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CHARACTERISATION AND EVALUATION TOWARDS SELECTION OF MAIZE LANDRACES WITH THE BEST *PER SE* PERFORMANCES

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ABSTRACT

The narrow genetic base of commercial maize varieties emphasise the necessity for conservation, characterisation and utilisation of germplasm stored within gene banks. Broad genetic variability preserved in the Maize Research Institute Zemun Polje (MRIZP) gene bank, which includes accessions which originated from the Western Balkan, as a part of European corn-belt, is an exceptional source of desirable traits for enriching breeders' working collections for maize breeding under temperate conditions. Preliminary screening for abiotic stress tolerance, which marked 321 maize landraces, served as the first step in stratification process of selecting a smaller number of accessions from the entire gene bank local collection. After classification of these landraces into eleven homogenous groups, the objective of this study was to continue the stratification process of selection (as a second step), based on evaluation of agro-morphological traits of interest for breeding. The conducted evaluation highlighted 40 landraces with the best *per se* performances, important for breeding. Out of them, 28 early-maturing flint landraces with stiff stalks, low positioned ears, high yield potential and good general ear assessment could be considered as valuable source for their introgression into elite flint germplasm pool.

Keywords: flints, gene bank, heterotic potential, pre-breeding, *Zea mays* L.

INTRODUCTION

Richness of genetic resources stored in gene banks is very little used in plant breeding. The huge gap between genetic variability maintained in gene banks and commercial breeding is caused by lack of documentation, of appropriate collection description and of information important for breeding, as well as by the fact that many samples have limited adaptability and seed quantities (Technow et al., 2014). Moreover, large number of gene bank accessions loaded with many undesirable properties from the aspects of modern cropping practices and breeding, require expensive and long-term process of their characterisation and improvement, while the final results, in a commercial sense, are uncertain.

The evaluation of five distinct Central European OP varieties, 85 hybrids and their parental components with the total of 55 SSR markers revealed that genetic variation within and among cultivars decreased significantly during the last five decades (Reif et al., 2005) and is almost certainly limited in comparison to the large genetic diversity available in gene banks (Le Clerc et al., 2005). Numerous unique alleles present in these OPVs, were lost in the elite flint pool, particularly in the last two decades, which impose a necessity to enlarge genetic base in breeding programmes that encompass only 3-5% preserved maize variability (Curry, 2017). Landraces adapted to local growth conditions could play significant role in the process of continuous introgression into commercial breeding material (Mitrović et al., 2016; Vega-Alvarez et al., 2017; Mikić et al., 2017).

The preservation of genetic resources diversity, significance of genetic variability for the survival of any species and its breeding, as well as previously mentioned actual difficulties for the efficient use of these resources, generated the pre-breeding concept (Ortiz et al., 2010).

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The implementation of long-term pre-breeding programmes is the most promising way of linking genetic resources and commercial breeding programmes. The concept encompasses programmes and activities for the purpose of identifying desirable properties and/or genes from non-adapted (exotic and semi-exotic) material, including adapted material that will be under certain selection pressure, as well as programmes and activities that improve gene bank materials up to the level acceptable to commercial breeding programmes (Shimelis and Laing, 2012). However, the fact that there are only few such programmes is a limiting factor for more efficient utilisation of landraces from gene bank collections (Carena, 2005). Successful examples of maize pre-breeding programmes are the Latin American Maize Project (LAMP), the Germplasm Enhancement of Maize (GEM) (Carena et al., 2009a) and EU GENRES (Gousnard et al., 2005).

Within Maize Research Institute Zemun Polje (MRIZP) gene bank, that encompasses 2217 local maize accessions, pre-breeding activities are carried out through the long-term stratification process of characterization and evaluation of gene banks accessions and the selection of a smaller number of genotypes with desirable properties, in order to introgress them into elite breeding material (Vančetović et al., 2015).

