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Maize (*Zea mays* L.) landraces in eastern Serbia: Characterization and the present cultivation

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Abstract

The evolutionary value of landraces is immense, especially when bearing in mind the constant diminishment of crop genetic variability and the development of intensive agriculture. The assessment of morphological and agronomic traits of landraces, together with molecular markers, is certainly a good basis for the efforts toward their protection. Ten maize accessions, originating from the eastern Serbia, were characterized in this study by the use of morphological and molecular analysis. Since the morphological characterization was performed on the plant material grown on two different locations, environmental influence on the development of morphological traits was also addressed. The accessions were significantly differentiated in most of the characteristics that were measured. However, the results have indicated that the plants grown in two experimental plots were also significantly different due to influence of the different environmental conditions. Another part of the study was represented by the field survey conducted in the eastern Serbia, in the Homolje region, in order to determine the presence of the maize landraces and the extent of their utilization in the region. Traditional agricultural practices related to the cultivation of the maize landraces were found to be important for the preservation of the local maize varieties, as well as the cultural diversity related to the cultivation of this crop. However, it was also found that the cultivation of the maize landraces has substantially decreased during the last two decades and, nowadays, it is performed only on small parcels. The preservation of the landraces is a complex task, which should be approached not only through the conservation of the agricultural practices and *in situ* conservation, but also through proper management and evaluation of existing seed collections. This necessity should be widely recognized as the activity of the national and international interest.

Keywords: maize, *Zea mays*, local varieties, characterization, conservation, zein proteins, electrophoresis, field survey, interviews, ethnobotany

Contents

Introduction

Maize landraces are considered to be a valuable resource because of their high genetic diversity. Furthermore, since they are most often connected to the traditional agricultural practices, preservation of the landrace crops and traditional agricultural practices is interconnected. The maize landraces presence and development in Serbia has a long tradition, since it has been cultivated there for centuries. During the last several decades, commercial maize varieties have become widely accepted in agriculture and their expansion suppressed cultivation of the landraces. The same process occurred in Serbia, and the areas where landraces were cultivated have severely diminished. Due to the underdeveloped commercial agriculture in the southern and eastern Serbia, it is believed that these regions represent a refuge area of the maize landraces, where their traditional cultivation is still being practiced. Unfortunately, there is a lack of studies that are dealing with this issue. Therefore there is a need for research activities that would identify the extent of the maize landraces preserved in these parts of Serbia. Moreover, it is also significant to evaluate and document the genetic diversity of existing collections of the maize landraces in the country. Two mentioned activities are important preconditions for the definition of the effective conservation measures and management of the maize landraces.

General information about the maize

The maize belongs to the family Poaceae, one of the most important families in the order Poales (Bremer *et al.* 2003). The family Poaceae has a world-wide distribution and it is economically the most important of all plant families. Members of this family are the three most important crops for the human food production: wheat, rice and maize (Mabberley 2008). Although the maize is one of the most domesticated plants, its origin and evolution are controversial, but it is accepted that the closest ancestor of the maize is the Mexican teosinte (*Z. mexicana*) (Mabberley 2008; INPhO 2003; Dowswell *et al.* 1996).

The maize is showing remarkable diversity, especially in the South America, its native continent. Maize cultivation extends from the equator to the high latitudes in the north and south, from the sea level to over 3 000 meters, and throughout different climate conditions (Global Crop Diversity Trust 2006). The existence of maize under such a wide variety of environmental conditions indicates that it possesses a great diversity of forms. The degree to which this is exhibited in the maize probably can not be found in any other crop (Mangelsdorf 1974).

Origin of maize landraces in Europe

The maize was brought to Europe for the first time at the end of the 15th century and nowadays it is grown in all the European countries. Due to different climate conditions of the European continent and the Middle and South America, the place of the maize origin, this crop needed to adapt to a cooler European climate with a different day period. The most important part of this process was that the new maize germplasm was introduced for a number of times after the 15th century. The North America flint maize had a key role in these introductions, since it originates from a more similar environment. This was especially important for the populations that were grown in the northern and central Europe (Rebourg *et al.* 2003). During the last five centuries of cultivation, plants brought in a new environment were subjected to a natural selection pressure, as well as to a selection pressure driven by man for traits that were useful in the light of farmers' needs for food, such as high yield. This has resulted in a creation of the new varieties, so called landraces, which were adapted to the regional agro-ecological environment (Reif *et al.* 2005).

Nevertheless, many studies (Gouesnard *et al.* 2005; Rebourg *et al.* 2003, 2001; Gauthier *et al.* 2002) have shown that the maize landraces in the northern and north-eastern Europe exhibit lower genetic diversity than those in the southern Europe. This is mostly due to the process of introduction, since most of the maize introductions have occurred in the southern Europe (Spain, Portugal). Beside this, maize populations in the northern parts were also under the stronger selection due to climate conditions, which has caused higher effect of genetic drift within these landraces. This suggests that the landraces form the north-eastern and the southern Europe have different origins. This is supported by the findings of Edwards & Leng (1965), whose results have shown that the maize landraces in the south-eastern Europe and those in Spain have different origins. The same authors have emphasized that similarity of some morphological characteristics between the maize landraces found in Balkan and the "American Coastal Tropical Flints" is remarkable. However, as Gouesnard *et al.* (2005) suggested, it is necessary to conduct more exhaustive survey of the diversity among the eastern European maize landraces, since most of the data used in the existing studies were related to the western European maize populations. This is important from a global point of view, since such survey would enable the establishment of a core collection that would be representative for the whole Europe (Gouesnard *et al.* 2005).

On the other hand, Leng *et al.* (1962) have concluded that the origin of maize in the south-eastern Europe is unclear, but suggested that the first maize has probably spread into the Mediterranean countries and south-eastern Europe few years after the voyages of Columbus and other explorers. They have also stated that this maize originates from Mexico or the northern South America.

This suggests the same origin of all maize landraces present in Europe. Furthermore, apart from different selection processes, these maize landraces naturally hybridized after the first introductions and the wide range of different ecotypes was developed. The same authors have claimed that maize was first introduced into Balkan by the Turks during expansion of their empire, starting from 14th century. This statement is supported by the similarity of the names used for the maize by all the nations that are settled in this region.

Importance of landrace collections and characterization

The maize is one of the three crops that are the most important as a food resource for the mankind (FAO 1996) and is the most widely produced crop in Serbia (SIEPA 2005). Many international agreements proclaim food security, which implies the conservation of plant genetic resources for food and agriculture. The landraces, as an important source of the genetic diversity and potential material that could be used to broaden the base for plant breeding, are among the main objects of these efforts. The on-farm conservation is a way of conserving landraces that implies traditional maintenance of a more diverse, locally adopted plant population (Bellon 2004). The study done by Louette & Smale (2000) has shown that farmers' agricultural management and seed selection practices protect the phenological integrity of the maize varieties grown in the Mexican community. This was confirmed through morphological and genetic analyses.

Continuity of the existence in the field and the traditional maintenance by farmers is resulting in the landrace's adaptability to environmental conditions. However, there is a concern that only *in situ* conservation will not be enough to preserve the genetic diversity that exists within the landrace populations. Perales *et al.* (2003) has implied that changes that occur with introduction of new varieties and new agricultural practices, lead to evolutionary change and even loss of the landraces. Commercial hybrids have become widely used because they give a higher yield in the short time perspective, and there is a tendency to displace the traditional varieties by these economically more profitable cultivars. The landraces are neglected and endangered, so it is necessary to make efforts to conserve all genetic resources of the crops that are important for food production, such as maize.

It is important to focus research of maize landraces on the regions where modern varieties have not yet been widely adopted and where the landraces production still has an important role in the rural society. These research activities are important for monitoring the threats to maize diversity and for a better understanding of social, cultural and economic factors that have impact on the maize diversity (Vaz Patto *et al.* 2007).

It can be concluded that the maize landraces are a valuable autochthonous source of potentially useful traits and a priceless "bank" of genetic diversity. This conclusion was given by Lucchin *et al.* (2003), who has also stated that the research of distinctive and characteristic traits of the maize landraces could be used to create core populations that would be used as basic nucleus for the maintenance of the landraces. Research of morphological and agronomic traits of the landraces, together with the molecular markers, can be a basis for recognition of geographic indication and thus used for protection.

Yamasaki *et al.* (2005) stated that there are two ways of gene diversity reintroduction into maize. One is the introduction of alleles from teosinte (*Z. mays* ssp. *parviglumis*) and the other is introduction of alleles from the landraces for improvement of the existing modern varieties. Adding these genes would certainly broaden the genetic base of maize breeding programmes and, according to Galarreta & Alvarez (2001, p. 391), "the effective use of maize landraces in breeding programmes is facilitated by a good taxonomic description." Reif *et al.* (2005) have also confirmed that it is important that landraces should not only be conserved in the gene banks. They should also be thoroughly characterized for efficient management and effective exploitation. The maize landraces that are present in the south-eastern Europe have been genetically isolated from the maize in North America. Selection processes that have occurred lead to development of advantageous agronomic traits of these landraces (Leng *et al.* 1962), which makes them worthy of attention in the future endeavours for conservation of the crop genetic diversity.

Maize landraces in eastern Serbia

The eastern Serbia is situated on the border of the Carpathian Mountains, what makes it a unique landscape. It represents a combination of the highland and mountain limestone ranges. This region is one of the least known and explored parts with unique natural beauties. Settlers of this region are also descendants of populations with the different geographical origin. Those people are still maintaining the customs and traditions of which many originate from the old times.

Nevertheless, some villages are geographically and socio-economically isolated, leading to the underdevelopment and suitability for survival of some native agricultural landraces. Those landraces are rare and recently became endangered by spreading of the more productive commercial varieties in the central parts of Serbia. This multiple isolation of the eastern Serbia region created favourable conditions for preservation of the agricultural landraces. In other words, along with conservation of tradition and culture, the old-custom agricultural practice has also been preserved.

In the mountainous regions, such as the eastern Serbia, cattle and 'primitive' agricultural practice are still present as a draft force for the field processing, because of the natural conditions and a low level of development (SIEPA 2005). It is a common practice that maize fields are harvested by hand and, during the harvesting, farmers are removing ears from the husk and leaving the husk on the plant. Harvested ears are then transported to the storage facilities and the rest of the plant is used as a fodder. Maize shelling is nowadays performed in electrical shellers. However, some households are probably still using the old mechanical shellers that are being run manually.

All specific conditions of the eastern Serbia region have contributed to the conservation of several maize landraces. These landraces are distinguished by their high capacity of tolerance to variations of biotic and abiotic conditions, resulting in the yield stability under a low input agricultural system. Constant evolution and adaptation to the environment, both the natural and human-made, resulted over time in the stable phenotype. One of the important characteristics of the eastern Serbia climate, particularly for the agricultural production, is the lack of precipitation and water supply during summer. Soil drought, induced by arid climate, leads to temporary or permanent water deficit, which clearly limits and decelerates plant growth and reduces the organic production (Dodig *et al.* 2005).

Hundreds of years of the maize cultivation in the eastern Serbia have given many varieties of landraces. An evolutionary value of such landraces is immense, especially when having in mind the constant diminishment of the crop genetic variability and the development of the intensive agriculture.

Objectives

The main objective of this study was to evaluate the genetic diversity of the ten accessions of maize landraces collected in the eastern Serbia, using both morphological and molecular methods. It is expected that all significant differences which are observed among traits in the material will reflect the diversity on a wider scale. The acquired data will be a necessary part of the national gene bank material, and a part of the global efforts to preserve agricultural genetic resources through the *ex situ* methods. Available landrace descriptors and their potential in the evaluation and description of the maize landraces have also been assessed.

Furthermore, the state of maize landraces in the eastern Serbia was assessed through a field study, with the aim to determine their presence and the extent of their utilization in this region. Traditional agricultural practices related to the cultivation of the maize landraces were also studied, in order to determine field conditions and the cultural diversity in their place of origin which led to their preservation.

Another objective of the conducted research was development of the specific recommendations for the maize landraces conservation through different national and international activities. The obtained data and results will be of interest for scientists and especially for breeders.

Material and methods

Maize landraces characterization

The ten maize landrace accessions collected in the eastern Serbia region, which are part of the seed collection in the Faculty of Agriculture of the Belgrade University, were used for setting up the field experiment. Although the origin of this collection is confirmed to be the eastern Serbia, more precisely the Homolje region, the exact locations and the time of collection are unknown. It is believed that this material was collected during the last two decades of the 20th century, when the social and economic turmoil at that time was the probable reason why the documentation was so poorly kept. Labels of these samples in the collection are: PF-ZM 4, PF-ZM 5, PF-ZM 10, PF-ZM 12, PF-ZM 18, PF-ZM 19, PF-ZM 26, PF-ZM 34, PF-ZM 37 and PF-ZM 39. In order to characterize and assess these accessions, a field experiment was set up on two locations, at the Maize Research Institute in Zemun Polje and the Institute for Vegetable Crops in Smederevska Palanka (Fig. 1).

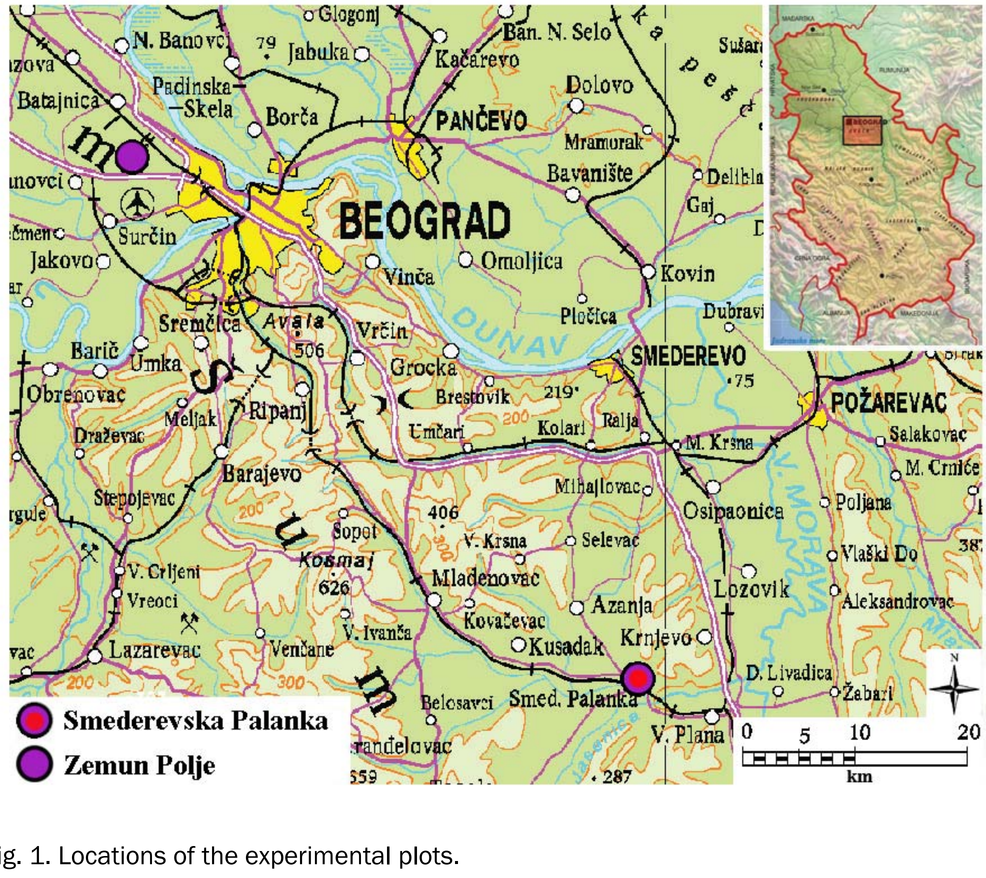


Fig. 1. Locations of the experimental plots.

