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Maize core collection for increased grain quality

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Abstract

Formation of the maize (*Zea mays* L) core collection for increased macronutrient content is presented. Among 3,443 populations from Maize Research Institute (MRI), Zemun Polje (ZP), a total of 352 accessions (around 10.2%) were chosen for the core collection. The criteria were increased protein, oil and/or starch content in the kernel of the accessions, as well as good combining ability (CA) that was tested with two Lancaster and one each BSSS and Iodent testers. Average values for protein, oil and starch content for the whole collection were 11.5%, 4.2% and 68.9%, respectively. On the other hand, averages for the core collection were 13.6% for proteins, 7.7% for oil and 74.1% for starch, and they were significantly higher than for the whole collection ($p<0.001$). Small negative, but highly significant correlations were found for protein content and latitude (-0.119; $p<0.01$), as well as for starch content and altitude (-0.090; $p<0.01$) of the collection sites for Western Balkan landraces. Positive small significant correlations were obtained for oil and altitude (0.069; $p<0.05$) and starch and latitude of (0.077; $p<0.05$) collection site, while medium highly significant positive correlation was found for protein content and altitude of collection site (0.237; $p<0.01$). Eighteen populations with increased protein and/or oil content and with good universal combining ability (with Lancaster, BSSS, and Iodent testers) were chosen to form the mini-core collection for grain quality, and they are planned for further detailed biochemical, phenotypic and genetic characterisation.

Keywords: grain quality, macronutrient, maize

Introduction

The main goals of plant genetic resource management are to acquire, maintain, distribute, characterize, regenerate, preserve, evaluate, and utilize the genetic diversity of crops and their wild relatives. In order to utilize genetic resources in plant breeding a concept of prebreeding was introduced (Nass and Paterniani, 2000). It represents a complex of activities aimed to identification of desirable traits and/or genes from unselected materials. In maize (*Zea mays* L), these activities may involve forming of new genetic pools for selection, as well as identification of new heterotic patterns (Vančetović et al, 2015).

A necessity to maintain a huge number of accessions in gene banks influenced the acceptance of the concept of core collections, which are formed to maximize the efficiency of germplasm evaluation and utilization, as well as genetic diversity maintenance in a collection. Brown (1989a) defined a core collection as a sub-sample of the large germplasm collection that contains chosen accessions representing as much as possible of the genetic variability of the original collection with a minimum repeatability. According to him, a core should contain around 10% of accessions representing about 70% of the genetic variability of the whole collection. Also, a core collection should not exceed 3,000 accessions. These core collections, though, should not influence the management of the whole collection that should be normally further maintained and multiplied.

Frankel and Brown (1984) and Brown (1989a; b) described how to assemble a core subset using the accessions morphological and agronomic characteristics. In CIMMYT, the core collections of maize were formed. Data about Tuxpeño core collection were summed by Crossa et al (1994) and Taba et al (1994). Radović and Jelovac (1995) represented a core collection from former Yugoslav populations, Abadie et al (1999) and Coimbra et al (2009) from Brazilian, and Malosetti and Abadie (2001) from Uruguay maize populations. Also, Li et al (2004) presented a formation of core collection from maize germplasm preserved in the Chines National Genebank. On the other hand, a total of 2,899 maize populations were used in the project named European RESGEN CT96-088 for forming the European maize core collection of around 100 accessions (Gouesnard et al, 2005), aimed at further evaluation for important agronomic traits.

Formation of a core collection is a good way of using the whole collection for identification of the best sources for the improvement of agronomical important traits. The next step is incorporation of these materials into elite genotypes. Data used for formation of core collections may include morphological traits, molecular marker data, agronomic traits or the geographical origin.

Maize breeding has been extremely successful in improving grain yield, but the grain quality was somewhat neglected. Large genetic macronutrient variability exists in maize kernel. This variability is the consequence of genetic and environmental factors. Maize

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is an excellent source of energy for food and feed due to its high starch content and composition. The second most abundant macronutrient in the maize kernel is protein. Popcorn and sweet maize usually have higher protein content than dent and flint maize. The third abundant macronutrient in maize kernel is oil.

Breeding for increased grain quality can provide better products for the end users. For example, high oil corn is important for better quality of poultry feed (Lambert, 1994). Maize oil is also an important renewable resource for biodiesel production and for dietary consumption by humans and livestock. High protein maize is important for ruminant animals and human food, while high starch maize serves as the basis for production of a variety of industrial products, including bioethanol.

A lot of researches have shown great variability of maize genetic resources for grain quality (Dunlap et al, 1995; Flint-Garcia et al, 2009; Pinto et al, 2009; Ignjatović-Micić et al, 2014; Vančetović et al, 2014; Ignjatović-Micić et al, 2015). Most of this germplasm has already undergone selection for specific traits (better taste, flavour and texture for staple food, better traits for feed, etc) by different cultures all over the world. On the other hand, breeding high yielding maize hybrids using the narrow genetic base gives rise to a high uniformity in kernel type and nutritive value of such derived hybrids. It is very likely that new grain quality traits not present in commercial genotypes could be found within genetic resources.

