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GRAIN QUALITY WITHIN *EX SITU* AND *IN SITU* CONSERVED TRADITIONAL WHITE MAIZE LANDRACES

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ABSTRACT

White maize had an important role in human nutrition throughout the Western Balkan region. A great number of farmers (approximately 86% in Serbia) still grow traditional white Open Pollinated Varieties (OPVs) for human diet. Out of 2217 maize landraces stored within Maize Research Institute „Zemun Polje" (MRIZP) gene bank, more than 700 are white kernel accessions. Eighteen white maize landraces, collected in the Western Balkan region in different periods, were selected for the present study. By the evaluation of agro-morphological performances and the basic chemical composition of grain, the objective was to determine the importance of landraces conserved under *in situ* and *ex situ* conditions, to be used as the initial material for breeding. The idea was to determine whether the varieties conserved under *ex situ* conditions (collected from the same or different regions) distinguish from varieties permanently grown in the region of western Serbia. It was found that varieties permanently grown in the western Serbia were more similar to varieties of the *ex situ* collection from eastern Bosnia and Herzegovina than to varieties collected in western Serbia 50 years ago. The comparison between OPVs and modern white maize hybrids for the basic chemical composition of grain showed the significant potential of OPVs for nutritive grain quality improvement. Increased organic production creates new possibilities for more intensive incorporation of OPVs into this agricultural system. New opportunities for the cooperation and share of responsibilities among breeders, farmers and genetic resources managers are initiated, to be used for *in situ* maize genetic resources conservation improvement. More detailed characterisation and evaluation of the white maize landraces will offer an explanation of what has motivated our farmers to maintain the OPVs production through centuries, despite the availability of modern hybrids.

Key words: open pollinated varieties (OPV), morphological characterisation, agronomic performance, maize hybrids.

INTRODUCTION

It is estimated that global white maize production ranges from 65 to 70 million tonnes annually. Compared to approximately 500 million tonnes of yellow maize, the white maize production seems negligible. In developed countries, white maize production is below 1% of total maize production. Moreover, it should be emphasised that white maize is exclusively produced for human consumption (Samayoa et al., 2016). It is particularly important in the countries of Sub-Saharan Africa and Central America, inhabited by about 400 million people (Badu-Apraku et al., 2013). In these countries, white maize is mainly grown under rain fed conditions with the limited application of contemporary cropping practices, and therefore achieved yield is low (1-2 t ha^{-1}).

In the past, white maize was very important in nutrition, especially for poorer human population of the Western Balkans. This is supported by the fact that the maize landraces collection from the former Yugoslavian regions, maintained at the Maize Research Institute „Zemun Polje" (MRIZP), contains nearly 700 white kernel accessions (out of total 2217 accessions) (Anđelković et al., 2017). The lower yield and a weaker response of OPVs to intensive cropping practices were the main reason for their replacement by hybrid varieties in the commercial maize production. Since modern varieties with all their benefits are available, it could not have been expected from the

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farmers to continue to grow old, traditional varieties. To prevent enormous genetic erosion in commercial breeding and agriculture production, the conservation of a broad diversity within OPV accessions in gene banks is of primary importance (Babić et al., 2012). The *ex situ* conservation has been designed to provide the preservation of the original (at the moment of collection) diversity, for a long period, although disabling changes in accordance with environmental alterations. Therefore, maize OPVs preserved in farmers' gardens under permanent variable conditions of the ecosystem, may have a great importance (Brush, 2004; Perales et al., 2005). Crossing with new introduced varieties and application of modern cropping practices could have negative impact on *in situ* conservation (Parales et al., 2003).

According to personal communication with Serbian farmers, it can be concluded that a great number of them still grow mainly white kernel OPVs, on small areas and for their own use, despite the availability of many high yielding hybrids in the market. Similar situation is in many European countries (Rodriguez et al. 2008; Bitocchi et al., 2009). Knežević et al. (2014) have studied the cultivation of old varieties in the region of Homoljske mountains (eastern Serbia) and found out that even 86% of interviewed farmers had grown at least one of old varieties. All of them have stated that the varieties were grown for their own use, while 15% had grown varieties for marketing. It is interesting that many farmers (44%) started to grow these varieties recently. OPVs seed originated from farm-saved seed through generations or by exchanging with friends and neighbours, while modern hybrids seed is produced and controlled by seed companies. Thus, introgression of the genetic material found in modern varieties can be a critical point for the *in situ* conservation system.