Experimental plot

Some of the agro-meteorological factors and other data for each location are shown in Table 1. These locations were chosen for setting the experiment because they belonged to the two renowned Institutes, which provided additional help in the arrangement and maintenance of the experiment.

Table 1. Agro-meteorological data for the two experimental sites during the vegetation period (April-September 2008)

Experimental plot location	Smederevska Palanka	Zemun Polje
Temperature (°C, Mean ± SD)*	18,7±4,6	18,7±4,1
Absolute max temperature (°C)*	38	38
Absolute min temperature (°C)*	1	1
Month amount of rainfall (mm, Mean ± SD)*	37±23,7	43,2±26,1
Number of rainy days (Mean ± SD)*	6±3,4	7,5±3,5
Max Standard Precipitation Index (SPI)*	-2,9 (Exceptional drought) in June	-2,5 (Extreme drought) in July
Soil type	vertisol (smonitza)	degraded chernozem
Altitude (m)	104	83
GPS data	N 44°21' 21'' E 20°57' 08''	N 44°51' 58'' E 20°20' 02''

*Source: Republic Hydro-meteorological Service of Serbia

The design of the experimental plot in each location is presented in Fig. 2 and the morphological characteristics were measured on randomly chosen ten or less plants per accession. Sowing on the experimental plots at the Maize Research Institute in Zemun Polje and the Institute for Vegetable Crops in Smederevska Palanka was conducted on 21 April and 29 April 2008, respectively. Each accession was sown in a separate row and the distance between sowing points was 40 cm. In each sowing point, four accession seeds were sown and, when the first two leaves were formed on each of the four sprigs, two sprigs were removed. The distance between rows was 70 cm.

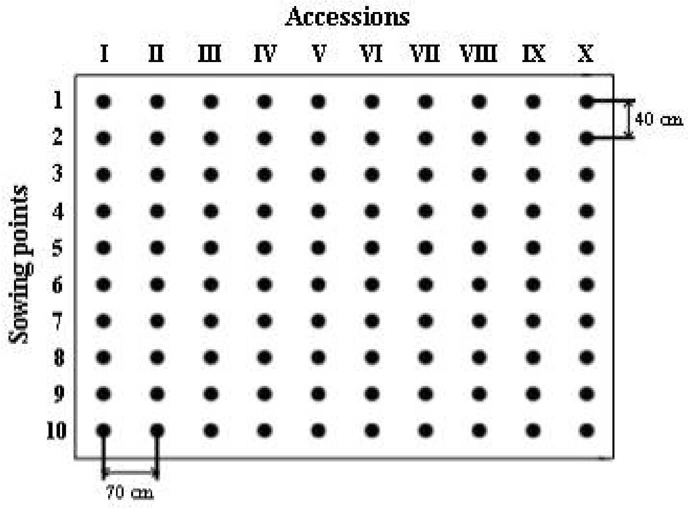


Fig. 2. Design of the experimental plot in each of the two locations.

An isolation of each accession (Fig. 3) in each field experiment was provided by placing a paper bag on each tassel of each plant and by placing plastic bags on each ear before silking (female flowering). After tasseling (male flowering), plastic bags were removed and the paper bags, full of pollen from tassels, were placed over the silk of the uppermost ear of the same plant, providing in that way the self-fertilization of each plant within the accession. In the Institute for Vegetable Crops in Smederevska Palanka, tassels were removed during the fertilization process, so data on tassel characteristics are lacking in this material. Nursery work on experimental plots was performed by technicians from both Institutes, according to the standard field conditions as a part of institute's seed collection regeneration. The experimental plots were visited on regular basis to check the development of the plants during the period from 15 July to 15 September 2008. The characterization of the vegetative parts of the plants was performed on 28 and 29 July 2008 on the experimental plot at the Institute for Vegetable Crops in Smederevska Palanka, and on 5 and 6 August 2008 on the experimental plot at the Maize Research Institute in Zemun Polje. All obtained data was first written on paper, and later transferred to the MS Excel spreadsheets.

Harvest of the material on the experimental plots at the Institute for Vegetable Crops in Smederevska Palanka and the Maize Research Institute in Zemun Polje were conducted on 12 and 15 September 2008, respectively. Further characterization of the plants was performed after drying of the harvested material. Kernels were removed from the cobs by hand after ear characterisation. The data was first recorded on paper and later transferred to the MS Excel spreadsheets.

Fig. 3. The experimental plot at Institute for Vegetable Crops in Smederevska Palanka – isolation of the plants (placing the paper bags on the tassel and the plastic bags over the silk) (permission to use this image granted by S. Prodanović).

Measurements of morphological traits

Plants were characterized for morphological and productive traits with the use of the Descriptors for maize (IBPGR 1991). The reason for choosing these descriptors was that they are commonly used for the characterisation of maize accessions in gene bank collections. The characterization of maize plants was performed with help of the technicians from both Institutes where field experiments were performed. The measured characteristics on the obtained material are presented in Table 2.

Table 2. Measured characteristics on the maize landrace plant material according to the Maize descriptors (IBPGR 1991)

Characteristic		Short description
	1. Vegetative data	
1.1	Plant height (cm)	From the ground level to the base of the tassel
1.2	Ear height (cm)	From the ground level to the node bearing the uppermost ear
1.3	Number of leaves above the uppermost ear	The leaves number above the node bearing the uppermost ear including ear leaf
1.4	Total number of leaves per plant	The leaves number on the whole plant
1.5	Number of ears per plant	The ears number on the plant
1.6	Leaf length (cm)	From ligule to the apex. Measure the leaf which subtends the uppermost ear
1.7	Leaf width (cm)	Mid-way along its length
1.8	Venation index	Divided the number of veins mid-way along the leaf by the leaf width
1.9	Tassel length (cm)	From the base of the tassel to the apex
1.10	Tassel peduncle length (cm)	From the base of the tassel to the lowermost primary branch on tassel
1.11	Number of primary branches on tassel	The number of primary ramifications
1.12	Number of secondary branches on tassel	The number of secondary ramifications
2.	Ear data	
2.1	Ear damage	The amount of the ear damage caused by ear rot and/or insects
2.2	Kernel row arrangement	On the uppermost ear
2.3	Number of kernel rows	The number of kernel rows in the central part of the uppermost ear
2.4	Number of kernels per row	Counted for two adjacent rows
2.5	Ear length (cm)	From the base to the apex
2.6	Ear diameter – top (cm)	Measured in the upper part of the uppermost ear
2.7	Ear diameter – middle (cm)	Measured in the central part of the uppermost ear
2.8	Ear diameter – basis (cm)	Measured in the lower part of the uppermost ear
2.9	Cob diameter (cm)	Measured in the central part of the uppermost ear
2.10	Rachis diameter (cm)	Measured in the central part of the uppermost ear
2.11	Cob colour	Observed on the cob of the uppermost ear
3.	Kernel data	
3.1	Kernel length (cm)	Measured on kernels from one row in the middle of the uppermost ear
3.2	Kernel width (cm)	Measured on kernels from one row in the middle of the uppermost ear
3.3	Kernel thickness (cm)	Measured on kernels from one row in the middle of the uppermost ear
3.4	Kernel hardness/type	Measured on kernels from one row in the middle of the uppermost ear
3.5	Kernel colour	Observed on kernels from one row in the middle of the uppermost ear
3.6	Endosperm colour	Observed on kernels from one row in the middle of the uppermost ear
3.7	1000 kernel weight (g)	Measured for each accession

Molecular analysis of maize landraces

After the characterization of the material harvested on the experimental plot in the Institute for Vegetable Crops in Smederevska Palanka, eight kernels from each of the ten accessions were randomly selected for a zein proteins profile determination by a gel electrophoresis. Zein proteins are storage proteins of the maize kernels and they account for more than 50 percent of all proteins in the endosperm (Yunchang *et al.* 2005). All kernels were soaked in water and incubated for an hour at 65°C, which enabled easy removal of a pericarp and germ. Afterwards, endosperm was dried at 65°C for 16 hours, according to the method described in Guimarães *et al.* (1995), and 100 mg of dried kernel endosperm was pulverized in a ball mill.

Protein extraction

Total extraction of proteins from a kernel meal was performed according to the protocol described in Wallace *et al.* (1990). The extraction buffer was added to each endosperm sample, followed by incubation with shaking at room temperature and centrifugation. After the centrifugation, ethanol was added into the first supernatant fraction to a final concentration of 70 percent in order to precipitate non-zein proteins. The supernatant obtained after another centrifugation comprised the total zein fraction, and was transferred to a new tube. The zein proteins concentration in the fractions was determined by absorbance at 280 nm using the NanoDrop ND-1000 Spectrophotometer.

Gel electrophoresis

A sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Figs. 4 & 5) was performed according to the protocol described in Fling & Gregerson (1986). Following the electrophoresis, gels were stained in Coomassie Brilliant Blue G-250 at room temperature with agitation and destained afterwards, prior to the further analysis. The gels were digitalized and densitometry was performed using the ImageJ software (Rasband 2008). Zein fractions of each sample from corresponding gels were quantified in the software analysis.

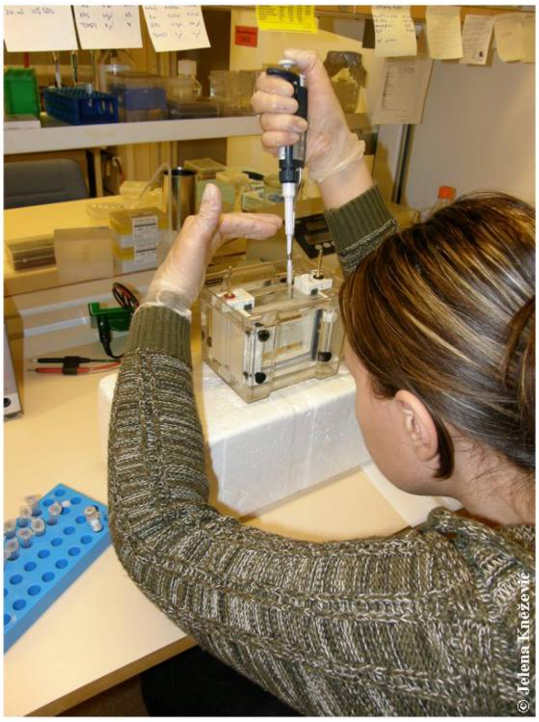


Fig. 4. Loading of lanes with samples of the maize endosperm for SDS-PAGE.

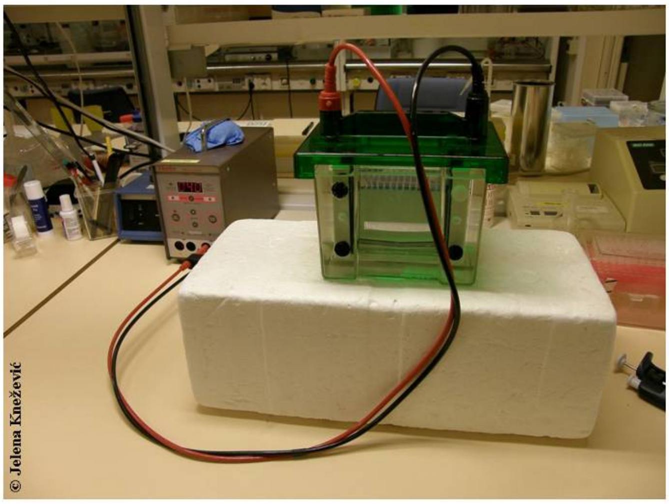


Fig. 5. SDS-PAGE used for analyzing zein proteins in the maize endosperm.

In order to compensate for the differences that existed between gels due to different factors, such as inconsistency of the staining process (Burstin *et al.* 1993), and in order to enable the gel comparison, scaling procedure was applied according to Consoli & Damerval (2001) and Leymarie *et al.* (1996). After densitometry, the integrated density of the bend *i* on the gel *j* was multiplied by a scaling factor (*F*). The scaling factor was calculated according to equation:

$$F_{ij}=m/m_j, \qquad (1)$$

Here, m is a mean integrated density of bands of the calibration set for all the gels, and m_j is a mean integrated density of bends of the calibration set for the gel *j*.

Statistical analysis

All statistical analyses were performed in the SPSS software (Version 15.0, SPSS Inc. 2006). Similar statistical methods were applied on the obtained values from both morphological and molecular analysis. Distributions of variables were evaluated using the Komogorov-Smirnov test for normality. Since the data obtained from morphological characterisation of plants generally had normal distribution ($p>0.05$), parametric tests were employed in the analysis. However, the dataset obtained by electrophoresis lacked the normality of distribution in some variables, so it was log transformed before the parametric statistical tests were applied. In order to assess the existence of differences among different accessions and two experimental plots, multivariate analysis of variance (MANOVA) was applied. Afterwards, the ANOVA and the sequential Bonferroni correction for multiple comparisons were used for comparison of each pair of accessions. Some plants lacked in yield, thus making the morphological measurements dataset unbalanced. Therefore, in order to obtain the most representative results of statistical analysis, most of the tests (MANOVA and ANOVA) were performed separately for vegetative characteristics as one group and ear and kernel characteristics as the other group of data. Nevertheless, all results will be presented together. In order to assess the differentiation of accessions based on all variables that were measured, the Canonical Discriminant Analysis was applied. Relationships among different variables of the molecular analysis were assessed by the use of the Pearson's Correlation test.

The cluster analysis was conducted on Euclidian distances between accessions after data standardization (subtracting with the mean of each variable and dividing by standard deviation). The Unweighted Pair Group Method (UPGMA) was used for the generation of the dendogram by the NTSYSpc (Rohlf 2008). The clustering was performed with complete dataset obtained at each experimental plot, in order to test whether the replications of the same accession could be recognized and grouped in the same cluster. Furthermore, clustering was also done with the mean values of all variables, in order to present relationships among the accessions.

Interview survey during field trips to Homolje region

Field trips to the eastern Serbia region were conducted in order to acquire information on the presence and utilization of the maize landraces in this region today, and on the traditional agricultural practices applied in the cultivation of these landraces. The route and visited locations during these field trips are presented in Fig. 6. Visited locations were situated in the Homolje region, which was the place of origin of the material that was used for the morphological and molecular characterization. Field trips were conducted during the three days (5 and 6 September and 2 October 2008), and the three major cities in the region and a number of villages were visited.