Previous screening for drought tolerance marked 321 local maize landraces as possible sources of tolerance (Babić et al., 2012a), and was exploited as the first step in stratification process. After classification of these landraces into eleven homogenous groups based on morphological similarity (Babić et al., 2015), the objective of this study was to continue the stratification process of selection (as a second step) of a smaller number (approximately 10-15% of representative landraces of each homogenous group) for the following pre-breeding activities. The selection of landraces was based on characterisation and evaluation of agronomic traits of interest for breeding, giving the priority to flint type.

MATERIAL AND METHODS

Plant material

Within the long-term pre-breeding activities, we focused on 321 MRIZP gene bank western Balkan landraces (https://mrizp.rs/emdb/default.htm), previously designated as possible sources of drought tolerance (Babić et al., 2012a). Due to the large number of genotypes (321), their assignment into homogeneous groups according to CIMMYT/IBPGR descriptors, was undertaken. Based on 27 morphological traits, along with the application of non-hierarchical and hierarchical cluster, correspondence and discriminant analyses, eleven homogenous groups were created (Figure 1), which were fully described in Babić et al. (2015). However, 11 landraces were excluded from further evaluation, due to too long/short vegetative period and high percentage of broken and barren plants.

Stratification process for selection of the best performing landraces, as a procedure which allows simultaneously retaining as much variability as possible, is presented through scheme of planned complete pre-breeding activities (Figure 2). Due to the large number of observed genotypes and economic aspects, the selection in the initial steps was performed on the basis of phenotypic characterisation.

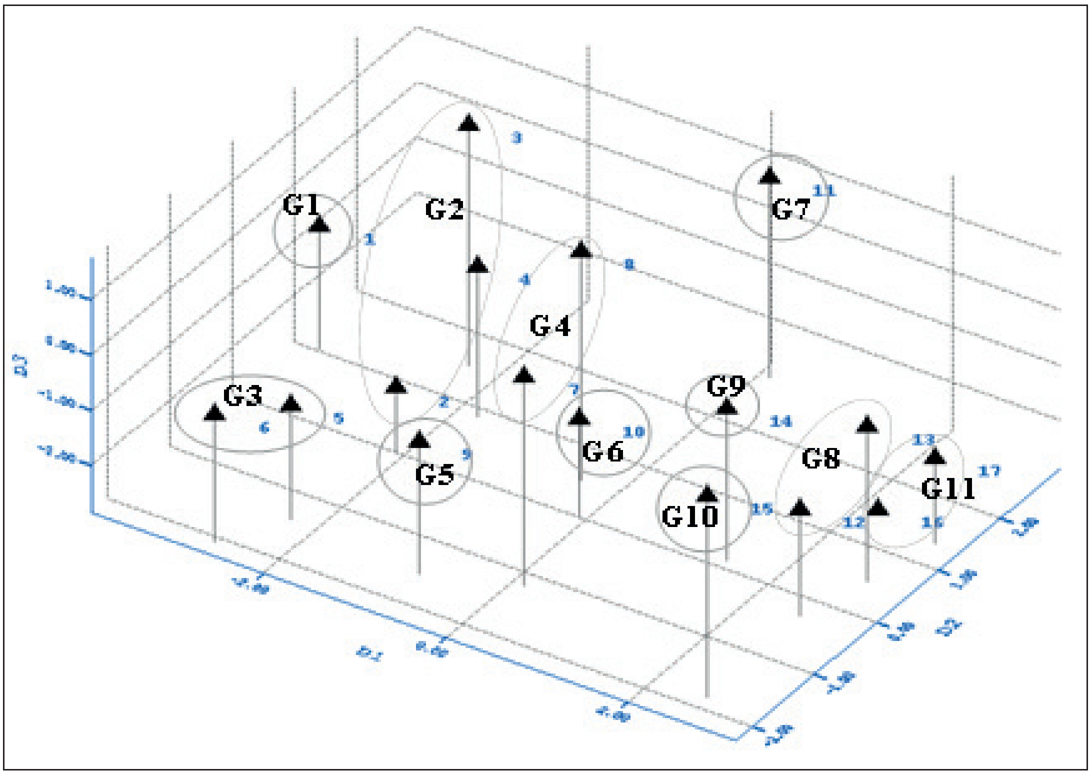

Figure 1. Distribution of cluster's centres, and groups for investigated local landraces in 3D discriminate space