For a long period, maize breeding has been focused on increased and stabile yield. Produced grain is usually very uniform in its properties and can't fully meet specific market requirements (Babić et al., 2012). According to results obtained by Radosavljević et al. (1995) on hybrids and maize landraces grain quality parameters, hybrid grain has a higher content of starch and a lower content of amylose. By comparing of grain quality parameters for five white hybrids with two white and one yellow landraces, Babić et al. (2016) observed that landraces had increased protein and reduced starch content. The main reasons for farmers to grow old varieties are tastier flour obtained from varieties, harder kernel more suitable for storage, early maturing and more resistant plant to unfavourable growing conditions. Although the grain quality is an important parameter for human consumption, parameters for good maize bread making quality have not yet been fully defined (Samayoa et al., 2016). Some grain parameters are generally accepted as quality criteria, such as grain uniformity, high grain density, absence of pests, diseases and grain damage (Alonso-Ferro et al., 2008; Watson, 1988). Moreover, flint maize flour is more desirable due to the better texture of dough made from such flour, consistence and flavour than dent maize flour.

Due to the inconsiderable economic importance, small efforts have been made to improve white maize through breeding. In Serbia, almost nothing has been done on the improvement of white OPVs. A large number of white maize accessions contained in the MRIZP gene bank hinder the efficient selection of varieties for both the improvement through breeding and the detailed biochemical characterisation. Additionally, preserved landraces usually have poor agronomic performances, they are susceptible to lodging, diseases and pests, making them very undesirable for modern agriculture. Recently, increased public interest in organic farming and high stability under low-input conditions, have emphasised the importance of maize OPVs use in this system of agricultural production (Kovačević et al., 2014; Revilla et al., 2015).

Sixteen white maize accessions from the MRIZP gene bank and two newly collected accessions (in 2014) were selected for the present study. These accessions are with similar geographical origin and/or displayed similar result of preliminary characterisation. The objectives of the study were to determine: i) whether the two newly collected accessions mutually differ, ii) their greatest similarity and difference with the landraces selected from the gene bank, iii) the extent of difference between the selected landraces and white commercial maize hybrids regarding the basic chemical composition of grain and grain yield, iv) more suitable initial material for breeding purposes within landraces maintained according to the principles of the *ex situ* conservation (from the gene bank) and/or landraces permanently grown under conditions of the dynamic (*in situ*) conservation.

MATERIALS AND METHODS

Plant material

In 2014, MRIZP gene bank acquired two samples of white maize landraces from Dragačevo, designated as Beli osmak (white eight-rowed type; ND1) and Beli staklarac (white vitreous kernel type; ND2), donated by farmer Mr. Dušan Ivanović. According to his statement, these are two different white maize OPVs that have been grown for human consumption in this region for centuries. Beli osmak is characterised by a small number of kernel rows (eight), large and somewhat softer kernels, while Beli staklarac has a higher number of kernel rows and harder kernels. It is assumed that these varieties were grown under dynamic conservation conditions and that they can have a special importance for white maize breeding material improvement.

First, it was necessary to multiply the received samples, which was conducted in 2015 according to the standard gene banks procedure (FAO, 2014). During the multiplication process, characterisation and preliminary evaluation (CPE) was done for the following traits: time of tasselling and silking, kernel row arrangement, kernel type and kernel colour.

Sixteen MRIZP gene bank landraces, originated from the region of Dragačevo (western Serbia), were collected approximately 50 years ago, when exclusively OPVs had been grown in the region. According to gene bank passport data, kernel samples of these landraces designated as "white eight-rowed type" and/or "white vitreous kernel type", were visually compared with kernel of newly received samples (ND1 and ND2).

Table 1. Passport data for maize landraces evaluated

No.	Accesion number	Local name	Collection data			
			Year	Country	Vicinity	Site
1	ND1	Beli osmak	2014	SRB	Lučani	Dragačevo
2	ND2	Beli staklarac	2014	SRB	Lučani	Dragačevo
3	530	Beli osmak	1963	SRB	Lučani	Rti
4	532	Beli staklarac	1963	SRB	Lučani	Viča
5	533	Staklarac osmak	1963	SRB	Čačak	Čačak
6	537	Staklarac	1963	SRB	Lučani	Kotraža
7	548	Staklarac beli	1963	SRB	Lučani	Kotraža
8	554	Staklarac	1963	SRB	Čačak	Mojsinje
9	570	Beli poluzuban	1963	SRB	Vlasotince	Novo Selo
10	707	Stodanka	1964	SRB	Gadžin Han	Ravna Dubrava
11	987	Beli staklarac	1966	SRB	Čačak	Jelica (planina)
12	1087	Beli tromak	1973	MAK	Tetovo	Tenovo
13	1261	Beli domaći	1975	MNE	Titograd	Brežine
14	2182	Bjelić brzak	1989	BIH	Doboj	Prisade
15	2186	Bjelić	1989	BIH	Doboj	Velika Bukovica
16	2195	Domaći bjelić	1989	BIH	Derventa	Bunar-Bukovac
17	2196	Bjeličić	1989	BIH	Doboj	Gornja Ostružnja
18	2203	Osmak	1989	BIH	Zavidovići	Vozuća-Maričići

AN-accession number; SER-Serbia; MAK-former Yugoslavian Republic of Macedonia; BIH-Bosnia and Herzegovina; MNE-Montenegro.