In total, 18 persons with some experience of the maize landraces cultivation were selected for the survey regarding presence and utilization of the maize landraces in the Homolje region. The main method that was used for information retrieval was a focused interview with open-ended questions (Fig. 7). The question list is presented in the Appendix 1. Since most of the interviews were not scheduled in advance, availability of the respondents for the interviewing differed, thus affecting the quality of a certain number of interviews. Therefore, in order to optimize the consistency of the responses, 14 interviews were used for further analysis. The number of respondents that were included in the survey was not large, so data obtained by these interviews were analysed by the use of an inductive coding method (Frankfort-Nachmias & Nachmias 1996; Auerbach & Silverstein 2003).

The answers to each of the questions were coded by two to four codes, whereas the lack of answer was always coded as zero. Following the method described by Auerbach & Silverstein (2003) and Coffey & Atkinson (1996), all coded responses within each question were arranged in the separate document, in order to acquire better overview of the data. Codes were then revised and organized into more general categories, according to their meaning and resemblance. Organizing the data into meaningful codes/categories was the first step in the qualitative data analysis. This was important because many answers comprised more information than it was indicated by the question. Finally, defined categories were used to develop the theoretical construction, which would help to observe the acquired data in a more understandable way. Development of the theoretical construction was a result of the revision of previously defined codes and categories. It was expected that theoretical construction might provide better understanding of the issues related to the study, such as how to approach the problem of the maize landraces preservation.

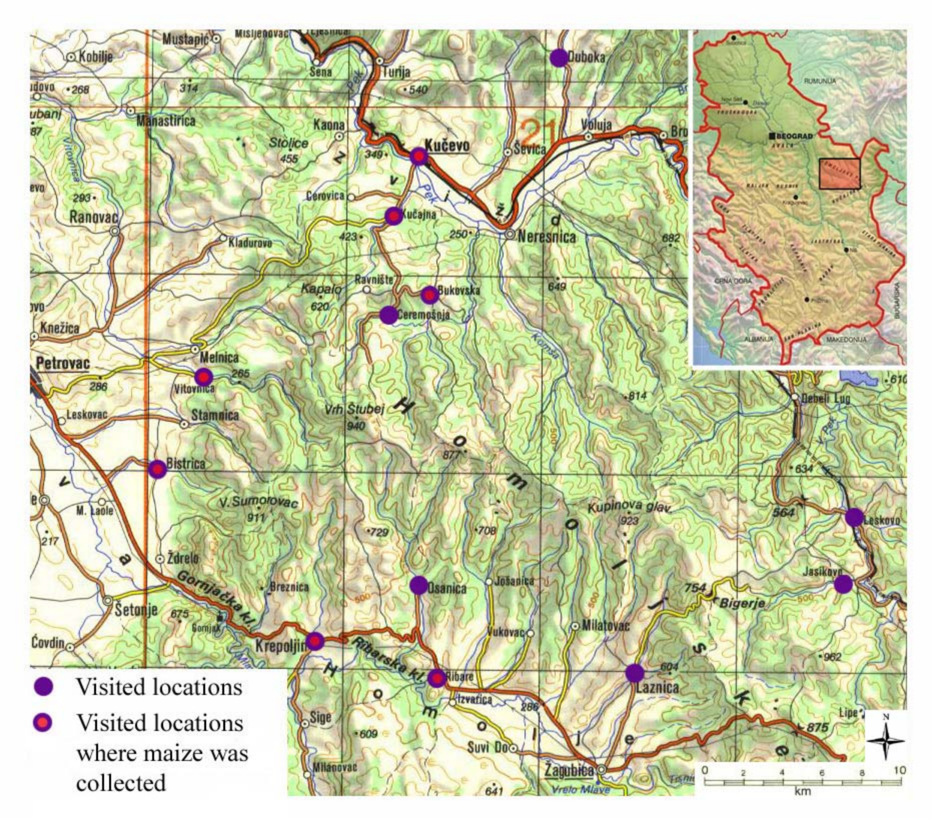


Fig. 6. Visited locations during the field trips to the eastern Serbia – the Homolje region.

Fig. 7. One of the respondents in the survey next to the field where he was growing the white eight-rowed maize.

Results

Maize landraces characterization

The values of the measured morphological characteristics of the accessions obtained from the experimental plots at Smederevka Palanka (Fig. 8) and Zemun Polje are presented in the Appendixes 2 and 3, respectively. Characteristics that were significantly different between two experimental plots for most of the accessions are presented in Fig. 9.

Fig. 8. Selected ears of the studied accessions obtained on the experimental plot at Smederevska Palanka (PF-ZM 4, PF-ZM 5, PF-ZM 10, PF-ZM 12, PF-ZM 18, PF-ZM 19, PF-ZM 26 – presented with kernels since ears were missing, PF-ZM 34, PF-ZM 37 and PF-ZM 39).

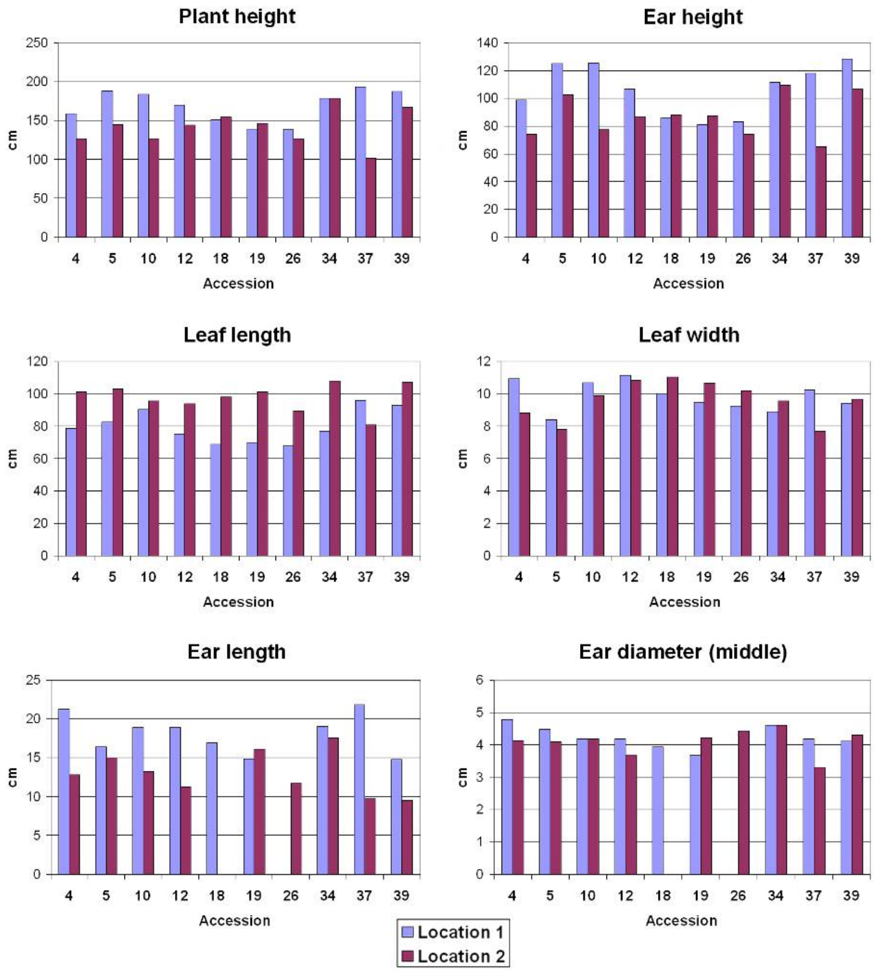


Fig. 9. Morphological characteristics that showed significant difference between two locations for most of the accessions (Location 1 – Smederevska Palanka, Location 2 – Zemun Polje).

The MANOVA test has revealed significant variation among the accessions, as it is shown by highly significant differences among them for all measured traits (Table 3). However, as shown in the table, the highest variation for some traits was influenced by the location, while its effect on some other traits was insubstantial.

Table 3. Multivariate analysis of variance of the ten accessions from the two locations

Variable	F-value		
	Accessions	Locations	Accessions*Locations
Plant height (cm)	9.255**	74.407**	9.064**
Ear height (cm)	11.132**	56.939**	6.173**
Number of leaves above the uppermost ear	2.863**	7.500**	0.878
Number of ears per plant	6.908**	8.538**	1.198
Leaf length (cm)	6.274**	143.848**	7.826**
Leaf width (cm)	10.476**	1.624	4.935**
Venation index	3.780**	0.930	0.874
Number of kernel rows	2.607*	0.043	2.679*
Number of kernels per row (1)	4.246**	0.096	1.963
Number of kernels per row (2)	3.515**	0.352	3.090**
Ear length (cm)	2.543*	44.273**	7.646**
Ear diameter – top (cm)	7.278**	6.540*	4.821**
Ear diameter – middle (cm)	6.667**	8.793**	4.397**
Ear diameter – basis (cm)	6.228**	2.891	5.674**
Cob diameter (cm)	6.775**	19.222**	6.721*8
Rachis diameter (cm)	2.257*	99.706**	1.473
Kernel length (cm)	5.588**	3.574	3.087**
Kernel width (cm)	2.222*	5.234*	4.000**
Kernel thickness (cm)	2.439*	25.517**	2.612*

** Significance at $p<0.01$
* Significance at $p<0.05$

Results of the ANOVA tests for the material obtained on the experimental plots in Smederevska Palanka and Zemun Polje are presented in the Appendixes 4 and 5, respectively. Accessions were significantly different in most of the characteristics that were measured. Although most of the registered variability was between the groups, some variables showed almost the same degree of variation within the groups. The multiple comparisons (Bonferroni adjustments) have shown which variables affected the most of the differentiation of certain accessions from the others. Accessions PF-ZM 18, 19 and 26 were mostly differentiated from the other accessions in the material obtained from the experimental plot at Smederevska Palanka. At the same time, the most differentiated accessions in the material from Zemun Polje were PF-ZM 34 and 37. The accession PF-ZM 34 had the most characteristics with the highest mean value in both experimental plots. Furthermore, accession PF-ZM 37 in the experimental plot at Smederevska Palanka was also noteworthy, as well as the accession PF-ZM 39 in the experimental plot at Zemun Polje.

The dendrogram (Fig. 10) is a result of the UPGMA cluster analysis for the ten accessions from both experimental plots, based on the matrix of Euclidian distances derived from 20 morphological traits. The two locations were clearly separated into two clusters, indicating strong differentiation of the plants grown in these two environments. Similarly, the ANOVA has confirmed that morphological traits were on average significantly differing between two sites in six accessions. The results of this ANOVA test have indicated that the strongest differentiation was present in the accession PF-ZM 37, followed by the accessions PF-ZM 4 and 19.

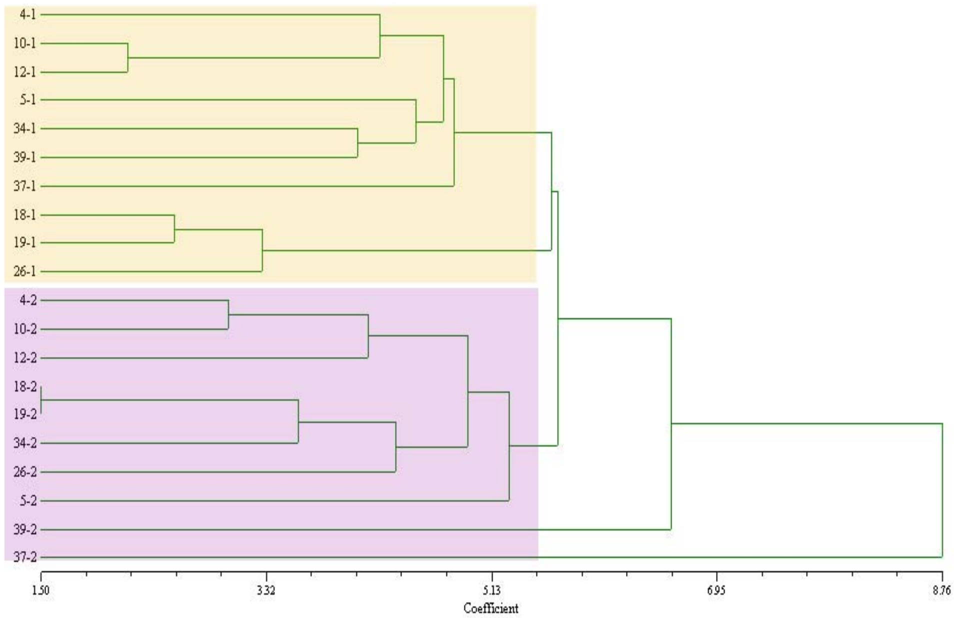


Fig. 10. Dendrogram of the ten studied accessions with the data obtained on two experimental plots (1 – Smederevska Palanka, 2 – Zemun Polje).

When the clustering was performed using separate data for each replicate, in order to check whether the replicates will be grouped in their corresponding accessions, dendrograms from each experimental site have shown similar relationships among the accessions. Most of the replicates of the accessions PF-ZM 18, 19 and 26 were clustered together in the cluster II, when the clustering was performed on the maize material from Smederevska Palanka (Fig. 11). Replicates of the other accessions were spread out over the dendrogram. Replicates presented in the cluster I in Fig. 11 had in average the highest values for the plant and ear height, leaf length and width, ear length and diameter, and kernel length. The cluster II comprised replicates with, on average, the lowest values for these characteristics.