The purpose of this research was to: a) analyse all populations maintained at Maize Research Institute Zemun Polje gene bank for grain macronutrient content and combining ability, and b) create a core collection that could be further used by maize breeders for simultaneous improvement of high yield and grain quality.

Materials and Methods

NIR analysis

A total of 3,443 populations from MRI Zemun Polje gene bank were screened for protein, oil and starch contents using Near Infrared Spectroscopy (NIR) transmittance analysis (Infratec 1241 Grain Analyser, FOSS, Sweden) with ANN calibrations. Populations comprised 2,149 landraces from Western Balkan (designated L) and 1,294 populations (landraces, synthetics and composites, designated IP) introduced from around 40 countries. The accessions belonging to a drought tolerant mini-core collection of MRIZP already screened for macronutrient content were excluded from the experiment (Ignjatović-Micić et al, 2014; Vančetović et al, 2014).

Regarding the fact that maize grain quality is influenced by xenia effect (Weingatner et al, 2004; Vančetović et al, 2009) NIR analyses were performed on the regenerated seed from gene bank storage. Namely, heterozygote accessions were multiplied by pair-crossing, and at least 80 successful ears were taken, dried, and approximately the same amount of seed from the middle of the ears was mixed and was stored in the cold chamber. This is particularly favourable for oil content analysis, since its maximum is in the kernels from the middle part of the ears (White and Weber, 2003).

All populations in this research were measured twice (in two replications) for macronutrients content. The selection intensity of 5% was chosen, i.e. 5% of the highest protein populations, 5% of the highest oil populations and 5% of the top starch populations were chosen for further research. Forty-five populations were in the top 5% for both protein and oil content and thus a total of 471 accessions were chosen for further analysis.

Combining ability

The 471 populations were crossed as mothers with elite inbred testers in spatial isolations in 2011. The testers were from three heterotic groups mostly used in Serbia: BSSS (named B tester), Iodent (named ID tester) and two Lancaster testers (L1 with better combining ability with ID germplasm, and L2 tester that better combines with B germplasm). A total of 120 kernels per population were sawn and thinned at the five to seven leaf stage, leaving 60 plants for the crossing. Only the top-crosses (TC) with 30 and more successful ears were used for combining ability tests (test-trials).

Next season (2012), TCs were tested at five locations in Serbia, alongside with two commercial hybrids used as checks (ZP341 and ZP505), in a randomized complete block design (RCBD) experiments with two replications. Each trial consisted of the two hybrid checks and 22 TCs. Mechanical planting and harvesting of the trials were done. Standard management for weed control and agronomical practices were used.

Data from the trials were statistically analysed by a two-way analysis of variance (ANOVA) for RCBD design. The selection criteria was the performance index over 85% or/and grain yield over 90% in comparison with the average grain yield of the two checks. Performance index was calculated by the formula: $PI = (GYTC \times CHMOIST)/(GYCH \times TCMOIST) * 100$; where *PI* is performance index; *GYTC* is grain yield of a particular TC; *CHMOIST* is the average grain moisture of the two checks in a trial; *GYCH* is the average grain yield of the two checks in a particular trial, and *TCMOIST* is the grain moisture of a particular TC.

In this way, a total of 352 populations were chosen as a source of increased macronutrient content and good combining ability. The average values for macronutrient contents of all 3,443 populations and core sub-sets for each macronutrient were calculated and compared by the t-test. For all landraces from Western Balkan that had data on latitude, longitude and altitude of the collection site, the Pearson's correlation coefficients between macronutrient contents

and these three parameters were performed.

In heterotic/macronutrient groups with four or more accessions, one-factorial analysis of variance (ANOVA) according to the RCBD was performed for macronutrient contents. All statistical analyses were done in MSTAT-C software. For each group number of accessions per group was given, minimum and maximum value of particular macronutrient, its range, average value, phenotypic coefficient of variation, standard deviation, mean square of genotypes from ANOVA, LSD value for accessions comprising the group at 0.05 level, Y/N sign for significant/not significant differences among accessions measured by LSD test, broad sense heritability, genotypic coefficient of variation and expected genetic gains for 5, 10 and 20% selection intensities (Sing and Chaudary, 1985) in the units of measurement (%), in percentage of the average value, as well as projected value of the macronutrient content after one cycle of selection with the three selection intensities.

