of the principal dilemmas for interpreting the cluster analysis involves deciding where to cut a dendrograms to find the right or natural groups. Use of statistical techniques such as the Multivariate Analysis of Variance (MANOVA) or the Discriminant Analysis, can facilitate the determination of the optimum number of

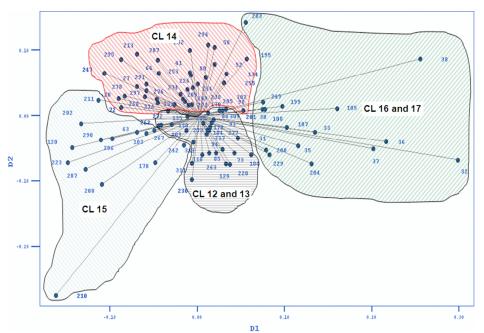


Figure 5. Correspondence analysis of morphological similarities of the TSC3 group of landraces.

clusters (Mohammadi and Prasanna, 2003). The assumption on the division into 17 divergent groups was tested by the Discriminant Analysis, indicating that 74% of the landraces were well classified, while 26% were inadequately classified by the cluster analysis. The first four discriminant functions encompassed 82% of the total data variability, where the first, as most important one, was correlated to the greatest extent to traits relating to ear size (EDM, EDT, EDB, EL, NK) and plant vigour (PH, LB, TNL, EH and WB). The second function was best correlated to cob (CD) and rachis diameter (RD). The best correlation was between the traits of NKR, KW, KT and KW1000 and the third function. The fourth discriminant function was the most strongly correlated with KH and KD, as well as with the length of lateral tassel branches (TBL). Furthermore, the analysis showed that the first 10 discriminant functions were significant, consequently, there was no justification for more than 11 homogenous groups.

A significant overlapping of territories and large centres proximity of particular clusters are observed on the discriminant territorial map (Figure 6). The territories of clusters 2, 3, and 4 were completely overlapped, although their centres were notably differentiated. On the other hand, the territories of clusters 12, 13, 14, and 17 were clearly separated, while centres for clusters 16 and 17, as well as for 12 and 13, were very close. The results indicated week discrimination among these groups of landraces.

Both analyses, correspondence and discriminant, indicate a week discrimination among clusters CL2, CL3 and CL4, CL5 and CL6, CL7 and CL8, CL12 and CL13, as well as between CL16 and CL17. Therefore, reclassification was done by merging accessions of these clusters into 11 groups (G1-G11), and that assumption was retested by the discriminant analysis. The discriminant territorial map (Figure 7) shows that territorial distribution of groups do not overlap, although the centre of the G5 is not placed within its territory, which points out to the lack of good discrimination between G5 and G4 populations. Merging the accessions led to increased number of well

99ааа *ааааааааааааааа

4.0

9 0 A * 9 10 11 centroid

G11

G8

98

ming all functions but the first two are zero

 35
 50

 35
 50

 35
 50

 35
 50

 35
 50

 35
 50

35

35

3554 344 34 34* 34*

24444449

26-66

277 G7 7A

8 8 G9

267*7777799AA

3333244 G4

33332222 224

G2*

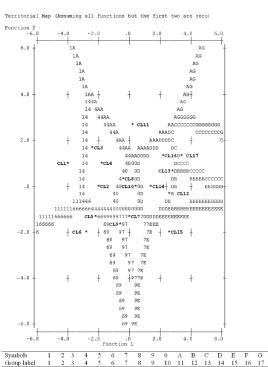


Figure 7. Discriminant territorial map (11 groups).

Territorial Map(As

Function

4.0

2.0

.0

-2.0

-4.0

-6.0

Group label

centroid

333

1113333

1111333333332222

1112222

12

12

12 12

1227

-2.0

-4.1

classified landraces (77%). Further enlarging of groups showed poorer results.

Average values of chosen phenotypic traits were presented only for 11 selected groups (Table 1). Data for LO, LA and AL were taken from MRIZP gene bank passport data. Although there is no strict regularity, a certain consistency in the distribution of selected groups depending on collection site data was noticed. Dent populations of a longer growing season mostly originated from lowlands, while flint populations of a shorter growing season prevailed at higher altitudes. A considerable morphological diversity of landraces, which are often transitional forms between dents and flints, is present in hilly-mountainous regions.