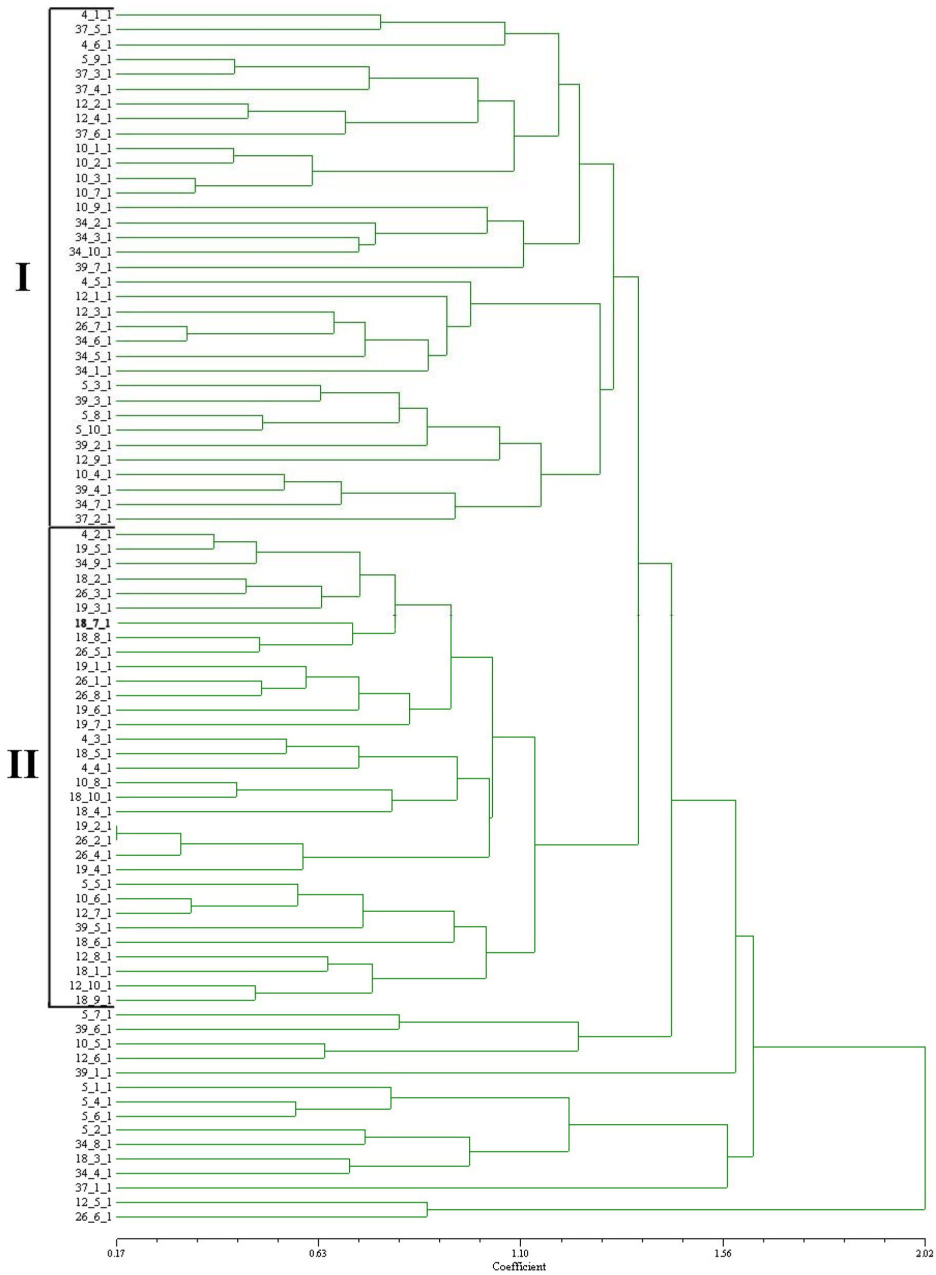


Fig. 11. Dendrogram of the ten studied accessions with data obtained on experimental plot in Smederevska Palanka.

In the dendrogram derived from the Zemun Polje dataset (Fig. 12), there were four main clusters that have successfully grouped replicates of certain accessions: the first cluster comprised most of the replicates of accessions PF-

ZM 4 and 12; the second one grouped the accessions PF-ZM 18 and 26, together with the more than 50 percent of replicates from the accessions PF-ZM 19 and 34; the third cluster comprised all replicates of the accession PF-ZM 5 and the remaining replicates of the accession PF-ZM 34; finally, fourth cluster encompassed all replicates of the accession PF-ZM 37. The clusters II and III in Fig. 13 comprised replicates with, on average, the highest values for plant and ear height, leaf length and width, ear length and diameter, and kernel length. The cluster III comprised replicates that had, on average, lower values for these characteristics, while the cluster IV included replicates with the lowest values.

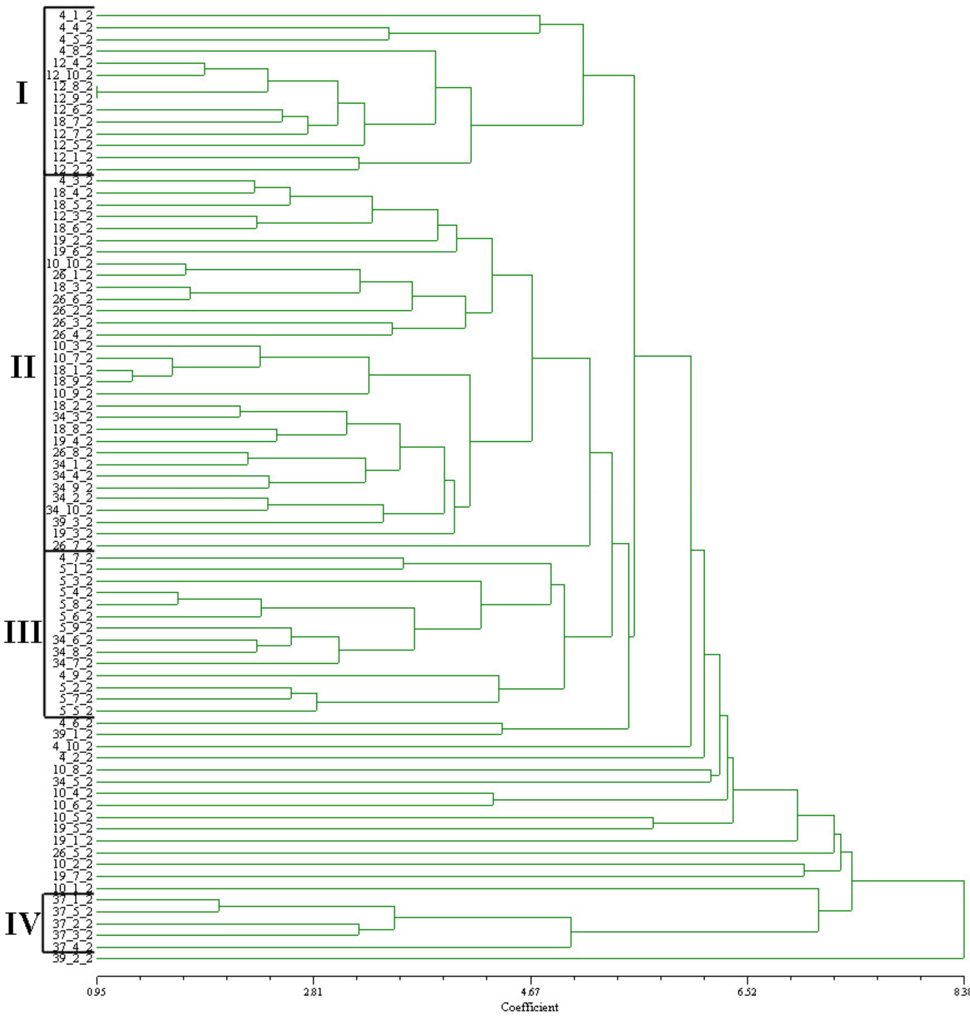


Fig. 12. Dendrogram of the ten studied accessions with data obtained on experimental plot in Zemun Polje.

The Canonical Discriminant Analysis of the measured characteristics on the plant material from Smederevska Palanka is presented in Fig. 13. The first two canonical functions described 72.4 percent of the existing variance. The plant height had the strongest effect in the Function 1, followed by the ear height, ear top diameter and cob diameter, while the Function 2 was mostly influenced by the ear length, kernel length and leaf width. The accessions PF-ZM 18 and PF-ZM 19 were separated from the other accessions along the Function 1, while the accessions PF-ZM 5 and PF-ZM 39 were shifted along the Function 2. The other accessions were fairly well grouped.

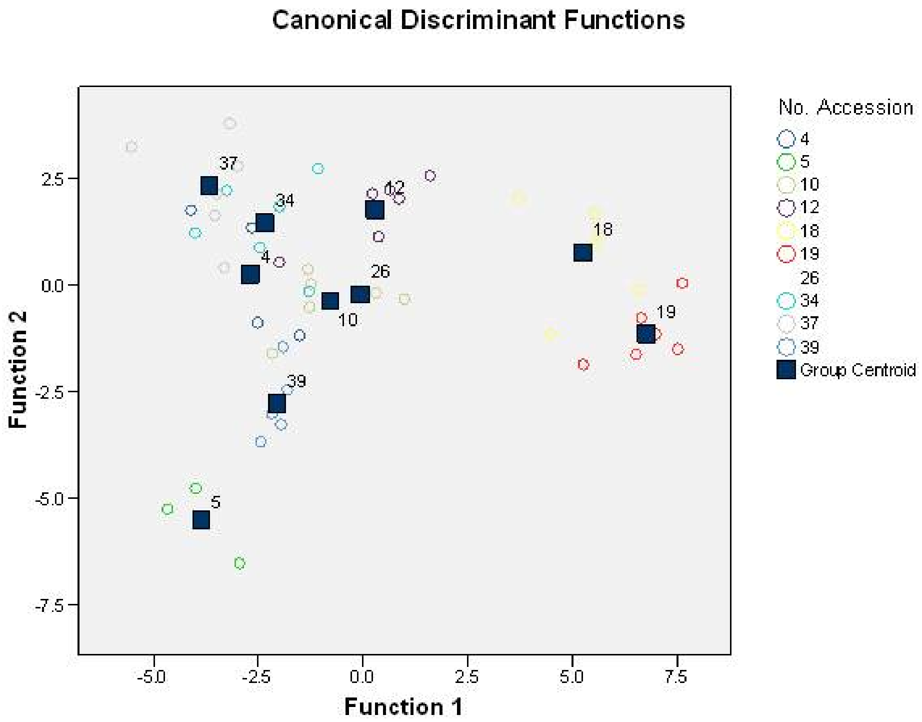


Fig. 13. Canonical Discriminant Analysis of the ten accessions grown in Smederevska Palanka.

The results of the Canonical Discriminant Analysis of the characteristic measurements obtained from the material grown in Zemun Polje are presented in Fig. 14. The first two canonical functions explained 67 percent of the total variance. The Function 1 was mostly influenced by the leaf width, number of leaves above the uppermost ear and leaf length. At the same time, the Function 2 was mostly influenced by the number of primary branches on the tassel, ear diameter at the middle, ear diameter at the basis and kernel length. The accessions were roughly divided into tree groups, where PF-ZM 4 and PF-ZM 5 were separated along the Function 1, and the accessions PF-ZM 12 and PF-ZM 37 were differentiated along the Function 2.

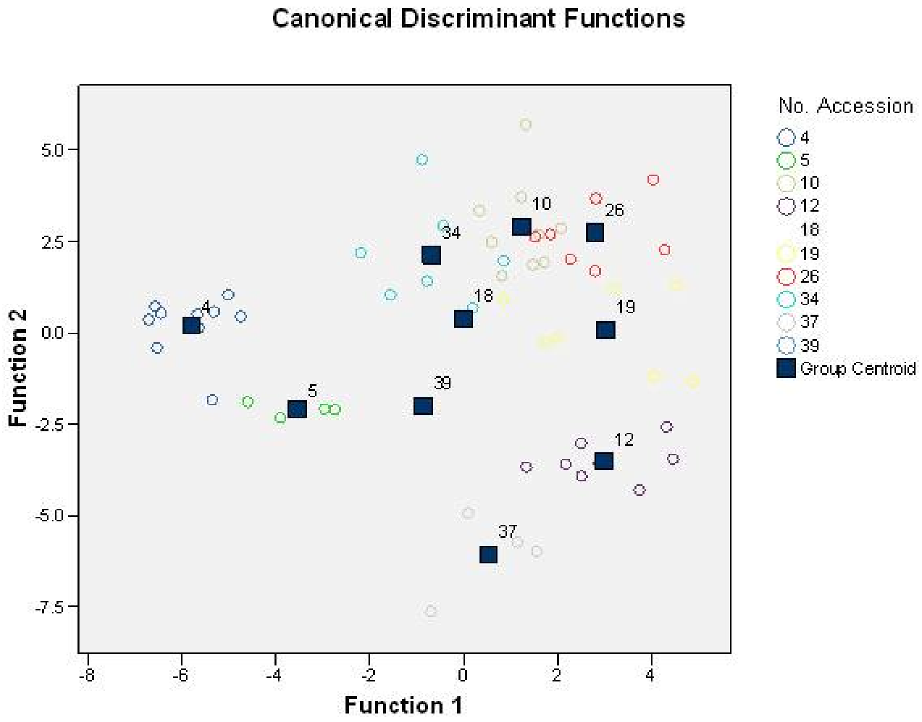
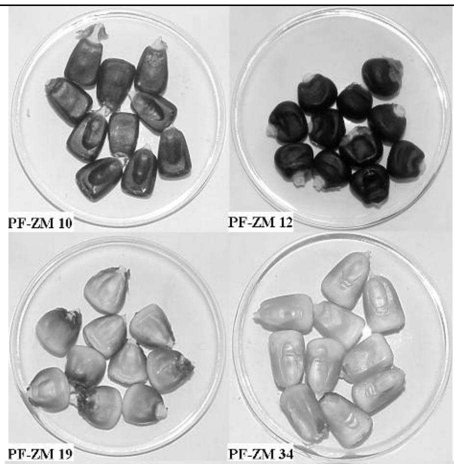
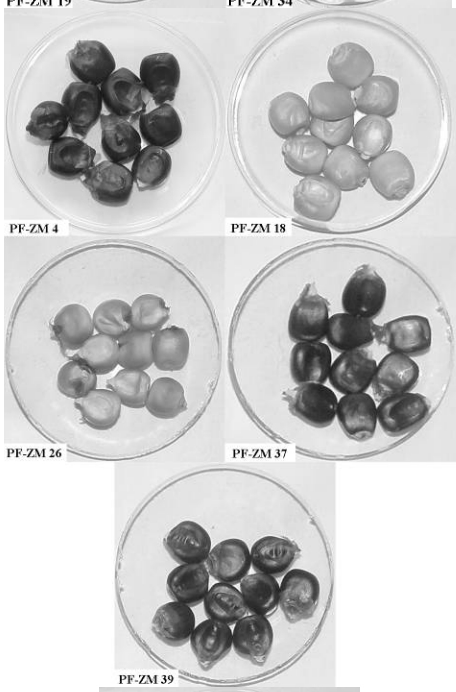


Fig. 14. Canonical Discriminant Analysis of the ten accessions grown in Zemun Polje.

According to estimated kernel characteristics of the ten studied accessions they belong to dent, flint and semi-flint types of kernel endosperm. Accessions PF-ZM 10, 12, 19 and 34 are dent, accession PF-ZM 5 is flint and accessions PF-ZM 4, 18, 19, 37 and 39 are belonging to semi-flint kernel type (Table 4).