The most similar landraces (530, 532, 533, 537, 548, 554 and 987) with ND1 and ND2, were finally selected for this experiment. Also, according to CPE, nine white MRIZP gene bank landraces originated out of western Serbia region, being most similar with ND1 and ND2, were additionally selected: two landraces from eastern Serbia (570 and 707), one from Montenegro (1261), one from Macedonia (1087) and five from Bosnia and Herzegovina (2182, 2186, 2195, 2196 and 2203). The names and passport data of all selected landraces are presented in Table 1.

For the comparative analysis of the grain basic chemical composition and grain yield, four commercial white maize hybrids (i.e. ZP-300b, ZP-620b, ZP-655b and ZP-718b) were chosen.

Field experiments

The experiment regarding agro-morphological characterisation of white maize landraces was conducted in 2016, in Zemun Polje, Serbia (44°52′N, 20°19′E, 81 m asl). Each landrace was sown in four rows with 20 plants per row, according the RCB design, in two replications.

For the characterisation according to CIMMYT/IBPGR descriptors for maize, the following traits were measured on 20 plants per landrace: tasselling and silking date, plant height, ear height, tassel node height, first tassel branch height, primary tassel branch number, number of leaves above the ear, ear leaf length and width, kernel row number, number of kernels per row, ear length and diameter, cob diameter, 1000-kernel weight, kernel hardness, total number of plants, number of lodged and broken plants at harvest, as well as grain yield. Traits evaluated were measured on plants from two central rows.

In next year (2017), 16 landraces and four commercial white maize hybrids were tested regarding grain yield evaluation. Genotypes were sown in four rows with 20 plants per row, according the RCB design, in two replications. Due to very poor agronomic performances, low yield and high susceptibility to diseases and pests, exhibited in previous field experiment, two landraces (532 and 570) were excluded from the experiment. The trial was sown and harvested manually. Two inner rows were used for yield estimation. Grain moisture at harvest was measured from five ears sample, while ten ears were used for the basic chemical grain composition assessment.

Laboratory experiment

Near-infrared spectroscopy (NIR; Infratec^{TM} 1241 grain analyzer, CHOPIN-Infraneo, Fosstecator, Sweden) was applied to determine the basic chemical composition of grain: proteins, starch and lipids.

Data analysis

All morphological parameters observed were analysed using two-factorial analysis of variance, and differences between mean values were tested by *F*-test. Mean values of measured parameters were used for the cluster analysis (Square Euclidian Distance, Complete Linkage). All statistical analyses were performed using programme the IBM SPSS Statistics 23.

RESULTS AND DISCUSSION

Table 2 shows that evaluated varieties expressed relatively low yield, ranging from 6.195 t ha^{-1} for the variety 12 (MB 1087) to 2.036 t ha^{-1} for the variety 4 (MB 532), respectively. The variety 1 (ND1) was a check and none of observed varieties had statistically higher yield than the check. The percentage of lodged and broken plants varied from 4.2% (variety 11) to 22.4% (variety 13), respectively. Landraces ND1 and ND2 exhibited 8.1% and 8.4% of lodged and broken plants respectively, being notably lower compared to the majority of observed landraces. Stalk quality of newly collected samples was improved to a certain extent in the long period of time by farmers selection considered as mass selection, in comparison to *ex situ* conserved accessions. It was observed that landraces ND1 and ND2, as well as varieties 11 and 16 expressed values for lodged and broken plants within the standards necessary for the registration on official varieties list. However, there was no significant improvement regarding yield. For instance, limit values in a trial carried out by the Serbian Commission for the Variety Releasing for standard grain quality maize hybrids FAO 300 maturity group amount to: 10.93 t ha^{-1} (yield), 20.4% (grain moisture), 3-5% (lodged and broken plants), depending on measured moisture. Required standards in the white maize hybrids trial are a bit lower: for FAO 300, limit yield amounted to 7.96 t ha^{-1}, limit moisture to 21.3%, while this value for lodged and broken plants was 7-9%. Additionally, the coefficients of variation in the OPVs trails were high, mainly due to high variability within and between varieties (Samayoa et al., 2016). Thus, the low yield and still poorer stalk quality are the main disadvantages, and should be improved during the white maize prebreeding activities.