The G1, G2, and G3 group of landraces (TSC1) originated from locations placed at higher altitudes, mainly from Montenegro, southern parts of Serbia, including Kosovo, and to a lesser extent, from FYR Macedonia and Bosnia and Herzegovina. The landraces belong to extra early and early maturity

groups. The first group (G1), with the average altitude of almost 850 m, had the earliest maturity. A certain number of landraces belonging to this group originated from locations placed at the altitude of 1,000 and above 1,000 m, mainly in m Montenegro. The G2 landraces, mainly grown in Montenegro, FYR Macedonia, and Kosovo, also originate from relatively higher altitudes (687 m). The landraces from Montenegro, especially from its northern parts, from Bosnia and Herzegovina, and from the southern parts of Serbia (mainly Kosovo and Metohija), prevailed among G3 landraces. At the same time, those groups of landraces were at the most eastern point.

Groups of G4, G5, and G6 landraces (TSC2) originate from locations at the altitudes ranging from 445 to 473 m. Group of G7 early maturity flint landraces, with robust plants and large kernels, stands out within TSC2. It is noticeable that landraces from this group were grown at higher altitudes (612 m), predominantly in Bosnia

Trait	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11
NDA $(days)^a$	46.6	51.9	51.2	56.1	57.2	56.3	53.5	61.4	58.6	62.9	62.8
PH (cm) ^{b}	140.8	159.0	152.6	182.2	185.5	182.0	182.0	206.5	198.1	222.0	204.7
EH (cm) c	37.7	53.5	49.3	64.2	65.2	66.6	61.7	78.9	72.2	79.1	82.0
$NLAE^{d}$	4.5	4.8	5.0	5.2	5.1	5.0	5.3	5.6	5.6	5.7	5.7
TNL ^e	12.5	13.5	13.9	14.8	14.8	14.7	14.3	16.3	16.0	16.5	16.2
NEP^{f}	1.3	1.3	1.4	1.3	1.2	1.2	1.2	1.3	1.2	1.3	1.3
LB $(cm)^{g}$	53.1	60.5	59.3	68.5	68.8	68.0	68.3	75.1	73.6	78.3	76.1
WB (cm) ^{h}	6.8	7.9	7.4	8.1	8.3	8.4	8.5	8.9	8.6	9.0	8.9
TL (cm) ^{i}	44.3	49.2	48.0	54.6	54.7	54.1	53.8	57.0	57.0	63.5	55.7
TPL (cm) ^{j}	19.5	20.2	18.9	20.9	22.4	22.5	23.0	22.6	21.3	24.1	25.0
$\text{TBL}(\text{cm})^k$	24.8	29.0	29.0	33.7	32.3	31.6	30.8	34.4	35.7	39.3	30.7
NPTB l	12.7	16.8	16.4	17.5	15.2	16.4	18.1	15.8	17.1	18.0	20.1
NSTB ^m	2.8	4.3	4.1	4.5	3.1	3.4	4.6	3.2	4.3	5.0	4.7
NKR ⁿ	12.0	12.2	10.5	10.4	10.1	13.0	13.0	13.2	12.7	10.6	15.2
NK1 ^o	20.1	23.9	26.3	28.0	32.2	30.1	25.1	35.8	34.3	36.8	33.1
$NK2^{p}$	20.6	24.6	26.7	28.7	32.5	30.7	25.8	36.5	34.9	37.7	33.8
$EL (cm)^{q}$	9.8	12.0	12.3	13.9	15.2	14.0	13.6	16.3	16.3	17.1	15.0
EDT (cm) ^{r}	3.0	3.1	2.8	3.3	3.1	3.3	3.6	3.8	3.5	3.5	3.9
EDM (cm) s	3.5	3.6	3.3	3.8	3.6	3.9	4.2	4.3	4.0	4.0	4.5
EDB (cm) ^{t}	3.8	4.0	3.6	4.2	3.8	4.2	4.6	4.6	4.4	4.3	4.9
$CD (cm)^{u}$	2.0	2.1	1.9	2.1	1.9	2.1	2.6	2.4	2.3	2.1	2.5
RD (cm) $^{\nu}$	1.7	1.8	1.5	1.8	1.6	1.8	2.2	2.0	1.9	1.8	2.1
KL (cm) w	0.9	0.9	0.9	1.0	1.0	1.0	1.0	1.1	1.0	1.1	1.2
KW (cm) ^{x}	0.9	0.9	0.9	1.0	0.9	0.8	0.9	0.9	0.9	1.0	0.8
$KT (cm)^{y}$	0.5	0.5	0.4	0.5	0.4	0.4	0.5	0.4	0.5	0.4	0.4
KH ^z	3.7	3.7	3.5	2.9	3.5	3.3	3.6	2.7	3.4	2.6	2.7
KD ^{aa}	2.3	2.3	2.5	3.0	2.5	2.7	2.4	3.2	2.6	3.4	3.2
KW1000 ^{ab}	267.3	285.9	264.7	356.5	321.3	283	349.7	338.5	318.5	380.6	319.7
ALA (degree) ac	42.9	42.6	42.7	42.5	43.4	43.8	43.1	43.9	44.0	43.5	44.8
ALO (degree) ad	18.5	19.3	19.7	19.5	19.0	17.8	17.7	18.5	17.8	18.4	17.6
AL $(m)^{ae}$	849.0	687.1	568.6	444.9	464.0	464.0	619.0	414.9	464.0	276.0	203.5