Table 4. Kernel types of the ten studied accessions and estimated characteristics that were used to define to which group the accession belongs

Type of endosperm	Texture of endosperm	Shape of upper kernel surface	Studied accessions
Dent	Hard and soft	Dented	
Semi-flint	Mostly hard	Slightly dented	
Flint	Mostly hard	Rounded	

Molecular analysis of maize landraces

The molecular analysis of endosperm proteins in the studied accessions, gave ten electrophoretograms/gels as a result. Each one was presenting both qualitative and quantitative structure of zein proteins for each accession (Fig. 15). The gels have shown the presence of five different fractions of zein proteins, which are classified as α-zeins and γ-zeins (Zhu *et al.* 2007; Azvedo *et al.* 2004). The α-zeins comprised in average 64 percent of the total registered zeins. Molecular weights of α-zein fractions identified on the gels were 17, 18, 21 and 24 kDa, and the only fraction identified as γ-zein was 27 kDa. The accessions PF-ZM 4, PF-ZM 5, PF-ZM 10, PF-ZM 12, PF-ZM 18, PF-ZM 19, PF-ZM 37 and PF-ZM 39 comprised all five fractions of zein proteins. At the same time, samples of the accession PF-ZM 26 comprised only the α-zein fractions of 24 and 21 kDa and the γ-zein fraction, while the accession PF-ZM 34 did not comprise the α-zein fraction of 18 kDa.

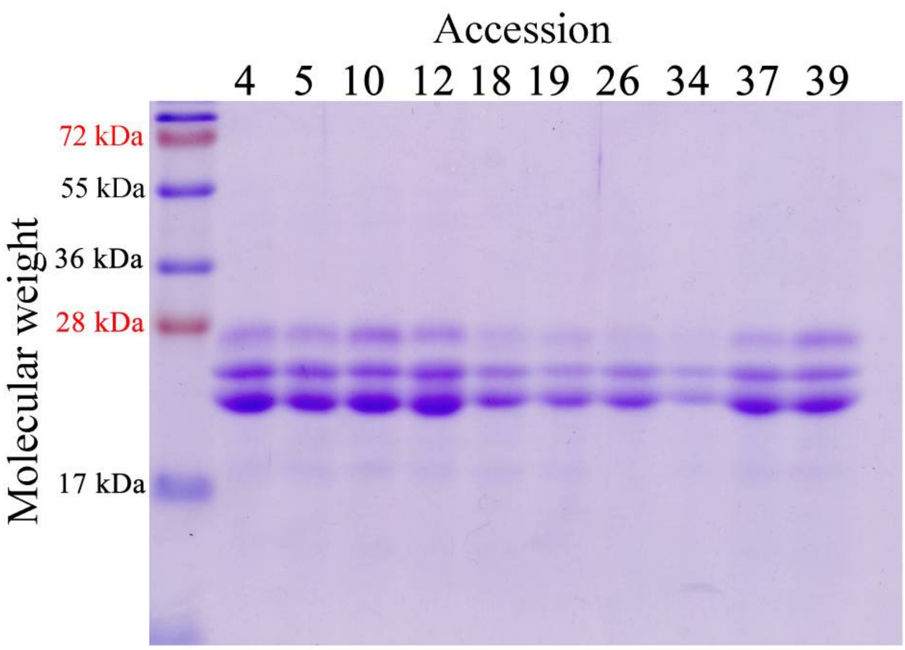


Fig. 15. SDS-PAGE gel comprising α- and γ-zein fractions from the ten analyzed maize accessions (densitometry was performed on the separate gels for each accession).

All detected zein fractions, except the 24 kDa fraction, were showing significant differences among the different accessions, as it is presented in Table 5. Variations of all zein fractions were higher between than within compared groups. The Bonferroni correction for multiple comparisons indicated that the accession PF-ZM 26 was significantly differentiated from all other accessions based on the γ-zein fraction of 27 kDa, as well as the α-zein fractions of 18 and 17 kDa. Similarly, the accession PF-ZM 34 was distinguished from the other accessions by 27 and 18 kDa fractions. This was most likely due to the absence of the 17 and 18 kDa fractions in the accession PF-ZM 26 and the fraction 18 kDa in the accession PF-ZM 34. However, the α-zein fraction of 17 kDa influenced significant the differentiation of the accession PF-ZM 37 from PF-ZM 5 and 18.

Table 5. Analysis of variance of zein protein fractions among different accessions

Zein fractions		Mean Square	F-value
	Between accessions	0.109	8.811*
	Within accessions	0.012	
24 kDa	Between accessions	0.014	0.857
	Within accessions	0.016	
21 kDa	Between accessions	0.037	2.864*
	Within accessions	0.013	
18 kDa	Between accessions	1.383	73.126*
	Within accessions	0.019	
17 kDa	Between accessions	0.667	33.180*
	Within accessions	0.020	

* Significance at $p<0.01$

Overall correlations (Pearson parametric correlation test) among the observed zein protein fractions were mostly significant, as it is shown in Table 6. The strongest correlation was between the fractions of 24 and 21 kDa. These two fractions of α-zeins are typically the most abundant in the maize endosperm and they comprised in average 54 percent from the total registered α-zeins.

Table 6. Pearson correlations of zein fractions

	27 kDa	24 kDa	21 kDa	18 kDa	17 kDa
27 kDa					
24 kDa	0.416**				
21 kDa	0.556**	0.732**			
18 kDa	-0.518**	0.127	-0.241*		
17 kDa	-0.271*	0.018	-0.063	0.662**	

** Correlation is significant at $p<0.01$
* Correlation is significant at $p<0.05$

The Canonical Discriminant Analysis showed a high differentiation of certain accessions (Fig. 16). Two canonical functions accounted for 95.6 percent of the total heterogeneity (the Function 1 stands for 69.6 percent and the Function 2 for 25.9 percent). The most influential variable presented in the Function 1 was the zein fraction of 18 kDa, followed by the 17 kDa zein fraction, while in the Function 2 the strongest effect was of the 17 kDa fraction. Obvious differentiation of the accessions PF-ZM 26 and 34 could be explained by the absences of certain fractions of α-zeins. The cluster analysis confirmed the result of the Canonical Discriminant Analysis, since the same two accessions were singled out into distinguished cluster, while the others were distributed with smaller grouping throughout the dendrogram.

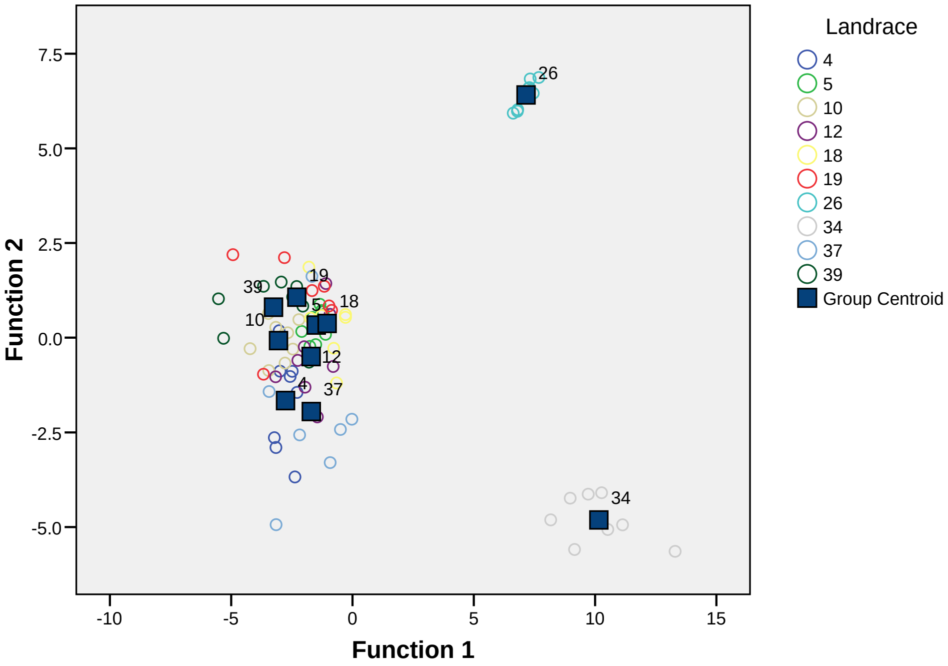


Fig. 16. Canonical Discriminant Analysis of zein fractions in the ten studied accessions.

Interview survey in eastern Serbia

As a result of the survey performed in 13 settlements in the eastern Serbia, in the Homolje region, an interview datasets were obtained. Since the respondents were chosen on the basis of their engagement in cultivation of maize landraces, most of them (12 out of 14) were growing at least one maize landrace. However, the extent of the area on which they were cultivating maize landraces was less than 0.1 hectare (5 respondents out of 11). Two respondents grow more than 1 hectare. "Eight-rowed white", "eight-rowed yellow" and "old domestic" maize are names of the old varieties that were cultivated by the respondents. The maize variety with red kernels, that was cultivated previously in this region, seems to have completely disappeared. All respondents that were engaged in the cultivation of maize landraces have claimed that their main motivation for its growing was to use them for their own flour consumption, followed by the cultivation for the preservation of the landrace (4 respondents out of 13) and the third reason was for commercial purposes (2 out of 13). Regarding the time span of the maize landrace cultivation, two different answers were obtained (9 out of 14): since always and recently - five and four responses, respectively. Furthermore, all persons that have stated that they are growing landraces since always, have also said that maize landraces were inheritance from their parents. Others have claimed that they had acquired seed from the neighbour villagers, but there is a general claim among most of the respondents that there is a strong practice of exchanging landraces seed material.

When respondents were asked to describe characteristics and to explain differences of maize landraces in comparison to commercial varieties, many interesting information were acquired. The most important characteristic/statement was that the flour of maize landraces is tastier than the one from commercial varieties. Along with this characteristic, there was an often emphasized fact that cobs and kernels of these landraces are drier and harder than other varieties. Moreover, old maize varieties are more resistant to unfavourable environmental conditions. Another important detail was that maize landraces cultivation is directly connected to the presence of the water–mills (Fig. 17). The grinding of maize landraces grain is practiced only in water–mills, which are fairly preserved on small mountainous streams within villages or in their vicinity. Regarding their opinion about how many people in the village is still cultivating maize landraces, most of the respondents (9 out of 10) answered that there are few households that are still practicing the small-scale cultivation, and only one person has confirmed the existence of the cultivation for commercial production. However, only one household was engaged in this type of production.

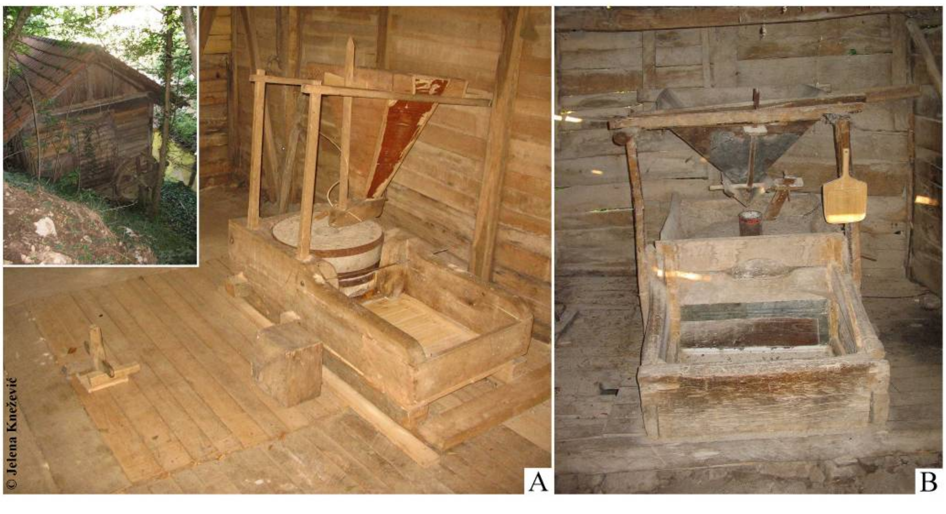


Fig. 17. Water-mills: repaired one in Ceremošnja (A) and the old one in Bistrica (B), these mills are still used for grinding of maize grain. Turning of the water wheel by the mechanical power of the river is causing the gear-wheels system to run. This process is making upper millstone turning while lower is fixed to the floor. Maize grain is grinded between the two millstones.

Application of the inductive coding method has provided further insight in the survey results. The answers obtained from the interviews were coded according to the previously identified repeating ideas in an attempt to organize and sum up relevant text. Next step was to recognize what was mutual for codes and, as a result of this process, three categories were defined: maize landraces cultivation for commercial benefit, cultivation for landraces preservation and, finally maintaining the tradition. The last two categories were without hesitation recognised as a single theoretical construction, named in this study as "Devotion to the traditional way of living". However, the first category, although it was implying different motivation, could be considered as a supporting category to this theoretical construction, due to its positive attitude towards the landraces preservation.

As a result of the field trips to the Homolje region, new samples of maize landraces were collected on seven locations (Bukovska, Vitovnica, Bistrica, Ribare, Kučajna, Kučevo and Krepoljin – Fig. 6). In total, 24 maize ears were collected and documented (Fig. 18), which have been deposited in the seed collection in the Faculty of Agriculture of the Belgrade University.

Fig. 18. Collected ears of eight-rowed and old domestic maize in the Homolje region (A, C, F, G – Kučajna; B – Kučevo; D – Bukovska, Bistrica and Vitovnica; E – Bukovska).

Discussion

Characterization of maize accessions

The overall variation between different maize landraces is normally caused by the two separate components – genetic, environmental, and their interaction (Ferro *et al.* 2007). The results obtained in this study support existence of the effect of all three components in the ten studied accessions. Results of the MANOVA and cluster analysis (Fig. 10), as well as the ANOVA that compared the accessions' performance in each site, have all indicated that there was certain differentiation among plants grown in the two different locations. Location, representing the environmental factors, is causing the highest variation (high F-values) for some traits, but its effect on some other traits is insubstantial. The Fig. 9 is showing that some of the measured characteristics were higher on one location, while the others were lower. These results are interesting, but in order to explain this variation, more tests are necessary.

Although neither of these two sites was similar to the environment where these maize landraces were originating from, the setup of the experiment enabled testing of the degree of differences in phenotypic expression of the same genotypes. Environmental conditions that were most different between these two locations were the humidity and soil type (Table 1). According to the results, the accession PF-ZM 37 was the most susceptible to the environmental conditions, since it showed significant divergence upon eleven morphological traits between locations. Moreover, the same accession had the most characteristics with the highest mean value on the experimental plot at Smederevska Palanka, what makes it more adapted to those micro-climatic conditions, in this case more expressed drought. Additionally, it is important to emphasize that the accession PF-ZM 34 was the most developed vegetative and yield characteristics on both experimental plots. As was already described in the Introduction chapter, the eastern Serbia region is characterized by exceptional drought periods, so the accessions PF-ZM 34 and 37 showed to be the most successful in similar conditions. Morphological characteristics that discriminated most of the accessions according to their response to different environment were the leaf length, plant and ear height and rachis diameter. The former two are in accordance with findings of Ferro *et al.* (2007).

Variability that was present within accessions was not as great as variability noted to exist between the accessions, regarding both morphological and molecular characteristics. Similar findings were recorded by other authors, such as Beyene *et al.* (2006) and Rebourg *et al.* (2001) who, beside the morphological traits, have also used the more sophisticated genetic analyses. These findings imply that these ten studied accessions were significantly diverse, even though they came from relatively small area. It would be a great advantage for this research if more accessions were included in the study, and if some of the commercial varieties that were cultivated in this region were used as well for making the comparisons. On the other hand, some studies, such as the one made by Lucchin *et al.* (2003), have found that the variation was much higher within than between populations of certain Italian maize landrace, and this was explained by the long practice of seed exchange among farmers and pollen dispersion between the fields. Environment is playing a key role in landraces genesis, together with the intensive farmer's practices, but which of these two processes will have dominance is yet to be identified.