Table 2. Rank for maize landraces grain yield in 2016

Rank	Genotype No.	Accession number	Lodged and broken plants (%)	Percentage of cob	Grain yield (t ha^{-1})
1	12	1087	12.9	18.0	6.195
2	17	2196	11.8	14.6	5.676
3	14	2182	13.6	18.5	5.258
4	5	533	10.4	16.2	4.986
5	15	2186	17.3	19.4	4.853
6	1	ND1	8.1	18.8	4.733^{check}
7	2	ND2	8.4	17.7	4.342
8	18	2203	13.3	18.7	4.219
9	13	1261	22.4	18.8	4.218
10	16	2195	8.3	13.7	4.117
11	8	554	16.9	16.8	3.675
12	6	537	15.5	17.0	3.656
13	9	570	13.0	16.4	3.513
14	7	548	18.2	17.7	3.477
15	3	530	9.6	14.4	3.441
16	10	707	12.6	15.9	3.146
17	11	987	4.2	26.1	2.282**
18	4	532	10.7	18.2	2.036**

$LSD_{0.05}$ = 1.664; $LSD_{0.01}$ = 2.286; CV(%) =19.25; ** - significant at 0.01 probability level.

The comparison of studied landraces at the phenotypic level, points out to the similarity between two newly collected samples (ND1 and ND2). Significant differences were obtained only for the number of kernels per row ($p<0.05$; Table 3a, b). According to the farmer's information, the variety Beli osmak is characterised by a small number of kernel rows (eight), large and somewhat softer kernels, while Beli staklarac has a greater number of kernel rows and harder, vitreous kernels. Analyses of variance did not show significant differences for these two traits between landraces ND1 and ND2. Intra-specific but not inter-specific differences for ear characteristics were found. It is possible that they used to be two different landraces that had been characterised by a different number of kernel rows and grain hardness, but in time, uncontrolled exchange of genes occurred (due to the lack in spatial or technical isolation). As a result, a new landrace with intermediate values was developed. Studying the scope of modern varieties-hybrids genes fluctuation into traditional OPVs, Bitocchi et al. (2009) indicated that "the specific conditions of cultivation (mostly the farmers' cropping practices) are the main factors influencing the level of introgression from maize hybrids into landraces. Therefore, the variable level of introgression from the modern maize hybrids suggests that by adopting appropriate practices, it is possible to avoid introgression from an undesired genetic source, and that the implementation of coexistence between different types of agriculture, including traditional landraces, is possible".

Aside of ND2, the comparison of the landrace ND1 with other landraces indicated that the lowest morphological differences were expressed regarding the landraces 15, 13 and 14 (MB2186, MB1261 and MB2182). It is interesting that neither of landraces was collected in the region of Dragačevo, but in the vicinity of Doboj, Bosnia and Herzegovina (14 and 15) and Podgorica, Montenegro (13). The landrace ND1 expressed the highest morphological differences with landraces from both western (landraces 3, 4, 5 and 7) and eastern Serbia (landraces 9 and 10), but also with one landrace from Bosnia and Herzegovina (landrace 17).

The similar trend was found for landrace ND2. Besides the minimal difference with the landrace ND1, ND2 expressed small differences with landraces 13 (Montenegro), 14 and 15 (Bosnia and Herzegovina), 12 (Macedonia) and 5 (Serbia).

The maximal morphological differences were noticed with landraces 3, 4, 6 and 11 from western Serbia, as well as with landraces 9 and 10 from eastern Serbia and with the landrace 16 from Derventa, Bosnia and Herzegovina.

Table 3a. Significant differences of plant morphological traits for evaluated maize populations