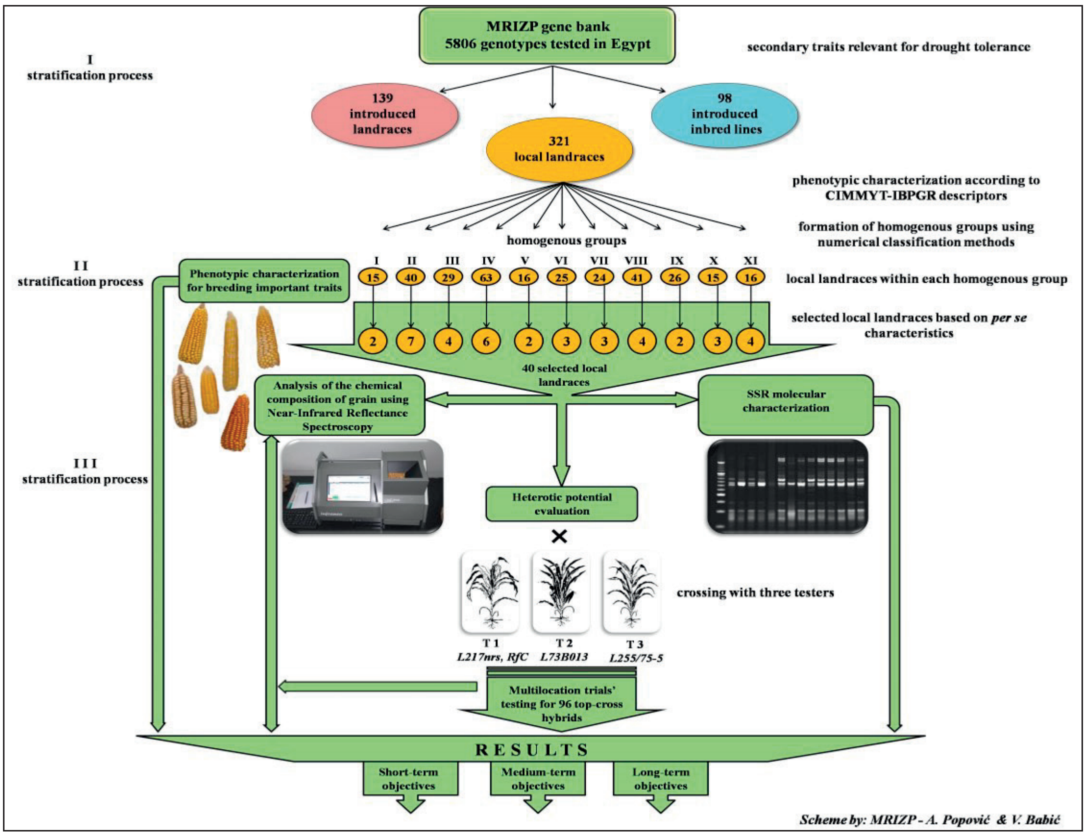

Figure 2. Selection of local maize landraces through stratification process

Field experiment

The experiment was carried out in 2013 and 2014 in Zemun Polje, Serbia (44°52´N, 20°19´E, 81 m asl). Landraces (310) were sown within eleven homogenous groups and further characterisation based on breeding important traits was conducted. The number of landraces per each homogenous group was presented in Figure 2. Each landrace was sown in two rows, 30 plants per row (15 hills were spaced at 40 cm, while the inter-row distance was 75 cm, two plants per hill). The experiment was set up according to the randomised complete block design, in two replications.