Table 1. Means of observed phenotypic traits over groups.

^{*a*} number of days to anthesis; ^{*b*} plant height; ^{*c*} ear height; ^{*d*} number of leaves above the ear; ^{*e*} total number of leaves; ^{*f*} number of ears per plant; ^{*g*} length of blade; ^{*h*} width of blade; ^{*i*} tassel length; ^{*j*} length of a tassel peduncle; ^{*k*} length of tassel branches; ^{*l*} number of primary tassel branches; ^{*m*} number of secondary tassel branches; ^{*n*} number of kernel rows; ^{*o*} number of kernels per row 1; ^{*p*} number of kernels per row 2; ^{*q*} ear length; ^{*r*} ear diameter (at the tip); ^{*s*} ear diameter (in the middle); ^{*t*} ear diameter (at the base); ^{*u*} cob diameter; ^{*v*} rachis diameter; ^{*w*} kernel length; ^{*x*} kernel width; ^{*y*} kernel thickness; ^{*z*} kernel hardness; ^{*aa*} kernel dentiness; ^{*ab*} kernel weight; ^{*ac*} average latitude; ^{*ad*} average longitude; ^{*ae*} altitude.

and Herzegovina, northern Montenegro, and in some regions of Croatia (Kompolje-Otočac, Štikovo-Drniš). From breeding aspect, this group of landraces could be extremely important, because of short maturity and favourable agronomic traits concerning ear size and plant height. The results pointed out that this group of early maturing flints probably originated from different introduced genotypes in relation to the rest of the analyzed early maturing flint populations. Groups of G8, G9, G10 and G11 landraces (TSC3) originatd from locations placed approximately one degree more to the west and north, in lowlands (from 174 to 464 m). The G9 group, placed at 464 m, encompassed a certain number of landraces from Slovenia, Croatia, Serbia and Bosnia and Herzegovina. The landraces from the G8 group were predominantly grown in Serbia, mainly collected from the Morava river valley, and Bosnia and Herzegovina, mainly from the vicinity of Ključ. The landraces from the G11 group were collected in the



Figure 7. Geographical distribution of landraces.

vicinity of Novi Sad (Vojvodina) and from the Croatian lowlands (Figure 8).

According to the results of all applied statistical analyses, it could be concluded that the most appropriate classification of 310 landraces (studied on the basis of the morphological description) would be classified into 11 homogeneous groups. The proper choice of core samples depends upon good passport and evaluation data to partition the collection. The stratified canonical sample increased phenotypic variance. The geographical origin of accessions provides indirect evidence of diversity and may be used in the absence of evaluation data on several characters to select useful core samples (Spagnoletti and Qualset, 1993).

Approximately 15% of the accessions from each of 11 groups were selected based on the plant architecture, stay green, stalk

lodging, and yield, while landraces with a larger number of kernel rows, with cylindrical and lower positioned ears, had an advantage. In this way, a total of 40 landraces were selected (three from G1; seven from G2; three from G3; seven from G4; two from G5; three from G6; three from G7; four from G8; three from G9; three from G10 and two from G11).

Further investigations will be towards testing the selected landraces with at least three commercial testers. There are two ways of using selected landraces: formation of broad based composites, from the landraces with similar morphology and heterotic pattern. Long-term methods of recurrent selection would be required for their improvement, considering the low level of favourable alleles for the most important agronomic traits in them. Faster way for improving selected landraces is application



of backcrossing method (Pollak, 2003) with related elite inbreds (through one or two backcrosses). Combination of these two approaches provides probably the best balance in achieving the short, medium and long term breeding goals. Developed synthetic populations will broaden the genetic base of elite breeding material and will be used as a source of new elite inbred lines with increased drought tolerance.