No	AN	PH	EH	NH	BH	TBN	NL	LL	LW
1	ND1	204.7^{b}	69.5^{cd}	143.0^{bc}	162.6^{bcd}	17.5^{def}	4.4^{def}	81.0^{abcd}	10.4^{bcde}
2	ND2	205.0^{b}	68.9^{cd}	143.0^{bc}	161.7^{bcd}	17.9^{def}	4.4^{def}	81.7^{abc}	10.4^{bcd}
3	530	173.2^{fg}	49.1^{e}	115.2^{fg}	138.9^{ef}	18.9^{cde}	4.7^{bcdef}	73.1^{ghi}	9.6^{efghi}
4	532	161.6^{g}	49.9^{e}	108.5^{g}	124.6^{g}	14.9^{fg}	4.5^{def}	77.8^{cdrfg}	8.4^{j}
5	533	181.7^{ef}	65.6^{d}	126.6^{def}	145.0^{e}	22.6^{ab}	4.7^{bcdef}	77.9^{cdef}	9.7^{cdefgh}
6	537	141.7^{h}	40.1^{f}	88.1^{h}	105.0^{h}	9.6^{h}	3.7^{g}	72.1^{hi}	7.3^{hi}
7	548	177.4^{ef}	61.8^{d}	125.8^{def}	143.7^{e}	19.4^{cde}	4.6^{cdef}	70.9^{i}	10.1^{bcdefgh}
8	554	200.3^{bcd}	61.6^{d}	143.1^{bc}	162.5^{bcd}	19.7^{bcde}	5.2^{ab}	79.9^{bcdef}	11.7^{a}
9	570	173.9^{fg}	67.6^{d}	127.3^{def}	145.7^{e}	16.6^{ef}	4.9^{abcd}	76.3^{efgh}	9.5^{fghi}
10	707	181.6^{ef}	63.7^{d}	127.2^{def}	142.0^{ef}	13.2^{g}	4.8^{bcde}	80.9^{abcde}	8.8^{ij}
11	987	162.1^{g}	48.2^{e}	111.6^{g}	129.5^{fg}	15.4^{fg}	4.8^{bcdef}	75.5^{fghi}	10.7^{b}
12	1087	210.4^{ab}	86.2^{a}	151.7^{ab}	172.2^{ab}	21.8^{abc}	5.1^{abc}	79.3^{bcdef}	10.3^{bcdef}
13	1261	186.6^{def}	69.0^{cd}	136.9^{cd}	150.1^{de}	17.9^{def}	4.7^{bcdef}	76.9^{defg}	9.4^{ghi}
14	2182	220.3^{a}	78.7^{ab}	159.8^{a}	179.5^{a}	17.3^{def}	5.4^{a}	84.8^{a}	10.5^{bc}
15	2186	186.9^{cde}	65.4^{d}	135.4^{cd}	150.4^{cde}	20.4^{abc}	5.0^{abc}	82.8^{ab}	10.7^{b}
16	2195	175.7^{efg}	66.0^{d}	120.6^{efg}	138.6^{ef}	20.2^{abc}	4.4^{ef}	77.0^{defg}	10.2^{bcdefg}
17	2196	186.9^{def}	65.1^{d}	128.9^{de}	145.9^{e}	19.1^{cde}	4.3^{f}	78.3^{bcdef}	9.5^{fghi}
18	2203	202.9^{bc}	76.9^{bc}	147.3^{abc}	163.8^{bc}	23.0^{a}	5.1^{abc}	80.8^{abcde}	9.6^{defghi}

AN - accession number, PH - plant height, EH - ear height, NH - tassel node height, BH - first tassel branch height; TBN - primary tassel branch number, NL - number of leaves above the ear, LL - leaf length, LW - leaf width.

Table 3b. Significant differences of ear morphological traits for evaluated maize populations

No	AN	KRN	NK	EL	ED	CD	KH	KW
1	ND1	9.9^{def}	10.2^{a}	18.7^{a}	3.73^{bcd}	1.84^{efg}	2.54^{ef}	253.8^{ef}
2	ND2	10.8^{bcde}	33.6^{b}	16.7^{ab}	3.62^{bcde}	1.99^{cde}	2.61^{e}	260.7^{e}
3	530	9.5^{f}	30.4^{bcd}	13.8^{cde}	3.46^{cde}	1.73^{fg}	2.12^{f}	211.9^{f}
4	532	10.9^{abcd}	25.5^{def}	13.4^{de}	3.43^{e}	1.93^{defg}	3.53^{ab}	352.5^{ab}
5	533	11.1^{abc}	31.2^{bc}	15.3^{bcd}	3.74^{abc}	2.09^{cd}	2.59^{e}	258.8^{e}
6	537	10.1^{cdef}	25.7^{def}	14.0^{cde}	4.02^{a}	2.40^{b}	2.74^{de}	273.8^{de}
7	548	10.1^{cdef}	26.4^{cde}	13.6^{de}	3.58^{bcde}	2.10^{cd}	3.49^{ab}	348.7^{ab}
8	554	11.9^{a}	24.6^{ef}	11.7^{e}	3.60^{bcde}	1.99^{cde}	3.33^{abc}	333.1^{abc}
9	570	10.9^{abcd}	26.2^{cde}	15.1^{bcd}	3.79^{ab}	2.14^{cd}	3.15^{bcd}	315.0^{bcd}
10	707	8.3^{g}	27.9^{cde}	16.0^{bc}	3.43^{e}	1.94^{def}	3.24^{abc}	323.8^{abc}
11	987	10.9^{abcd}	20.5^{f}	11.8^{e}	3.83^{ab}	2.86^{a}	3.56^{ab}	355.6^{ab}
12	1087	10.1^{cdef}	27.3^{cde}	15.2^{bcd}	3.70^{bcde}	1.91^{defg}	3.70^{a}	370.0^{a}
13	1261	9.6^{f}	27.0^{cde}	16.1^{bc}	3.74^{ab}	1.99^{cde}	2.90^{cde}	290.0^{cde}
14	2182	9.6^{f}	30.7^{bcd}	16.7^{ab}	3.72^{bcd}	2.20^{bc}	2.69^{de}	269.4^{de}
15	2186	9.8^{ef}	29.8^{bcde}	15.3^{bcd}	3.72^{bcd}	1.94^{def}	3.29^{abc}	328.8^{abc}
16	2195	11.3^{ab}	26.0^{cde}	14.2^{cd}	3.76^{ab}	1.96^{cdef}	3.26^{abc}	326.3^{abc}
17	2196	9.9^{def}	30.7^{bcd}	15.1^{bcd}	3.44^{de}	1.69^{g}	3.48^{ab}	348.1^{ab}
18	2203	11.1^{abc}	30.2^{bcd}	15.6^{bcd}	3.73^{bc}	2.10^{cd}	3.33^{abc}	332.5^{abc}

AN - accession number, KRN - kernel row number, NK - number of kernels per row, EL - ear length, ED - ear diameter, CD - cob diameter, KH - kernel hardness, KW - 1000 kernel weight.