Beginning and mid-flowering dates (i.e. 10% and 50% tasseling, anthesis and silking) were recorded. The plant and ear height were measured on 15 plants per landrace, in both replications. The intensity of leaf green colour was estimated on the 1-3 scale: 1 - light green, 2 - medium green and 3 - dark green.

At harvest, the following traits were recorded: the total number of plants per row, the number of lodged and broken plants, as well as the number of ears per landrace. The yield per plot was determined. A sample of five ears was used to determine the grain moisture content. Visual ear evaluation based on shape, length, diameter, number of kernel rows, seed set and general ear assessment (1 - poor, 2 - medium, 3 - good) was estimated. The grain type was also determined in the same sample according to the International Union for the Protection of Cultivated Varieties of Plants (UPOV) Descriptor: 1 - flint, 2 - flint-like, 3 - intermediate, 4 - dent-like, 5 - dent, 6 - sweet, 7 - pop. Plants and ears were photographed.

In parallel, five commercial inbred lines (potential testers) were sown, in order to evaluate the date of flowering under the same environmental conditions, as important information for subsequent crossings.

Statistical analysis

IBM SPSS Statistics 20 programme was used for assignment of maize landraces into 11 homogenous groups, which include application of nonhierarchical, hierarchical cluster, correspondence and discriminant analyses (Babić et al., 2015). Descriptive statistics were used for all agro-morphological traits measured. Two-way ANOVA was used for determination of differences in grain yield within each homogenous group.

RESULTS AND DISCUSSION

Despite the extensive genetic potential contained in gene bank collections, the valuable variations remain mostly unused by plant breeders (Carena et al., 2009b; Masuka et al., 2017). Lack of information significant for breeding is frequently a limiting factor when using gene bank material. Although there were 27 traits of selected landraces characterised by the CIMMYT/IBPGR Descriptor, there was no information on stalk quality, combining ability and grain yield, the traits important for breeding. Since agro-morphological characterization of germplasm accessions is fundamental in order to provide information for plant breeding programs, the following traits were tested during the preliminary evaluation: early and final growth, tasseling and silking, plant and ear height, lodged and broken plants, ear number per plant, endosperm type, kernel colour, ear quality and yield (Nascimento et al., 2011; Badu-Apraku and Fakorede, 2017). Therefore, in this experiment, the selected landraces (310) were sown according to defined groups and the preliminary evaluation for breeding important traits was done.

The plant and ear heights are presented in Figure 3A. The lowest plant and ear height, as well as the ratio for these two parameters were noticed in the first three groups of populations. Somewhat higher average values of the same parameters were recorded in landraces from the groups IV, V, VI and VII. Study of plant architecture, phenology and yield components on 336 maize lines, revealed that yield components distinguished dents from lower yielding genetic groups (Bouchet et al., 2017). This was in line with our findings that the higher participation of dents is followed by the higher average plant height, and it was observed in the groups VIII and XI. These groups comprised higher yielding medium to late maturing dent maize landraces. Also, it was noticed that ascendant group number of a homogenous group exhibited the trend of the constant increase of ratio between PH and EH, ranging from 24% (for the group I) to 39% (for the group XI).

The lowest number of days to 50% tasseling was recorded in landraces from the group I (47.4), while the number of days in landraces from groups X and XI ranged from 58.3 to 59.1, respectively (Figure 3B). The highest anthesis-silking interval (ASI) was in the group XI (3.8), and the lowest in the groups I and II (0.1 and 0.6, respectively). More pronounced variation in ASI (even up to four days) was observed on individual landraces level. However, the 4-days interval difference could be considered as continuous, undisturbed pollination, if it occurs under optimal conditions (i.e. under conditions without extreme temperatures that lead to pollen abortiveness or without the combination of high temperatures and humidity that disturb physiological role of silk up to 40%) (Cerović et al., 2014).