ACKNOWLEDGEMENTS

Part of this work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia through the Project TR 31028. Authors would like to thank the Ministry for the support.

REFERENCES

- Abadie, T., Magalhaes, J. R., Parentoni, S. N., Cordeiro, C. and Andrade, R. V. D. 1999. The Core Collection of Maize Germplasm of Brazil. *Plant Gen. Res. Newsletter*, 117: 55-56.
- Agrama, H. A., Yan, W. G., Lee, F., Fjellstrom, R., Chen, M. H., Jia, M. and McClung, A. 2009. Genetic Assessment of a Mini-core Subset Developed from the USDA Rice Genebank. *Crop. Sci.*, 49: 1336-1346.
- Assenov, B., Andjelkovic, V., Ignjatovic-Micic, D., Vancetovic, J., Nikolic, A., Christov, N. K., Tsonev, S., Abu-Mhadi, N., Vassilev, D., Muhovski, Y., Ilchovska, M. and Todorovska, E. 2013. Identification of SNP Mutations in MYBE-1 Gene Involved in Drought Stress Tolerance in Maize. *Bulg. J. Agric. Sci.*, **19**: 181-185.
- Babic, M., Anđelkovic, V., Mladenovic-Drinic, S. and Konstantinov, K. 2011. The Conventional and Contemporary Technologies in Maize (*Zea mays* L) Breeding at Maize Research Institut Zemun Polje. *Maydica*, 56: 155-164.
- Babic, V., Vancetovic, J., Prodanovic, S., Andjelkovic, V., Babic, M. and Kravic, N. 2012a. The Identification of Drought Tolerant Maize Accessions by Two-Step

Cluster Analysis. Rom. Agric. Res., 29: 53-61.

- Babic, V., Andjelkovic, V., Babic, M., Pavlov, M., Kaitovic, Ž. and Filipović, M. 2012b. Role of Genetic Resources in Diversity Increment of Commercial Maize Hybrids. *Proceeding of Third International Scientific Symposium: AGROSYM 2012*, Jahorina, Bosnia and Herzegovina, PP. 97-103.
- 1997. 7. Berthaud, J. Strategies for Conservation of Genetic Resources in Relation with their Utilisation. In: "Special Issue: EUCARPIA Meeting on Tropical Crop Breeding", (Eds.): Glaszmann J. C., Clerget Β. and Schwendiman J.. Montpellier, France, 11-15 March. Euphytica, 96: 1-12.
- Blasius, J. and Greenacre, M. 1998. Visualisation of Categorical Data. Academic Press, San Diego. PP 107-324.
- Bridges, Jr. W. C. and Gardner, C. O. 1987. Foundation Populations for Adapted by Exotic Crosses. *Crop Sci.*, 27: 501-506.
- Brown, A. H. D. 1989. Core Collections: A Practical Approach to Genetic Resources Management. *Genome*, **31**: 818-824.
- Bull, J. K. and Hogarth, D. M. 1990. The Implications of Genotype×Environment Interactions for Evaluation of Sugarcane Families. Genotype-by-environment Interaction and Plant Breeding. Louisiana State University Agricultural Center LA 70803-2110, USA, 52 PP.
- Cavalli-Sforoza, L. L., Menozzi, P. and Riazza, A. 1994. *The History and Geography of Human Genes*. Princeton University Press, USA. PP 374-376.
- Crossa, J., DeLacy, I. H. and Taba, S. 1995. The Use of Multivariate Methods in Developing a Core Collection. In: "Core Collections Sources", (Eds.): Hodgkin, T., Brown, A. H. D., Hintum, Th. J. L., Morales, E. A. V., John Wiley and Sons, New York, 77-92.
- 14. Everitt, B. S. 1980. Cluster Analysis. *Quality and Quantity*, 14: 75-100.
- 15. Gallais, A. and Monod, J. P. 1998. La Gestion des Resources Genetiques du Mais en France: De leur Caracterisation Jusqu aux Premiers Stades de leur Valorisation. *Comptes Rendus del Academic Agriculture de France*, 84: 173-181.
- Greenacre, M. 1988. Corespondence Analysis of Multivariate Categorical Data by

Weighted Least-squares. *Biometrika*, **75(3)**: 457-467.