The cluster analysis performed for each of the two locations has revealed significant similarity of the accessions PF-ZM 18, 19 and 26, while the molecular analysis has indicated that the accessions PF-ZM 26 and 34 were closer to each other and differentiated from the other accessions. Noted similarity among accessions is probably due to the proximity of sites where they were collected, or the possible reason could be also that there was certain crosspollination during the previous regenerations of those accessions (Vigouroux *et al* 2008). Significant shortcoming in this study was that the records related to the exact locations where these accessions were collected from, as well as their on-farm cultivation history are missing due to the bad management practice of seed collection in the past, probably because of the social and economic circumstances in the country.

However, many studies have used the cluster analysis to integrate morphological/phenotypic and geographical differences of maize populations or, in other words, to express the maize landrace's integrity – its place of origin (Corral *et al.* 2008; Magorokosho *et al.* 2005; Rebourg *et al.* 2001; Gouesnard *et al.* 1997). Some authors (Gerić *et al.* 1989; Leng *et al.* 1962; Stojaković *et al.* 2000) were studying maize landraces in Southeast Europe and even more localized areas that were also object of this study as well. Taking into account the findings of this study, studied accessions could be identified as the group of "derived flints" mentioned by Gerić *et al.* (1989) and Leng *et al.* (1962), which was found most probably to be the result of hybridization between rare Mediterranean flints and other flint races. Distribution of this group was found to be wide and it was morphologically heterogeneous. However, identified resemblance that grouped these maize populations together was probably caused by similarity of environmental conditions since they were coming from mountainous areas. Since the two mentioned studies were conducted several decades ago, it would be useful if there were more recent studies that are dealing with this issue.

Another result of the cluster analysis, as well as of the Canonical Discriminant Analysis, was that some of the accessions analyzed in this study were more differentiated than the others. The molecular analysis has clearly distinguished the accessions PF-ZM 26 and 34, and the Bonferroni adjustment of the ANOVA test has indicated the differentiation of the accession 37 from PF-ZM 5 and 18. Likewise, morphological characteristics showed significant variation among accessions within the same location (Appendixes 4 and 5). The Canonical Discrimiant Analysis (Fig. 13 & 14) has indicated the most influential morphological traits for the differentiation of the accessions. This moves us to the question about the maize characteristics that are the most suitable for characterization of seed collections. As was shown in this study, the most selective morphological traits within the group of vegetative characteristics were the plant and ear height, leaf length and width, while within the group of the ear related characteristics – ear length and diameter and kernel length. Similar findings were indicated by Ilarslan *et al.* (2002). Furthermore, Galarreta & Alvarez (2001) and Rebourg *et al.* (2001) have suggested that the traits that have a high heritability, such as ear related characteristics (length, diameter, row number) and vegetative characteristics (days to flowering, plant and ear height) are more appropriate for characterization and classification of maize landraces. Nevertheless, Sánchez *et al.* (1993) were implying that there was a lack of knowledge regarding the characters that are the most suitable for the study of racial differences among maize populations, adding that some of the characters have not been tested to determine the relative importance of the influences of the genotype, environment and their interactions.

Robutti *et al.* (2000) addressed the problem that the ear and kernel phenotypes may be affected by the environment and that molecular analysis would be more representative for racial classification and characterization. Other authors (Carvalho *et al.* 2004; Rebourg *et al.* 2001; Sanchez *et al.* 2000) have also suggested that genetic analyses are an attractive method to evaluate polymorphism among the accessions in the seed collection, and that they would perform even better if it would be used in combination with morphological characterisation. This study could not compare results obtained by analyses of the morphological and molecular data, since the maize kernels that have been used in molecular analysis were taken from the bulked seed material. However, it would be a great advantage if these different types of analysis could be used at the same time, to generate complex picture of relationships among the seed collection material in general. Robutti *et al.* (2000) has implied that the association of storage proteins, such as zeins in maize, together with the genotype, could be a useful molecular marker for future analysis. Furthermore, Denić (1983) has stated that the storage proteins, due to their role in the crop ontogeny, are not under the strict genetic control such as that for the enzymes, and they are not affected by the environmental conditions as other phenotypic characters.

Zein proteins in maize endosperm

Zeins are the main storage proteins in the endosperm of maize kernels. They account for 50-70 percent of the proteins that are present in kernels (Azvedo *et al.* 2004). Zeins are classified into four groups: α-, β-, γ- and δ-zeins, according to their genetic characteristics and amino acid sequences (Rodriguez-Nogales *et al.* 2006). Researches conducted by Landry *et al.* (2001) and Jimenez-Flores & Bleck (1996) have determined that the α-zeins group accounts for 70 percent of the total zeins in a mature kernel. Correspondingly, the analysis of zeins in the maize accessions that was applied in this study has shown that the most abundant group of zeins was α-zeins, comprising in average 64 percent of the total registered zein proteins. As was indicated in the Results section, fractions of α-zein proteins with molecular weight 24 and 21 kDa were the most dominant ones (with averagely 54 percent of total zeins) and strongly correlated. This has also been confirmed by other authors, such as Zhu *et al.* (2007) and Liu & Rubenstein (1993).

Some studies, such as those of Landry *et al.* (2004) and Mestres & Matencio (1996), have found that the zein content in maize kernels is in strong correlation with the endosperm vitreous and kernel hardness. The zein content was found to be twice larger in vitreous than in the floury endosperm, especially α-zein fractions. These studies have compared different maize genotypes, including the wild genotype, and found that the zein content was higher in wild maize genotype in both vitreous and floury endosperm. Maize accessions that were analyzed in this study were determined to belong to the groups of flint or semi-flint and dent maize, which is supported by statement of Dallard (2007), who has claimed that all European maize landraces have either flint or dent kernel type. Although the relationship between the kernel hardness and zein content was not the object of this study, findings of larger content of certain zein fractions in flint kernels should probably be expected. Furthermore, results based on the field survey in eastern Serbia indicate that kernel hardness was favourable characteristic and the characteristic which was making maize landraces more resistant. Study by Rodriguez-Nogales *et al.* (2006) and other previously mentioned authors have also confirmed that the amount of zein proteins is highly affecting kernel texture and its susceptibility to pests and mechanical damages.

Many studies were using SDS-PAGE for identification and quantification of zein proteins in maize (Wilson 1986; Wallace *et al.* 1990). However, some authors, such as Parris *et al.* (1997), have claimed that this method has many drawbacks (e.g. it is labour and time consuming, provides low reproducibility and accuracy), and that some other methods that are used to analyze proteins in other cereals could be more appropriate. Decision to use SDS-PAGE for molecular analysis in this study was made due to the time and money constrains. Moreover, the only available material for this analysis was kernel samples (vegetative samples were not available).

In situ preservation

Many authors (Soleri *et al.* 2006; Bellon & Berthaud 2004; Doebley 2004) have expressed concern that the processes that are threatening diversity are more complex than it is usually described. Replacement of landraces by modern varieties and contamination of landraces by transgenes is only one part of the problem. Another aspect of this problem is the abandonment of maize landraces cultivation by farmers, due to their migration to more urban areas or shifting to other crops, the aging of the farming population and the lack of interest by young people to continue with the traditional practices. Efforts to conserve the genetic diversity of crops should be also directed to maintaining traditional farming practices by investing into small-scale farming. On-farm conservation of landraces is a dynamic system that could provide help in maintaining technical, social, cultural and environmental framework within which they have evolved. Generally, maize landraces are not only an important source of potentially useful characteristics, but also unique banks of adapted and coevolved genotypes (Lucchin *et al.* 2003).

Fig. 19. Maize fields in the landscape of Homolje region in the eastern Serbia.

Since the commercial varieties and hybrids are widely cultivated due to the present market demands, most of the respondents were growing small parcels of maize landraces which they called "eight-rowed white", "eight-rowed yellow" and "old domestic" maize in their gardens. The main reason that this small-scale cultivation of landraces is still present is that the farmers want to get hold of enough maize to produce flour, which is used by themselves and their relatives for their own consumption (Fig. 19). Growing of maize landraces is directly associated with presence of the old wooden water-mills, since the flour is obtained exclusively there. These are some of the illustrations of features which interviewed persons said about maize landraces (Fig. 20). There were two most striking facts that were encountered during interviews. The first one is that, although most of the respondents have claimed that these maize landraces are hard for processing due to the hardness of their ears and kernels, the same characteristic was making maize landraces more favourable for consumption, such flour seems to be much tastier and easier to preserve for long time. Secondly, the only maize landraces that were registered in the field had white or yellow kernel colour, while the red coloured maize is apparently not present anymore in this region. Presence of the red coloured maize landraces among the accessions that were characterized in this study makes them even more valuable, since they have most probably disappeared as cultivated variety in the eastern Serbia region.

Fig. 20. The golden decorations on walls are used to enrich the interior.

Similarly to the findings of Bellon *et al.* (2003), the most repeated positive characteristic of maize landraces in the present study was related to the consumption. Respondents have claimed that the flour of maize landraces was much tastier than of any commercial variety, and they have also emphasized its suitability for the preparation of some traditionally made recipes, such as maize porridge and cornbread. These facts seemed to have large influence on farmers' choice to cultivate maize landraces, as the farmers were accustomed to this type of food, inheriting this tradition along with the landraces' seed from their parents. Another positive characteristic which was specified by the respondents (although with the exception of one respondent) was that the maize landraces are more resistant to unfavourable environmental conditions, such as drought and pest infestations, but their weaker yield is making them far surpassed by the commercial varieties in the large-scale cultivation. However, it was suggested by one respondent that, although it is usually low, yield is showing certain stability.

As it is indicated by the information gathered during the survey in eastern Serbia, Serbian farmers did not completely abandon maize landraces, despite the wide acceptance of the modern varieties for cultivation. Similar findings were recorded by Bellon & Brush (1994). This was due to the fact that all respondents seem to be accustomed to maize landraces as a component of their lives, which was the main reason for preservation of these varieties. Maize landraces resistance to complete extinction was also explained by García (2007), who has found that the small-scale farming was the most contributing factor to the maize biodiversity conservation. The fact that is worthy for consideration, related to preservation of maize landraces in Homolje region, is the possibility to relate maize landraces to the overall efforts to conserve the traditional agricultural practices, as the cultural heritage of the region.

As was mentioned earlier, *in situ* conservation of landraces is a complex task but still a necessary one in order to preserve the crop's genetic diversity. According to the survey data in this study, some of the traits with potential interest for breeders that are still present among the local maize varieties are the resistance to drought, earlier maturity and storage durability. These characteristics were developed and are still maintained in specific agro-ecological conditions, so the efforts for their conservation should be aimed towards the preservation of these environments – the villages with the maintained agricultural practices, as it was recommended by Pressoir & Berthaud (2004). Smale *et al.* (2003) and Dhillon *et al.* (2004) have discussed possible solutions to improve farmers' positions in current social and economical circumstances. The agricultural potential of the landraces and local varieties is laying in organic agricultural production (Prodanović & Šurlan-Momirović 2006), due to their stability in low-input agricultural systems and increasing public interest for this type of production. This aspect should not be neglected in the development of strategies for conservation of this type of biodiversity (Fig. 21).

Fig. 21. One of the respondents in the survey cultivated four different old maize varieties (yellow and white eight-rowed, white ten-rowed and small Croatian maize).

However, it is important to assess the genetic diversity that is present nowadays in the field, and furthermore to compare it with the one conserved in seed collections. Since a number of ears of maize landraces were collected during the field trips within the present study, it would be of great interest to compare their characterization with the accessions characterized in this study. Furthermore, beside the collection of the new maize landrace accessions, which will enable their *ex situ* preservation, information about the villages where these varieties are still cultivated was also recorded, so any further investigation or collection efforts will be facilitated using these data.

Additionally, analyses performed on the collected maize material would produce data that might resolve uncertainty regarding the collection locations. Furthermore, comparison of the results of these studies could explain more about relationship among the ten studied accessions and reveal trends in morphological and molecular characteristics. Future molecular analyses should, apart from analysis performed in this study, also include application of genetic markers. This would describe diversity among the accessions on another level, but it would also enable comparison of the two sets of the results, genetic variability and variability of proteins (zeins).

As it was previously discussed, the on-farm conservation represents one of the most important efforts for the maintaining and preservation of landraces. In future projects, it is necessary to put more attention on the improvement of the local biodiversity sustainable management. Considering the problems found in this study, regarding the preservation of maize landraces, it is important to motivate farmers to engage more in conservation efforts. This could be achieved by introducing incentives that would encourage and support farmers to continue with the cultivation of the old varieties. Studies that deal with the popularization of the consumption of products derived from these varieties could contribute to the improvement of the conservation. Of course, development of a national policy for on-farm conservation should be a priority, because it would establish the basis for further social and economical development.

Conclusions

The ten maize accessions studied here have shown a significant difference according to both morphological and molecular comparison. Since the same accessions were grown on two sites, the analysis showed that these two environments caused certain differentiation of the morphological traits assessed in the study. Some of the accessions were shown to be more similar, which might imply the same origin and/or possible proximity of sites where they were collected. At the same time, some of the accessions were more differentiated from the others, indicating which characteristics express the most variability among the accessions. In accordance to findings of the other authors, this study indicated that the most selective morphological traits within the group of vegetative characteristics were the plant and ear height, leaf length and width, while within the group of the ear related characteristics – ear length and diameter and kernel length. The molecular analysis of the storage proteins – zeins, performed in this study could be a suitable method to scrutinize variability among maize accessions. In combination with the morphological characterization, molecular analyses are able to provide enough information about origin and diversity of the accessions within collections.

Interview survey in the eastern Serbia has revealed that the extent of maize landraces cultivation has significantly diminished during the last few decades, and that some maize types (such as the red-coloured maize) seem to have completely disappeared from this region.

Efforts to conserve the genetic diversity of this crop should also be directed to maintaining traditional farming practices by investing into small-scale farming, since this was the only way that these local maize varieties continued to exist. Important outcome of this study was that, during field trips, new samples of maize landraces were collected and added to the seed collection kept in the Faculty of Agriculture in Belgrade. Gathered information about the agricultural practice and traditional values related to the maize landraces are interesting opportunity for future ethno-botanical research.

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References

Auerbach, C.F. & Silverstein, L.B. 2003. *Qualitative data: An introduction to coding and analysis*. New York University Press, New York and London.

Azvedo, R.A., Damerval, C., Lea, P.J., Landry, J., Bellato, C.M., Meinhardt, L.W., Guilloux, M.L., Delhaye, S., Toro, A.A., Gaziola, S.A., Varisi, V.A. & Gratão, P.L. 2004. Genetic control of lysine metabolism in maize endosperm mutants. *Functional Plant Biology* 31: 339-348.