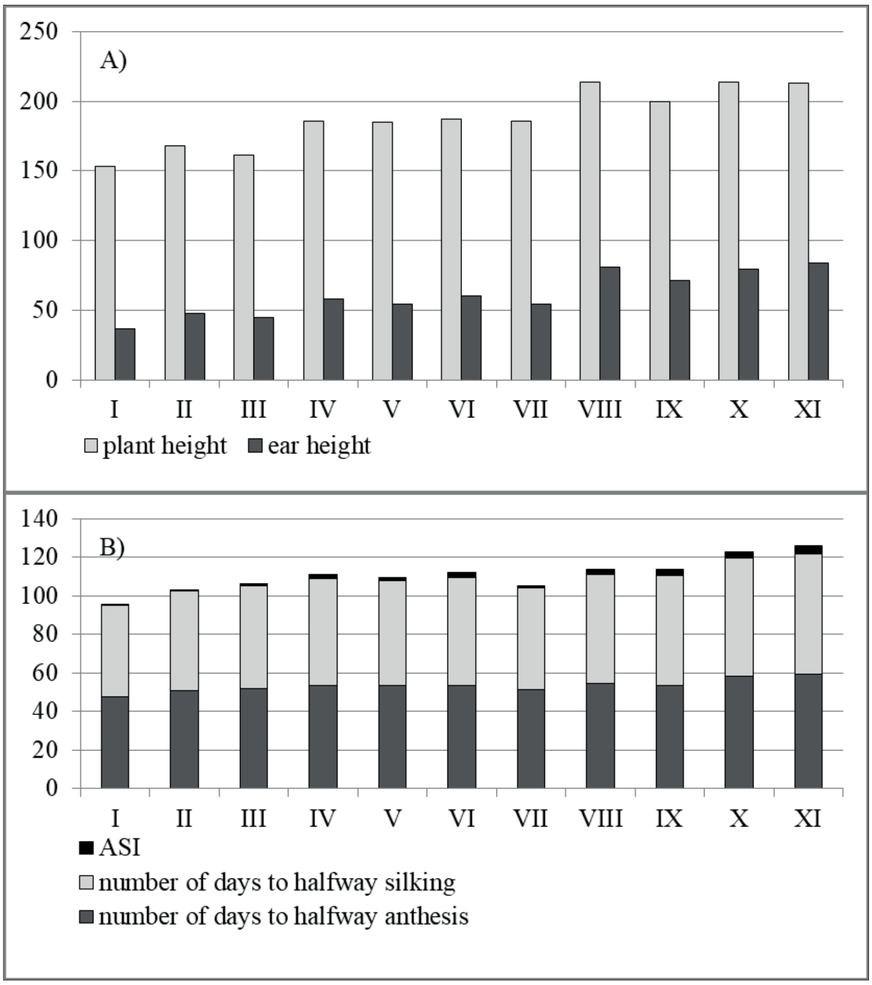

Figure 3. Average values of: A) plant and ear height, B) number of days to flowering and ASI, over homogenous groups

The grain type was evaluated according to the UPOV Descriptor, and then the percentage of participation of different kernel types per homogenous group was estimated (Figure 4A). The group I exclusively comprised extra-early maturing flint landraces originated from the sites where landraces were grown on rather isolated fields. Inability to cross with other maize landraces might be reflected in preservation of original introduced form.

Flints and flint-like landraces, originating from the hilly regions, prevail in groups II and III (90 and 89%, respectively), are similar to those from the group I, although with a slight percentage of derived and semi-dent kernel types and a certain number of pop maize landraces. Even a slight participation of dent germplasm in landraces from groups II and III contributed to their more successful spreading and this is confirmed by a higher number of collected accessions. Flints and flint-like kernel types, which prevail in the groups IV (63), V (16), VI (25) and especially in the group VII (24), differ from the flints of the first three groups. These are the early-maturing flint landraces, with more robust and higher plants, wider leaves and longer ears. However, certain percentages of dent-like and dent kernel types were recorded in the groups IV and VI.