- Grillo, O., Miceli, C. and Venora, G. 2011. Computerised Image Analysis Applied to Inspection of Vetch Seeds for Varietal Identification. *Seed Sci. Technol.*, **39(2)**: 490-500.
- Hadi, G. 2005. Maize Varieties Grown in Eastern Central Europe between 1938 and 1983. Acta Agron. Hung., 52: 421-438.
- Hu, J., Zhu, J. and Xu, H. M. 2000. Method for Constructing Core Collections by Stepwise Clustering with Three Sampling Strategies Based on the Genotypic Values of Crops. *Theor. Appl. Genet.*, **101**: 264-268.
- 20. IBPGR 1991. Descriptors for Maize. International Maize and Wheat Mexico Improvement Center, City/International Board for Plant genetic Resources, Rome. Available on: http://www.bioversityinternational.org/elibrary/publications/detail/descriptors-formaizedescriptores-para-maizdescripteurspour-le-mais/
- Kannenberg, L. W. 2001. HOPE, a Hierarchical, Open-ended System for Broadening the Breeding Base of Maize. In: *"Broadening the Genetic Base of Crop Production"*, (Eds.): Cooper, H. D., Spillane, C. and Hodgkin, T.. CABI Publishing, Oxon, UK, 311 PP.
- 22. Khodarahmpour, Z., Choukan, R., Bihamta, M. R. and Majidi Hervan, E. 2011. Determination of the Best Heat Stress Tolerance Indices in Maize (*Zea mays* L.) Inbred Lines and Hybrids under Khuzestan Province Conditions. *J. Agr. Sci. Tech.* **13**: 111-121.
- Kovačić, J. Z. 1998. Multivarijaciona Analiza. Ekonomski Fakultet, Beograd. *St.* 131-175, PP. 206-240. (on Serbian)
- Kravic, N., Markovic, K., Andjelkovic, V., Hadži-Taškovic Šukalovic, V., Babic, V. and Vuletic, M. 2013. Growth, Proline Accumulation and Peroxidase Activity in Maize Seedlings under Osmotic Stress. *Acta Physiol. Plant.*, 35: 233-239.
- 25. Le Clerc, V., Bazante, F., Baril, C., Guiard, J. and Zhang, D. 2005. Assessing Temporal Changes in Genetic Diversity of Maize Varieties Using Microsatellite Markers. *Theor. Appl. Genet.*, **110**: 294-302.
- 26. Leng, E. R., Tavčar, A. and Trifunović, V. 1962. Maize of Southeastern Europe and Its

Potential Value in Breeding Programs Elsewhere. *Euphytica*, **11(3)**: 263-272.

- 27. Malosetti, M. and Abadie, T. 2001. Sampling Strategy to Develop a Core Collection of Uruguayan Maize Landraces Based on Morphological Traits. *Gen. Res. Crop Evol.*, **48**: 381-390.
- 28. Milligan, G. W. and Cooper, M. C. 1985. An Examination of Procedures for Determining the Number of Clusters in Data Set. *Psychometrika*, **50**: 159-179.
- Mohammadi, S. A. and Prasanna, B. M. 2003. Analysis of Genetic Diversity in Crop Plants-salient Statistical Tools and Considerations. *Crop Sci.*, 43(4): 1235-1248.
- Nass, L. L. and Paterniani, E. 2000. Prebreeding: A Link between Genetic Resources and Maize Breeding. *Sci. Agric.*, 57(3): 581-587.
- Pollak, L. M. 2003. The History and Success of the Public-private Project on Germplasm Enhancement of Maize (GEM). *Adv. Agron.*, 78: 45-87.
- 32. Radovic, G. and Jelovac, D. 1994. The Possible Approach in Maize Core Collection Development. Evaluation and Exploitation of Genetic Resources: Pre-breeding. *In Proc.* of the Genetic Resources Section Meeting of Eucarpia, 15-18 March 1994, Clermont-Ferrand, France, 1096 PP.
- 33. Reif, J. C., Hamrit, S., Hechenberger, M., Schipprack, W., Maurer, H. P., Bohn, M. and Melchinger, A. E. 2005. Trends in Genetic Diversity among European Maize Cultivars and Their Parental Components during the Past 50 Years. *Theor. Appl. Genet.*, 111: 838-845.
- 34. Sevilla, R., Salhuana, W., Rubio, J., Avila, G., Paratori, O., Cardenas, F., Zhu, L. H., Pollak, L., Bejarano, A., Santos, M. X., Diaz, C., Fuentes, M., Sanchez, H., Ferrier, M. and Vivo, G. 1994. Latin America Maize Project: A Cooperative Genetic Resources Evaluation Project. In: "Evaluating and Exploitation of Genetic Resources, Prebreeding", (Eds.): Balfourier, F. and Perretant, M. B.. Proc. of the Genetic Resources Section Meeting of Eucarpia, 15-18 March, Clermont Ferrand, France, 289 PP.
- 35. Shaboon, A. E. M. 2004. Genetic Improvement via Selection in Maize. Dissertation, Faculty of Agriculture, University of Cairo.