Obtained results point out not entirely credible donors' statement that the landraces had been grown in the region of western Serbia for centuries. Although the landrace ND2 was morphologically similar to landrace 5, collected in western Serbia more than 50 years ago, the rest of similar populations were not collected in that region. It is very likely that received samples ND1 and ND2 had not been exclusively derived from landraces cultivated in western Serbia in the 1950s. Due to established similarities, it can be assumed that these landraces originated from additional reintroductions, i.e. from the regions of eastern Bosnia. This could be a logical conclusion, because the OPVs remained longer in the production in regions with extensive farming, such as highland regions in Bosnia and Herzegovina. Furthermore, observed samples from Bosnia and Herzegovina were collected 25 years later. This is in line with Bitocchi et al. (2015), that "according to information from the farmers, modern maize landraces from central Italy appear to be derived from the landraces cultivated in the same area before 1950. However, the farmer information might be incorrect or could be incomplete; thus, we cannot exclude other possible explanations".The same authors stated that modern landraces may be derived from reintroductions from other regions, or can be developed from modern hybrids by seed multiplication from year to year.

The coefficients of variation were estimated for all observed traits within each landrace (data not presented). For newly collected landraces, they varied within the similar range as for the rest of landraces. Such a result may indicate that a high level of genetic diversity was maintained within the variety. Bitocchi et al. (2009) obtained the similar results and stated that "this lack of genetic erosion indicates *in situ* conservation as an efficient strategy for preserving genetic diversity".

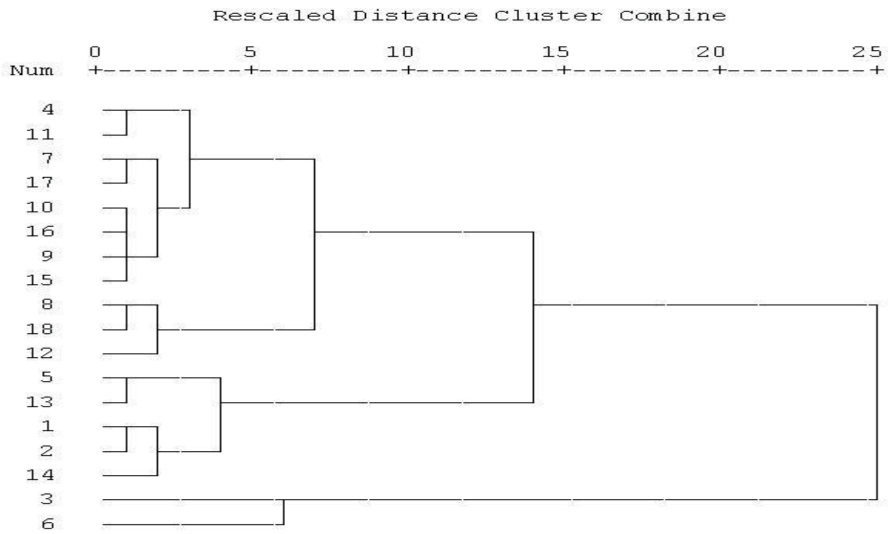

Figure 1. Cluster analysis of maize OPVs for morphological traits observed

The cluster analysis was performed on the basis of phenotypic characterisation (Figure 1). Newly collected landraces ND1 and ND2 were clustered only with the landraces 14 (MB2182) from Bosnia and Herzegovina, 13 (MB1261) from Montenegro and 5 (MB533) from Serbia. Based on results of the morphological analysis, it is difficult to draw very reliable conclusions. It can be assumed that the exchange of the genetic material occurred in a broader territory within the former Yugoslavia and that therefore there are similarities among samples of landraces collected in different geographical regions but also in various periods. On the other hand, there are significant differences among samples collected in the same region but in the different time intervals. It could be speculated that certain changes occurred in OPVs during their 50-year cultivation in the region of Dragačevo due to: 1) uncontrolled gene fluctuation among various varieties, occurring under the lack of crosses prevention measures; 2) possible reintroduction of new varieties by seed exchange among farmers; 3) occurrence of changes as a result of natural and/or artificial selection. Resulting changes may be positive, such as higher stalk quality of newly collected varieties, but also unfavourable, such as lower protein content in relation to the *ex situ* maintained varieties. Moreover, it is possible that the fluctuation of genes from modern hybrid varieties into open-pollinated populations occurred in the long time period of the *in situ* conservation. Despite of all shortcomings, the *in situ* conservation is a very useful form of genetic variability conservation. If adequate measures are applied during cultivation, undesirable effects on OPVs can be prevented and efficient coexistence of different types of agriculture can be provided (Lorenzana and Bernardo, 2008; Urechean and Bonea, 2017).