In the group VIII (41 landraces) predominate dent and dent-like kernel types (80.2%), which is followed by 12.2% of intermediate, and 7.6% of flint and flint-like kernel types. These landraces probably originated from the fourth introduction (at the end of 19th century) of American Corn Belt dents, crossed with already existing flints (Babić et al., 2012b). The group IX (26 landraces) encompasses 50% flint and flint-like types, 11.5% intermediate and 38.4% dent-like and dent kernel types. In the group X prevailed dent-like and dent landraces (53.3%), with 33.3% of intermediate kernel type. Sixteen landraces of the group XI, predominantly collected in Pannonian Plain, consist of dents morphologically the most similar to dents from South America.

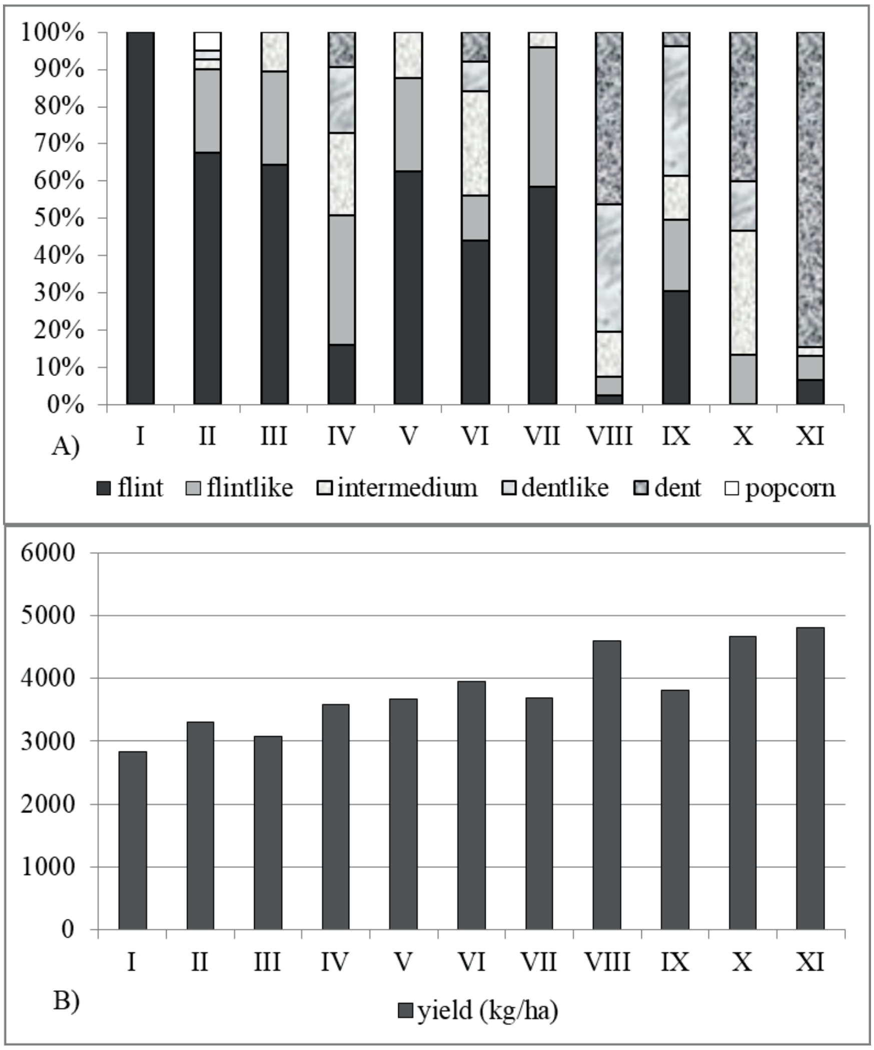

Figure 4. Average values of: A) kernel types B) grain yields over homogenous groups

By comparing average yield of these eleven groups, it can be concluded that the first three homogenous groups represent early-maturing, mainly yellow-orange flints, characterised with lower yield and similar morphological properties (Figure 4B). The yield in the homogenous groups IV, V, VI, VII and IX is approximately equal, ranging from 3.59 t ha^{-1} to 3.96 t ha^{-1}. The yield of landraces from the groups VIII, X and XI, which are predominantly late-maturing dents and their derived types, is over 4.5 t ha^{-1}.