- Smykalova, I., Grillo, O., Bjelkova, M., Pavelek, M. and Venora, G. 2013. Phenotypic Evaluation of Flax Seeds by Image Analysis. *Ind. Crop. Prod.*, 47: 232-238.
- 37. Smith, O. S., Smith, J. S. C., Bowen, S. L., Tenborg, R. A. and Wall, S. J. 1990. Similarities among a Group of Elite Maize Inbreds as Measured by Pedigree, Fl Grain Yield, Grain Yield Heterosis and RFLPs. *Theor. Appl. Genet.*, 80: 833-840.
- Southworth, J., Randolph, J., Harbeck, M., Doering, O., Pfeifer, R., Rao, D. and Johnston, J. 2000. Consequences of Future Climate Change and Changing Climate

Variability on Maize Yields in the Midwestern United States. *Agric. Ecosyst. Environ.*, **82**: 139-158.

- Spagnoletti Zeulli, P. L. and Qualset, C. O. 1993. Evaluation of Five Strategies for Obtaining a Core Subset from a Large Genetic Resource Collection of Durum Wheat. *Theor. Appl. Genet.*, 87: 295-304.
- Upadhyaya, H. D., Bramel, P. J., Ortiz, R. and Singh, S. 2002. Developing a Mini Core of Peanut for Utilization of Genetic Resources. *Crop Sci.*, 42(6): 2150-2156.
- Ward, J. H. 1963. Hierarchical Grouping to Optimize an Objective Function. *Am. Stat. Ass. J.*, 56: 236-244.

دسته بندی عددی مقاومت به خشکی در ارقام بومی ذرت (.Zea mays L) غرب بالکان

و. بابيك، ج. وانشتوويك، س. پرودانويك، ن. كراويك، م. بابيك، و و. آندلكويك

چکیدہ

گرم شدن جهان و پیش بینی تغییرات جوی توجه به برنامه های بهنژادی ذرت برای مقاومت به خشکی را هر چه بیشتر در کانون توجه قرار داده اند. مطالعات گسترده ای روی مواد جمع آوری شده و موجود در بانک ژن موسسه تحقیقات ذرت "Zemun Polje" انجام شده که هدف آن شناسایی و ایجاد منابع اولیه برای توسعه ذرت درون زاد مقاوم به خشکی بوده است. همه نمونه های ثبت شده (در حدود 6000) در مصر در معرض تنش خشکی کنترل شده قرار داده شدند. از این تعداد، تقریبا 8٪ از ژنوتیپ های آزمون شده انتخاب شدند. در این پژوهش، 21 رقم ذرت غرب بالکان که با شرایط رشد مناطق معتدل و طول روز متوسط ساز گار بودند مورد توجه بودند. به این منظور، داده های به دست آمده از شناسایی ویژگی های ریخت شناسی ذرت مطابق توصیف گرهای RDRYT/IBPGR ممراه با روش دسته بندی عددی برای گروه بندی یکنواخت ارقام بومی ذرت برمبنای مشابهت های ریختی به کار گرفته شد. نتایج به دست آمده از تجزیه های ترتیبی و غیر ترتیبی، شکل گیری 11 گروه انشعابی را آشکار ساخت.. بر مبنای عملکرد دانه و امتیازهای مشاهده خوابیدگی ساقه و سبز گیاه، نزدیک به 15 ٪ نمونه های ثبت شده از هر یک از 11 گروه انتخاب شدند. بررسی های بیشتر در جهت تعیین الگوی نمونه های ثبت شده از هر یک از 11 گروه انتخاب شدند. بررسی های بیشتر در جهت تعیین الگوی مونو یو تیک و امکان کار برد از آن در ایجاد و بهبود جامعه های مصنوعی(سنتیک) خواهد بود.