Flour made of white maize is almost exclusively used in human nutrition. The NIR method was applied to determine the basic parameters of grain chemical composition in evaluated OPVs and four white maize hybrids in 2017. In previous studies on traditional maize varieties (Babić et al., 2016), obtained results indicated a higher content of protein and a lower content of starch in OPVs grain. Radosavljević et al. (1995) established not only a lower content of starch in landraces grain, but also a higher portion of the amylose component. Pronounced differences in protein, starch and lipid content between OPVs and hybrids were observed in Figure 2.

Figure 2. Protein, starch and lipids content (% of absolute dry matter) in grain for maize OPVs and hybrids observed in 2017

OPVs grain posses higher protein and lower starch content compared to the hybrids grain. Analysis of variance showed that protein content of all OPVs is significantly higher compared to the protein content of hybrids grain ($p<0.01$; LSD=1.494). Starch content in hybrids ZP 718b and ZP 655b was significantly higher than in all OPVs ($p<0.01$; LSD=3.219). Even the hybrid ZP 620b had higher starch content than all OPVs, with the exception of landrace ND2 ($p<0.05$; LSD=2.335). At this probability level, hybrid ZP 300b also had higher starch content than OPVs, except ND1, ND2 and MB554. Such a clear diversification was not observed regarding lipid content, except in case of hybrid ZP 620b, which lipid content didn't significantly differ from OPVs ($p<0.05$; LSD=0.4133).

Grain yield and protein yield per ha of tested OPVs and hybrids were presented on Figure 3a. As it was expected, hybrids grain yield obtained (ranging from 6.462 t ha^{-1} for ZP 300b, up to 8.929 t ha^{-1} for ZP 620b) was higher in compare to OPVs grain yield (ranging from 2.523 t ha^{-1} for OPV 532, up to 5.593 t ha^{-1} for ND1). Due to higher grain yield per ha, hybrids ZP 620b, ZP 655b and ZP 718b, produced higher protein yield per ha, although having lower grain protein content. Early maturing and lower-yielding hybrid ZP 300b exhibited similar protein yield per ha as ND2, MB533, MB1087, or even lower compared to ND1 and MB1261 (Figure 3b). Compared to *ex situ* OPVs, newly collected *in situ* varieties ND1 and ND2 achieved higher grain yield due to possible existence of certain selection pressure.