Besides good *per se* performances, the initial breeding population must have good combining ability with another population, or with a group of populations or inbreds, that will serve as a second parent in the process of hybridisation (Vančetović et al., 2015). The main idea of this work was to select the most representative landraces of each of eleven homogenous groups to be crossed with elite commercial testers in order to define their heterotic pattern.

Flowering synchrony between male and female parents of maize hybrids is an important driver for improved kernel set and grain yield (Worku et al., 2016). In order to evaluate the date of flowering under the same environmental conditions, five commercial testers were sown. Three inbred testers were chosen for subsequent testing: L225/75-5 (Lancaster germplasm), B73B013 (BSSS x Iowa dent) and L217 (Iowa dent). Due to extremely poor agronomic traits (poorly developed tassels, short pollen shading period and small amount of pollen), inbreds F2 and Polj17 were excluded.

When selecting the initial material adapted to the testing conditions, a high level of desirable traits is important, as it provides better chances to obtain superior inbreds through direct selection (self-pollination) from the landrace and/or requires a lower number of selection cycles to achieve the desired level of traits within the landrace (Gorjanc et al., 2016). In this evaluation, landraces with small ASI values, low positioned ears, high yield *per se*, dark green leaves, a low number of lodged and broken plants, as well as a good general estimation of ears were preferable. In order to be selected, landrace should obtain yield significantly higher than the average value of yield from the homogenous group to which the landrace belongs. This requirement was not achieved by the landraces 589, 1379 and 2144. However, they were selected for the following reasons: landraces 589 and 1379 are early-maturing genotypes with favorable plant architecture, cylindrical shape of ear and pests/disease damage-free. The landrace 2144 was characterized by low positioned, well developed cylindrical and long flinty ear.

In accordance with the set goals, 40 landraces (10-15% from each homogenous group) were selected, such as follows: two, seven, three, seven, two, three, three, four, two, three, four over groups I to XI, respectively (Table 1). The proportional selection of representative landraces from each of eleven groups leads to the equal inclusion of the available gene pool variability. Typical representatives of each homogenous group were presented in Figure 4.

The narrow base of existing breeding collections imposes the necessity of revising flint landraces (Böhm et al., 2014). Considering the high genetic variation found within the Western Balkan flint landraces, it seems promising to use the very best of them for programmes of introgression in order to enlarge the genetic base of the elite flint germplasm pool in Central Europe.