Bellon, M.R. 2004. Conceptualizing interventions to support on-farm genetic resource conservation. *World Development* 32(1): 159-172.

Bellon, M.R. & Berthaud, J. 2004. Transgenic maize and the evolution of landraces diversity in Mexico. The importance of farmers' behavior. *Plant Physiology* 134: 883-888.

Bellon, M.R., Berthaud, J., Smale, M., Aguirre, J.A., Taba, S., Aragón, F., Díaz, J. & Castro, H. 2003. Participatory landrace selection for on-farm conservation: An example from the Central Valleys of Oaxaca, Mexico. *Genetic Resources and Crop Evolution* 50: 401-416.

Bellon, M.R. & Brush, S.B. 1994. Keepers of maize in Chiapas, Mexico. *Economic Botany* 48(2): 196-209.

Beyene, Y., Botha, A.M. & Myburg, A.A. 2006. Genetic diversity in traditional Ethiopia highland maize accessions assessed by AFLP markers and morphological traits. *Biodiversity and Conservation* 15: 2655-2671.

Bremer, K., Bremer, B. & Thulin, M. 2003. *Introduction to Phylogeny and Systematics of Flowering Plants*. Symbolae Botanicae Upsalienses 33:2, Uppsala.

Burstin, J., Zivy, M., De Vienne, D. & Damerval, C. 1993. Analysis of scaling methods to minimize experimental variations in two-dimensional electrophoresis quantitative data: application to the comparison of maize inbred lines. *Electrophoresis* 14: 1067-1073.

Carvalho, V.P., Ruas, C.F., Ferreira, J.M., Moreira, R.M.P. & Ruas, P.M. 2004. Genetic diversity among maize (*Zea mays* L.) landraces assessed by RAPD markers. *Genetics and Molecular Biology* 27(2): 228-236.

Coffey, A. & Atkinson, P. 1996. *Making sense of qualitative data.* SAGE Publications, Inc., Thousand Oaks.

Consoli, L. & Damerval, C. 2001. Quantification of individual zein isoforms resolved by two-dimensional electrophoresis: Genetic variability in 45 maize inbred lines. *Electrophoresis* 22: 2983-2989.

Corral, J.A.R., Puga, N.D., González, J.J.S., Parra, J.R., Eguiarte, D.R.G., Holland, J.B. & García, G.M. 2008. Climatic adaptation and ecological descriptors of 42 Mexican maize races. *Crop Science* 48(4): 1502-1512.

Dallard, J. 2007. Maize: Implementation of the European network for evaluation, conservation and utilization of European maize landraces genetic resources. In European Commission, Directorate-General for Agriculture and Rural Development (ed.) *Genetic resources in agriculture*, p. 53-55. Office for Official Publications of the European Communities, Luxembourg.

Denić, M. 1983. Genetic basis of storage protein synthesis in maize. In Gottschalk, W. & Müller, H.P. (eds.) *Seed proteins: Biochemistry, genetics, nutritive value*, p. 245-270. Martinus Nijhoff/Dr W. Junk Publishers, Hague.

Dhillon, B.S., Dua, R.P., Brahmi, P. & Bisht, I.S. 2004. On-farm conservation of plant genetic resources for food and agriculture. *Current science* 87(5):557-559.

Dodig, D., Aleksić, V., Spasov, P., Petrović, R. & Miletić, R. 2005. Climate change in eastern Serbia and their influence on plant production and ecosystem. http://www.wg-crop.icidonline.org/8doc.pdf [accessed in March 2009]

Doebley, J. 2004. The genetics of maize evolution. *Annual Review of Genetics* 38:37-59.

Dowswell, C.R., Paliwal, R.L. & Cantrell, R.P. 1996. *Maize it the Third World.* Westview Press, Inc., Boulder.

Edwards, R.J & Leng, E.R. 1965. Classification of some indigenous maize collections from southern and southeastern Europe. *Euphytica* 14: 161-169.

FAO 1996. Report on the state of the world's plant genetic resources for food and agriculture. Food and Agriculture Organization of the United Nations, Rome.

Ferro, R.A., Brichette, I., Evgenidis, G., Karamaligkas, Ch. & Moreno-González, J. 2007. Variability in European maize (*Zea mays* L.) landraces under high and low nitrogen inputs. *Genetic Resources and Crop Evolution* 54: 295-308.

Fling, S.P. & Gregerson, D.S. 1986. Peptide and protein molecular weight determination by electrophoresis using a high-molarity tris buffer system without urea. *Analytical Biochemistry* 155: 83-88.

Frankfort-Nachmias, C. & Nachmias, D. 1996. *Research methods in the social sciences*, 5th ed. Arnold, London.

Galarreta de, J.I.R. & Alvarez, A. 2001. Morphological classification of maize landraces from northern Spain. *Genetic Resources and Crop Evolution* 48: 391-400.

García, J.B. 2007. Maize adoption and biodiversity conservation in Mexico. Conference proceedings, "Micro Evidence on Innovation in Developing Economies (MEIDE)", 31 May – 1 June 2007. UNU-MERIT, Maastricht, Netherlands.

Gauthier, P., Gouesnard, B., Dallard, J., Redaelli, R., Rebourg, C., Charcosset, A. & Boyat, A. 2002. RFLP diversity and relationships among

traditional European maize populations. *Theoretical and Applied Genetics* 105: 91-99.
Gerić, I., Zlokolica, M., Gerić, C. & Stuber, C.W. 1989. Races and populations of maize in Yugoslavia. Isozyme variation and genetic diversity. *Systematic and Ecogeographic Studies on Crop Genepools 3*. International Board for Plant Genetic Resources, Rome.
Global Crop Diversity Trust 2006. *Priority crops: Maize*. http://www.croptrust.org/main/priority.php?itemid=30 [accessed in May 2009]
Gouesnard, B., Dallard, J., Bertin, P., Boyat, A. & Charcosset, A. 2005. European maize landraces: Genetic diversity, core collection definition and methodology of use. *Maydica* 50: 225-234.
Gouesnard, B., Dallard, J., Panouillé, A. & Boyat, A. 1997. Classification of French maize populations based on morphological traits. *Agronomie* 17: 491-498.
Guimarães, C.T., de Barros, E.G., Vasconcelos, M.J.V. & Paiva, E. 1995. Characterization of South American exotic maize (*Zea mays* L.) populations with opaque phenotype. *Brazilian Journal of Genetics* 18(2): 259-264.
IBPGR 1991. *Descriptors for Maize*. International Maize and Wheat Improvement Center, Mexico City/International Board for Plant Genetic Resources, Rome.
Ilarslan, R., Kaya, Z., Kandemir, I. & Bretting, P.K. 2002. Genetic variability among Turkish pop, flint and dent corn (*Zea mays* L. ssp. *mays*) races: Morphological and agronomic traits. *Euphytica* 128: 173-182.
INPhO 2003. *Compendium on Post-harvest Operations*. Information Network on Post-harvest Operations. http://www.fao.org/inpho/content/compend/toc_main.htm [accessed in May 2009]
Jimenez-Flores, R. & Bleck, G.T. 1996. Biotechnology. In Nakai, S. & Modler, H.W. (eds.) *Food proteins: properties and characterization*, p. 505-534. VCH Publishers, Inc., New York.
Landry, J., Delhaye, S. & Damerval, C. 2004. Protein distribution pattern in floury and vitreous endosperm of maize grain. *Cereal Chemistry* 81(2): 153-158.
Landry, J., Delhaye, S., Philippeau, C. & Michalet-Doreau, B. 2001. Isolation and quantitation of zeins in waxy and amylose-extender and wild flint and dent maize endosperm using new solvent sequence for protein extraction. *Journal of Agricultural and Food Chemistry* 49: 164-169.
Leng, E.R., Tavčar, A. & Trifunović, V. 1962. Maize of southeastern Europe and its potential value in breeding programs elsewhere. *Euphytica* 11: 263-272.
Leymarie, J., Damerval, C., Marcotte, L., Combes, V. & Vartanian, N. 1996. Two-dimensional protein patterns of *Arabidopsis* wild-type and auxin

intensive mutants, axr1, axr2, reveal interactions between drought and hormonal responses. *Plant Cell Physiology* 37(7): 966-975.

Liu, C.N. & Rubenstein, I. 1993. Transcriptional characterization of an α-zein gene cluster in maize. *Plant Molecular Biology* 22: 323-336.

Louette, D. & Smale, M. 2000. Farmers' seed selection practices and traditional maize varieties in Cuzalapa, Mexico. *Euphytica* 113: 25-41.

Lucchin, M., Barcaccia, G. & Parrini, P. 2003. Characterization of a flint maize (*Zea mays* L. convar. *mays*) Italian landrace: Morpho-phenological and agronomic traits. *Genetic Resources and Crop Evolution* 50: 315-327.

Mabberley, D.J. 2008. *Mabberley's plant book*, 3rd edn. Cambridge University Press, Cambridge.

Magorokosho, C., Bänziger, M. & Betrán, F.J. 2005. Characterization of local maize landraces from Zimbabwe, Zambia and Malawi. In Jarvis, D., Mar, I. & Sears, L. (eds.) *Enhancing the use of crop genetic diversity to manage abiotic stress in agricultural production systems*, p. 25-30. Proceedings of a workshop, 23–27 May 2005, Budapest, Hungary. International Plant Genetic Resources Institute, Rome, Italy.

Mangelsdorf, P. 1974. *Corn, its origin, evolution and improvement*. The Belknap Press of Harvard University Press, Cambridge, Massachusetts.

Mestres, C. & Matencio, F. 1996. Biochemical basis of kernel milling characteristics and endosperm vitreousness of maize. *Journal of Cereal Science* 24: 283-290.

Parris, N., Dickey, L. & Craig, J. 1997. Quantitative analysis of corn zeins by capillary electrophoresis. *Cereal Chemistry* 74(6): 766-770.

Perales, H.R., Brush, S.B. & Qualset, C.O. 2003. Dynamic management of maize landraces in Central Mexico. *Economic Botany* 57(1): 21-34.

Pressoir, G. & Berthaud, J. 2004. Population structure and strong divergent selection shape phenotypic diversification in maize landraces. *Heredity* 92: 95-101.

Prodanović, S. & Šurlan-Momirović, G. 2006. *Genetički resursi biljaka za organsku poljoprivredu* [*Plant genetic resources for organic production*]. Faculty of Agriculture at University of Belgarde, Belgrade – Zemun. (In Serbian)

Rasband, W.S. 2008. ImageJ. Version 1.41o. U. S. National Institute of Health, Bethesda, Maryland, USA. http://rsb.info.nih.gov/ij/ [accessed in March 2009]

Rebourg, C., Gouesnard, B. & Charcosset, A. 2001. Large scale molecular analysis of traditional European maize populations. Relationships with morphological variation. *Heredity* 86: 574-587.

Rebourg, C., Chastanet, M., Gouesnard, B., Welcker, C., Dubreuil, P. & Charcosset, A. 2003. Maize introduction into Europe: the history reviewed in the light of molecular data. *Theoretical and Applied Genetics* 106: 895-903.

Reif, J.C., Hamrit, S., Heckenberger, M., Schipprack, W., Maurer, H.P., Bohn, M. & Melchinger, A.E. 2005. Genetic structure and diversity of

European flint maize populations determined with SSR analyses of individuals and bulks. *Theoretical and Applied Genetics* 111: 906-913.

Robutti, J.L., Borrás, F.S., Ferrer, M.E. & Bietz, J.A. 2000. Grouping and identification of Argentine maize races by principal component analysis of zein reversed-phase HPLC data. *Cereal Chemistry* 77(2): 91-95.

Rodriguez-Nogales, J.M., Garcia, M.C. & Marina M.L. 2006. High-performance liquid chromatography and capillary electrophoresis for the analysis of maize proteins. *Journal of Separation Science* 29: 197-210.

Rohlf, F.J. 2008. NTSYSpc: Numerical Taxonomy System, Ver. 2.11L. Exeter Publishing, Ltd.: Setauket, New York.

Sanchez, G.J.J., Goodman, M.M. & Stuber, C.W. 2000. Isozymatic and morphological diversity in the races of maize of Mexico. *Economic Botany* 54(1): 43-59.

Sánchez, G.J.J., Goodman, M.M. & Rawlings, J.O. 1993. Appropriate characters for racial classification in maize. *Economic Botany* 47(1): 44-59.

SIEPA 2005. *Facts about Serbia*. Serbia Investment & Export Promotion Agency, Belgrade.

Smale, M., Bellon, M.R., Aguirre, J.A., Rosas, I.M., Mendoza, J., Solano, A.M., Martínez, R, Ramírez, A. & Berthaud, J. 2003. The economic costs and benefits of a participatory project to conserve maize landraces on farms in Oaxaca Mexico. *Agricultural Economics* 29: 265-275.

Soleri, D., Cleveland, D.A. & Cuevas, F.A. 2006. Transgenic crops and crop varietal diversity: The case of maize in Mexico. *Bioscience* 56(6): 503-513.

Stojaković, M., Jocković, Dj., Bekavac, G., Nastić, A., Vasić, N. & Purar, B. 2000. Characteristics of maize inbred lines originating from local populations. *Cereal Research Communications* 28(3): 299-306.

Vaz Patto, M.C., Moreira, P.M., Carvalho, V. & Pego, S. 2007. Collecting maize (*Zea mays* L. convar. *mays*) with potential technological ability for bread making in Portugal. *Genetic Resources and Crop Evolution* 54: 1555-1563.

Vigouroux, Y. Glaubitz, J.C., Matsuoka, Y., Goodman, M.M., Sánchez, J.G. & Doebley, J. 2008. Population structure and genetic diversity of new world maize races assessed by DNA microsatellites. *American Journal of Botany* 95(10): 1240-1253.

Wallace, J.C., Lopes, M.A., Paiva, E. & Larkins, B.A. 1990. New methods for extraction and quantitation of zeins reveal a high content of γ-zein in modified opaque-2 maize. *Plant Physiology* 92: 191-196.

Wilson, C.M. 1986. Serial analysis of zein by isoelectric focusing and sodium dodecyl sulfate gel electrophoresis. *Plant Physiology* 82: 196-202.

Yamasaki, M., Tenaillon, M.I., Bi, I.V., Schroeder, S.G., Sanchez-Villeda, H., Doebley, J.F., Gaut, B.S. & McMullen, M.D. 2005. A large-scale screen for artificial selection in maize identifies candidate agronomic loci domestication and crop improvement. *The Plant Cell* 17: 2859-2872.

Yunchang, G., Zhongdong, L., Hongjie, A., Minqian, L. & Jun, H. 2005. Nano-structure and properties of maize zein studied by atomic force microscopy. *Journal of Cereal Science* 41: 277-281.

Zhu, F., Kale, A.V. & Cheryan, M. 2007. Fractionation of zein by size exclusion chromatography. *Journal of Agricultural and Food Chemistry* 55: 3843-3849.