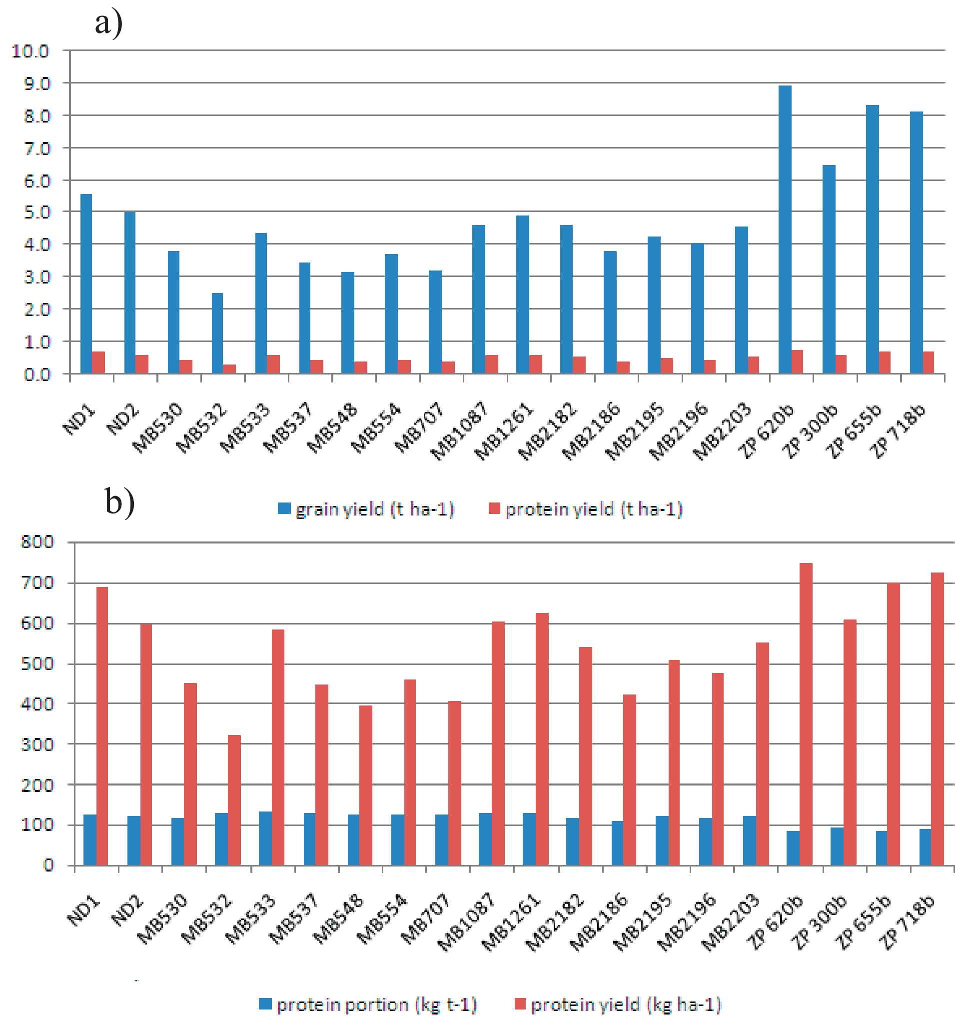

Figure 3. Grain yield, protein yield and protein portion in maize OPVs and hybrids grain observed in 2017

Farmers are prone to select larger ears for the succeeding sowing, which probably resulted in a more frequent selection of ears similar to dent types, being more robust and yielding in comparison to flint types. This has led to a slight shift within varieties towards dent types. The assumption is confirmed by the fact that newly collected varieties ND1 and ND2 ranked 1st and 2nd, i.e. 17th and 15th regarding ear length, i.e. kernel hardness, respectively. This is in line with the founding of N'Da et al. (2015) that the farmers would choose as seed to renew their crop, the biggest and the most beautiful ears from the previous harvest.

Modern plant breeding has been historically oriented toward high agronomic yield rather than the nutritional quality (Morris and Sands, 2006). Such trend indicates that it is necessary to balance between yield and quality in breeding process. Nevertheless, obtained results indicate that OPVs maintained in gene bank, as well as OPVs continuously grown on fields represent potential source for development of good initial material, thus providing grain quality enhancement in white maize breeding collections. The increased incidence of intolerance and allergies to gluten and gluten-containing products has opened up new possibilities for the use of maize flour and maize-based products (Padalino et al., 2011). Since traditional habits in the consumption of white maize still exists, there is a need for its more intensive breeding, but also for the development of new products made of white maize flour and their popularisation among consumers. It is necessary to take certain pre-breeding activities in order to develop a good initial material for white maize breeding. After a certain improvement of poor agronomic traits, old maize varieties could take an important place in the chain within organic agricultural production. We have to bear in mind that the varieties development is not only the result of commercial breeding, but also of farmers voluntary activities, being the very first breeders (Mirić, 2013). The introduction of the organic agricultural system resulted in a possibility of a broad cooperation and sharing of responsibilities in the genetic resources conservation. The farmer becomes one of the actors in the creation and maintenance of the present diversity (Berthaud, 1997). In addition to the *ex situ* genetic resources conservation preformed within gene banks as institutions, the *in situ* conservation may have a certain role in this process through building-creating new relationships among breeders, farmers (especially those involved in organic farming) and genetic resources managers.

CONCLUSIONS

Since stem quality and grain yield of the OPVs tested were below standards required for registration on the List of released varieties, special attention should be paid on these two traits improvement during white maize pre-breeding activities.

At morphological level, landraces (ND1 and ND2) continuously grown in Western Serbia region, expressed high mutual similarity and high similarity with certain *ex situ* maintained OPVS collected out of this geographical area.

Grain protein content of OPVs was significantly higher ($p<0.01$; LSD=1.494) compared to the hybrids evaluated. Although grain protein content in ND1 and ND2, as actual OPVs, were lower compared to the majority of OPVs, differences were not significant, suggesting right direction in the local farmer's system of maintaining.

In comparison to OPVs, hybrids obtained significantly higher grain yield per ha. Despite the lower grain protein content, hybrids ZP 620b, ZP 655b and ZP 718b, achieved higher protein yield per ha. Protein yield per ha obtained by early maturing ZP 300b, as lower-yielding hybrid, was at the level of ND2, MB533 and MB1087, and below ND1 and MB1261, emphasizing high potential of these landraces towards improved grain quality.

Therefore, OPVs maintained both under *in situ* and *ex situ* conservation represent a good source for existing MRIZP gene pool of white maize breeding materials improvement, and to a large extent, can play a significant role in production of high quality food for human nutrition.

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