Table 1. Basic passport data and observed parameters in selected landraces

HG	AN	CC	AL	PH (cm)	EH (cm)	PH/EH (%)	LBP	Y (t ha^{-1})	GEA	GC	KT
1	1869	CRO	850	147	30	20.47	5	3.46	2	3	1
	1890	CRO	600	147	41	27.68	4	4.27	3	2	1
2	594	MAC	661	175	62	35.62	1	3.04	2	2	1
	773	SRB	500	188	52	27.74	2	4.23	3	3	1
	1185	MNE	215	160	38	23.59	3	3.45	3	3	1
	589	MAC	661	168	56	33.02	2	2.39	3	1	1
	1379	BIH	650	169	44	26.42	6	2.12	2	1	1
	1381	BIH	700	190	53	28.33	5	4.50	3	2	2
	1267	MNE	500	202	54	27.07	0	4.53	2	2	3
3	13	MNE	900	174	54	31.23	3	3.14	3	3	1
	467	BIH	500	159	34	21.19	2	3.68	3	3	1
	144	SRB	900	181	67	36.81	1	4.39	3	2	1
4	871	MAC	105	164	43	26.30	3	3.55	3	3	2
	1960	BIH	750	178	46	25.83	4	4.84	2	3	3
	1276	MNE	800	195	57	29.28	3	4.97	2	3	5
	846	BIH	700	182	48	26.32	10	3.35	3	3	3
	1384	BIH	650	185	50	27.07	7	3.99	3	3	3
	2144	CRO	620	175	45	25.77	3	3.41	3	2	1
	1534	SRB	174	197	68	34.51	1	5.44	3	3	1
5	642	SRB	533	184	45	24.77	4	4.29	2	3	1
	1798	SRB	550	195	70	35.76	0	5.36	3	2	3
6	1895	CRO	500	181	56	30.53	3	5.14	3	2	1
	2033	CRO	50	198	71	35.80	2	5.47	3	3	3
	2006	BIH	800	206	81	39.37	3	5.48	3	2	1
7	2230	BIH	700	189	53	28.14	2	4.84	3	3	2
	2236	BIH	750	205	59	28.87	2	4.20	3	3	2
	2176	SLO	620	210	55	26.28	4	4.19	3	1	1
8	2249	BIH	800	230	90	39.08	2	7.74	3	2	5
	2036	BIH	800	205	72	34.54	3	5.48	3	2	4
	1665	MAC	700	218	91	41.90	0	5.79	3	2	4
	632	SRB	400	220	94	43.19	4	5.34	2	2	5
9	1945	BIH	700	204	72	35.02	5	4.45	3	3	1
	2047	CRO	180	209	88	42.27	3	4.69	2	1	4
10	1346	MNE	200	225	80	35.60	6	5.88	3	3	5
	877	BIH	41	206	68	33.18	1	5.36	3	3	3
	1569	SLO	103	261	121	46.23	3	5.52	3	3	5
11	197	SLO	200	213	77	36.03	1	4.65	3	3	5
	288	SRB	80	221	85	38.32	3	5.23	3	1	5
	1509	SLO	200	216	87	40.07	5	6.92	3	3	5
	1450	CRO	300	233	98	41.69	8	5.73	3	3	5

HG – homogenous group; AN – accession number; CC – country of collection; AL – altitude of collection site; PH – plant height; EH – ear height; PH/EH – plant and ear height ratio; LBP – lodged and broken plants; Y – yield; GEA – general ear assessment; GC – leaf green colour; KT – kernel type; CRO – Croatia; SRB – Serbia; BIH – Bosnia and Herzegovina; MAC – Republic of North Macedonia; SLO – Slovenia.

CONCLUSIONS

Broad genetic variability preserved in the MRIZP gene bank accessions, which originated from the Western Balkan region, generally considered as a part of European corn-belt, is an exceptional source of desirable traits for enriching breeders' working collections for maize breeding under temperate conditions.

Applied stratification process could be recommended as efficient method for selection of small number of landraces from entire gene-bank collections, allowing the preservation of as much gene pool variability as possible. Finally, this method could provide precise information necessary to develop synthetic populations as an initial material in accordance to short-, medium- and long-term goals of the commercial maize breeding programmes under temperate conditions and fulfil so-called missing places in commercial breeding gene pool (particularly with early-maturing flint genotypes).

As a result, forty landraces with the best *per se* performances important for breeding were selected out of 310 landraces. In particular, twenty-eight early-maturing landraces with flint (AN - 1869, 1890, 594, 773, 1185, 589, 1379, 13, 467, 144, 2144, 1534, 642, 1895, 2006, 2176 and 1945), flint-like (AN - 1381, 871, 2230 and 2236) and intermediate kernel types (AN - 1267, 1960, 846, 1384, 1798, 2033 and 877) could be considered as genotypes of special importance for the commercial breeding programme.

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