Appendix 1 Interview questions

The question list used for the interviews performed during the field trips to the eastern Serbia:

1) Do you grow maize landraces?
2) Which maize landraces do you grow?
3) How large is the area on which you grow maize landraces?
4) Why do you grow maize landraces?
5) Since when do you grow maize landraces?
6) How did you get the seeds and do you exchange seeds with others?
7) What are the characteristics of the maize landraces?
8) What are their advantages/disadvantages comparing to the modern maize varieties?
9) Do people you know grow maize landraces?

Appendix 2 Measurements of maize material at Smederevska Palanka

Mean values with the standard deviation of measurements obtained from the maize material grown at Smederevska Palanka (1000 kernel weight is related to the accession)

Characteristic	PF-ZM 4	PF-ZM 5	PF-ZM 10	PF-ZM 12	PF-ZM 18	PF-ZM 19	PF-ZM 26	PF-ZM 34	PF-ZM 37	PF-ZM 39
Plant height (cm)	159,3±18,7	188±10,6	184,2±13,6	169,5±17,6	151±6,1	139,3±14,3	138,1±21	178,5±19,7	193,3±17,8	187,9±9,9
Ear height (cm)	98,9±15,3	125±14,1	125,6±21,1	106,5±17	86±8,1	81,4±13,1	83,1±24,9	111,5±17,2	118,3±9,3	128,6±19,3
Number of leaves above the uppermost ear	4,7±1,4	5,8±1,1	5±0,5	4,9±0,9	5,2±1,1	5,1±0,4	5±0,8	5,1±0,9	4,8±1,6	4,9±1,2
Total number of leaves per plant	11,1±1,3	13,3±1,1	12,7±1,2	11,2±1,2	10,8±1,5	11±1,3	11,4±0,9	11,9±1,1	12,3±0,8	11,6±0,8
Number of ears per plant	1,5±0,8	1,4±0,5	2±0,5	1,4±0,5	1,9±0,6	2,3±0,5	2,3±0,7	2,3±0,7	1,7±0,5	2,1±0,7
Leaf length (cm)	78,9±13,4	82,7±8,2	90,3±13,4	75,1±7,6	68,8±8,3	69,9±8,4	67,9±7,8	77,1±6,9	95,9±15,3	92,9±10,4
Leaf width (cm)	10,9±0,9	8,4±1,7	10,7±1,1	11,1±1,3	10±1,3	9,5±1,1	9,3±1,1	8,9±1,1	10,2±1,8	9,4±1,3
Venation index	2,2±0,3	2,5±0,5	2,3±0,3	2,3±0,5	2,4±0,3	2,4±0,1	2,5±0,3	2,6±0,3	2,3±0,3	2,4±0,2
Number of kernel rows	15±2,6	14,7±2,3	14,3±2	14,3±2	12,3±2,1	11,3±2,1		15,3±2,7	14,3±1,5	13,2±1,8
Number of kernels per row (1)	25±4,5	27,3±4,6	26±9,6	26±9,6	28,4±7,4	24,7±10,9		24,8±6,4	16,7±6,8	23,4±4
Number of kernels per row (2)	24,5±7	26,3±4,6	27±11,6	27±1,6	27,7±7,1	22,8±11,3		23,8±8,5	16,2±6,4	23,6±4,5
Ear length (cm)	21,3±4,8	16,4±5,3	19±1,8	18,9±1,8	16,9±2,4	14,9±2,3		19,1±3	21,9±2,4	14,8±2
Ear diameter – top (cm)	4,1±0,3	3,7±0,4	3,4±0,3	3,4±0,3	3,2±0,2	3±0,2		3,8±0,3	3,7±0,3	3,5±0,4
Ear diameter – middle (cm)	4,8±0,3	4,5±0,4	4,2±0,5	4,2±0,5	3.,9±0,3	3,7±0,3		4,6±0,6	4,2±0,3	4,1±0,3
Ear diameter – basis (cm)	4,5±0,4	4±0,2	4,2±0,5	4,2±0,5	3,7±0,4	3,5±0,2		4,5±0,5	4,1±0,3	3,9±0,3

Cob diameter (cm)	2,8±0,6	3±0,4	2,5±0,1	2,5±0,1	2,3±0,2	2,3±0,2		2,7±0,2	2,8±0,3	2,6±0,3
Rachis diameter (cm)	1,5±0,2	2±0,3	1,7±0,1	1,7±0,1	1,5±0,3	1,5±0,2		1,6±0,2	1,6±0,4	1,7±0,3
Kernel length (cm)	1±0,1	0,9±0,1	1,1±0,1	1,1±0,1	1,1±0,1	1±0,1	0,9±0,1	1,2±0,1	1,1±0,1	1±0,1
Kernel width (cm)	0,9±0,1	0,9±0,0	0,8±0,0	0,8±0,0	0,9±0,1	0,9±0,1	0,9±0,0	0,9±0,1	0,9±0,1	0,9±0,1
Kernel thickness (cm)	0,5±0,1	0,6±0,1	0,5±0,1	0,5±0,1	0,5±0,0	0,6±0,1	0,6±0,1	0,5±0,1	0,6±0,1	0,5±0,1
1000 kernel weight (g)	385	375	320	335	315	285	160	385	410	335

Appendix 3 Measurements of maize material at Zemun Polje

Mean values with the standard deviation of measurements obtained from the maize material grown at Zemun Polje (1000 kernel weight is related to the accession)

Characteristic	PF-ZM 4	PF-ZM 5	PF-ZM 10	PF-ZM 12	PF-ZM 18	PF-ZM 19	PF-ZM 26	PF-ZM 34	PF-ZM 37	PF-ZM 39
Plant height (cm)	126,7±22,7	144,8±22,7	126,6±25,5	143,9±20	154,2±14,5	146,4±36,3	126,9±25,2	179±15,6	101,8±10,9	166,7±25,2
Ear height (cm)	74,1±11,6	102,8±11,2	77,5±22,8	86,7±9,2	88,3±11,2	87,1±10	74,4±21,3	109,5±10,9	65±13,7	106,7±15,3
Number of leaves above the uppermost ear	4,4±1,1	5,8±1	5,1±0,7	5,5±1	6±0,9	6±0,0	5,4±0,5	6,1±0,6	5±0,1	5,3±0,6
Number of ears per plant	1,3±0,5	1,1±0,3	1,7±0,5	1,2±0,4	1,9±0,6	2,4±0,8	1,5±0,5	1,6±0,5	1±0,0	2,3±1,5
Leaf length (cm)	101,4±6	103,1±4,9	95,5±15,6	93,8±4,7	98,1±8	101,4±7,6	89,3±8,7	107,8±5,7	81±4,6	107,3±9,4
Leaf width (cm)	8,8±1,2	7,8±1,2	9,9±0,7	10,9±0,6	11,1±0,6	10,6±0,8	10,2±0,7	9,6±0,6	7.7±0,7	9,7±1
Venation index	2,2±0,3	2,6±0,3	2,2±0,2	2±0,2	2,3±0,2	2,2±0,2	2,5±0,5	2,5±0,2	2,6±0,2	2,3±0,2
Tassel length (cm)	58,7±9,4	67,6±5,2	62,5±8,8	56,6±4,6	59,4±7,7	57,9±8,1	60,6±5,7	67±7,5	48±4,5	56,7±5,8
Tassel peduncle length (cm)	21,7±5,5	27,1±5,4	22,3±2,8	20,3±4	21,7±2,6	22,9±5,2	23,8±3,5	28±4,3	29±2,2	24±1,7
Number of primary branches on tassel	11,7±2,4	6,7±1,2	16,7±4,3	8±3,1	12,8±3,8	15,3±4,1	17,3±4,2	15,5±3,6	5,4±0,9	16,7±5,1
Number of secondary branches on tassel	1,4±1,2	1,2±1	2±1,4	0,6±0,7	2±1,3	2,3±0,9	1,9±1,4	2,7±1,1	0,2±0,4	3,3±0,6
Number of kernel rows	13,8±1,5	13,5±1	13,3±2	12,1±1,4		14,6±2,2	15,7±1,4	15,4±1,5	13±2,6	17±1,4
Number of kernels per	20±5,7	28±9	24,3±8,4	22,4±5,6		31,7±2,6	21,1±4,4	30,3±5,1	13±5	16,5±13,

row (1)										4
Number of kernels per row (2)	19,9 ±6,3	28± 8,2	23,7 ±8,6	19,6 ±6,5		31,7 ±2,9	20,3 ±3,8	29,9 ±5,2	13± 5,4	16± 12,7
Ear length (cm)	12,9 ±3,7	15± 4	13,3 ±4,2	11,3 ±1,6		16,1 ±1,9	11,7 ±2	17,6 ±2	9,8± 0,9	9,5± 7,8
Ear diameter – top (cm)	3,4± 0,4	3,6± 0,5	3,4± 0,5	3,2± 0,2		3,4± 0,3	4±0, 3	3,9± 0,4	2,8± 0,3	3,1
Ear diameter – middle (cm)	4,1± 0,4	4,1± 0,6	4,2± 0,4	3,7± 0,1		4,2± 0,3	4,4± 0,1	4,6± 0,2	3,3± 0,5	4,3± 0,6
Ear diameter – basis (cm)	3,9± 0,4	4±0, 7	4,1± 0,6	3,9± 0,1		4,3± 0,4	4,4± 0,1	4,6± 0,2	2,9± 0,3	4,4± 0,7
Cob diameter (cm)	2,6± 0,2	2,7± 0,3	2,4± 0,2	2,5± 0,2		2,5± 0,2	2,5± 0,1	2,5± 0,2	2±0, 1	3±0, 8
Rachis diameter (cm)	2,2± 0,2	2,3± 0,4	2±0, 2	2,2± 0,2		2,2± 0,1	2,2± 0,1	2,2± 0,1	1,9± 0,2	2,6± 0,5
Kernel length (cm)	1,1± 0,2	1,1± 0,1	1,2± 0,1	1±0, 1		1,2± 0,1	1,2± 0,1	1,2± 0,1	0,9± 0,2	1±0, 1
Kernel width (cm)	0,9± 0,1	0,8± 0,1	0.9± 0,1	0,9± 0,0		0,9± 0,1	0,9± 0,1	0,9± 0,1	0,7± 0,1	0,8± 0,0
Kernel thickness (cm)	0,5± 0,0	0,4± 0,1	0,4± 0,0	0,5± 0,0		0,5± 0,1	0,5± 0,0	0,4± 0,0	0,4± 0,1	0,1± 0,2
1000 kernel weight (g)	346	291	356	306		336	341	356	236	246

Appendix 4 Measurements of Morphological characteristics – Smederevska Palanka

The analysis of the variance of the morphological characteristics –Smederevska Palanka

Characteristic	Variability	Mean Square	F
A plant height (cm)	Between accessions	3359.203	14.146**
	Within accessions	237.470	
A ear height (cm)	Between accessions	2723.659	9.751**
	Within accessions	279.311	
Number of leaves above the uppermost ear	Between accessions	0.837	0.816
	Within accessions	1.026	
Total number of leaves per plant	Between accessions	5.914	4.407**
	Within accessions	1.342	
Number of ears per plant	Between accessions	1.153	3.186**
	Within accessions	0.362	
Leaf length (cm)	Between accessions	791.449	8.056**
	Within accessions	98.244	
Leaf width (cm)	Between accessions	7.325	4.386**
	Within accessions	1.670	
Venation index	Between accessions	0.114	0.969
	Within accessions	0.118	
Number of kernel rows	Between accessions	10.187	2.269*
	Within accessions	4.489	
Number of kernels per row (1)	Between accessions	87.503	1.789
	Within accessions	48.919	
Number of kernels per row (2)	Between accessions	118.485	2.075
	Within accessions	57.112	
Ear length (cm)	Between accessions	34.493	4.405**
	Within accessions	7.831	
Ear diameter – top (cm)	Between accessions	0.613	6.520**
	Within accessions	0.094	

Ear diameter – middle (cm)	Between accessions	0.593	4.072**
	Within accessions	0.146	
Ear diameter – basis (cm)	Between accessions	0.650	4.068**
	Within accessions	0.160	
Cob diameter (cm)	Between accessions	0.281	3.599**
	Within accessions	0.078	
Rachis diameter (cm)	Between accessions	0.082	1.441
	Within accessions	0.057	
Kernel length (cm)	Between accessions	0.027	3.828**
	Within accessions	0.007	
Kernel width (cm)	Between accessions	0.014	2.396*
	Within accessions	0.006	
Kernel thickness (cm)	Between accessions	0.012	2.234*
	Within accessions	0.005	

** Significance at $p<0.01$
* Significance at $p<0.05$

Appendix 5 Measurements of Morphological characteristics – Zemun Polje

The analysis of the variance of the morphological characteristics – Zemun Polje

Characteristic	Variability	Mean Square	F
Plant height (cm)	Between accessions	3525.048	6.919*
	Within accessions	509.445	
Ear height (cm)	Between accessions	1638.004	7.917*
	Within accessions	206.887	
Number of leaves above the uppermost ear	Between accessions	2.597	4.116*
	Within accessions	0.631	
Number of ears per plant	Between accessions	1.516	4.813*
	Within accessions	0.315	
Leaf length (cm)	Between accessions	438.187	6.417*
	Within accessions	68.282	
Leaf width (cm)	Between accessions	10.951	14.968*
	Within accessions	0.732	
Venation index	Between accessions	0.320	4.533*
	Within accessions	0.071	
Tassel length (cm)	Between accessions	219.810	4.279*
	Within accessions	51.364	
Tassel peduncle length (cm)	Between accessions	71.853	4.183*
	Within accessions	17.177	
Number of primary branches on tassel	Between accessions	146.783	12.601*
	Within accessions	11.649	
Number of secondary branches on tassel	Between accessions	5.407	4.452*
	Within accessions	1.214	
Number of kernel rows	Between accessions	11.564	3.926*
	Within accessions	2.946	
Number of kernels per row (1)	Between accessions	198.629	5.171*
	Within accessions	38.410	
Number of kernels per row (2)	Between	212.654	5.214*

	accessions		
	Within accessions	40.785	
Ear length (cm)	Between accessions	40.993	4.422*
	Within accessions	9.271	
Ear diameter – top (cm)	Between accessions	0.748	5.221*
	Within accessions	0.143	
Ear diameter – middle (cm)	Between accessions	0.842	6.763*
	Within accessions	0.125	
Ear diameter – basis (cm)	Between accessions	1.222	7.192*
	Within accessions	0.170	
Cob diameter (cm)	Between accessions	0.242	5.820*
	Within accessions	0.042	
Rachis diameter (cm)	Between accessions	0.139	3.603*
	Within accessions	0.038	
Kernel length (cm)	Between accessions	0.065	5.010*
	Within accessions	0.013	
Kernel width (cm)	Between accessions	0.016	3.193*
	Within accessions	0.005	
Kernel thickness (cm)	Between accessions	0.010	3.134*
	Within accessions	0.003	

* Significance at $p<0.01$