Heritability in broad sense (H) was estimated as: $H=\sigma^2_g/\sigma^2_p x100$; where σ^2_g and σ^2_p are the genotypic and phenotypic variance components derived from ANOVA, respectively. Phenotypic and genotypic coefficients of variation were calculated as: $CV_p=(\sigma_p/\overline{X})$ x100 and $CV_g=(\sigma_g/\overline{X})$x100; where CV_p and CV_g are phenotypic and genotypic coefficient of variation, respectively, σ_p and σ_g phenotypic and genotypic standard deviation, respectively, and $\overline{X}$ is the overall mean.

Expected genetic gain is given as: $\Delta G=K \times \sigma_p \times H$, where K is the standardized selection differential (K = 2.06 for 5%, 1.75 for 10%, and 1.40 for 20% selection intensity, respectively), σ_p is phenotypic standard deviation and H is the broad sense heritability.

Results

Data for the whole grain quality core collection of MRIZP gene bank are given in Supplementary Table 1. These data include: accession type (L or IP), accession number in the main collection, drought tolerance (DT) data - if the particular accession was chosen in the first test for drought tolerance done in manage water stressed environment in Egypt in 2007 (Babić et al, 2015), combining ability (CA) estimate, testers with which crosses in 2011 failed for some accessions, macronutrient (chemical compound - CH) as the first selection criterion (before CA testing), values for protein, oil and starch content, as well as the rank of each accession for these three macronutrients within a total of 3,443 populations measured, FAO group, origin, population name and population type (landrace, synthetic or composite).

Average values for protein, oil and starch content for the whole collection were 11.5%, 4.2%, and 68.9%, respectively. On the other hand, averages for the core collection were 13.6% for proteins, 7.7% for oil and 74.1% for starch, and they were significantly higher than for the whole collSmall negative, but highly significant correlations were found for protein content and latitude (-0.119; $p < 0.01$), as well as for starch content and altitude (-0.090; $p < 0.01$) of the collection sites for Western Balkan landraces. Positive small significant correlations were obtained for oil and altitude (0.069; $p < 0.05$) and starch and lati-

Table 1 - High protein core collection including heterotic groups comprised of at least four accessions.

	Heterotic Group										Ø
	L1/L2	L2	L2/ID	L1/L2/ID	L1/L2/B	L1/L2/B/ID	L1/B	L2/B	L2/B/ID	B	
no.of accessions	11	18	5	4	13	5	5	19	4	8	9.2
min	12.16	13.16	13.44	13.25	13.15	13.26	13.17	13.21	13.19	13.24	13.22
max	14.67	14.43	13.91	14.58	15.37	14.04	14.26	14.79	12.46	14.95	14.45
Range	1.51	1.27	0.47	1.33	2.22	0.78	1.09	1.58	0.27	1.71	1.22
AV	13.32	13.46	13.70	13.64	13.64	13.49	13.53	13.66	13.33	13.73	13.55
CVp	2.47	2.86	3.01	1.07	1.94	2.47	6.51	3.41	3.34	2.86	2.99
SD	0.46	0.36	0.29	0.56	0.60	0.36	0.60	0.53	0.28	0.58	0.46
MS	0.387***	0.185*	0.083ns	0.811***	0.726***	0.224ns	0.419ns	0.454***	0.042ns	0.683***	0.401
$LSD_{0.05}$	0.577	0.633	0.895	0.363	0.452	0.719	1.903	0.763	1.098	0.725	0.813
Y/N	Y	Y	N	Y	Y	Y	N	Y	N	Y	
H	70.48	34.55	20.19	96.84	88.82	53.95	5.15	54.95	39.29	75.80	54.00
CVg	3.48	1.90	0.88	5.43	5.02	2.44	1.39	3.44	2.45	4.63	3.11
ΔG5	0.69	0.26	0.09	1.28	1.13	0.42	0.07	0.61	0.36	0.97	0.59
ΔG10	0.59	0.22	0.08	1.09	0.96	0.36	0.06	0.52	0.30	0.83	0.50
ΔG20	0.47	0.18	0.06	0.87	0.77	0.29	0.05	0.42	0.24	0.66	0.40
ΔG5(%)	6.01	2.30	0.81	11.00	9.74	3.69	0.61	5.23	3.18	8.27	5.08
ΔG10(%)	5.11	1.96	0.69	9.35	8.28	3.14	0.52	4.46	2.65	7.08	4.32
ΔG20(%)	4.09	1.56	0.55	7.48	6.62	2.51	0.43	3.60	2.12	5.63	3.46
AV5%	12.01	11.72	11.79	12.92	12.77	11.91	11.60	12.27	11.69	12.70	12.14
AV10%	11.91	11.68	11.78	12.73	12.60	11.85	11.59	12.18	11.63	12.56	12.05
AV20%	11.79	11.64	11.76	12.51	12.41	11.78	11.58	12.08	11.57	12.39	11.95

*,*** - statisticaly significant at 0.05 and 0.001 level, respectively; ns - statistically non-significant; AV - average value per heterotic group; CVp - phenotypic coefficient of variation; SD - standard deviation; MS - mean square of genotypes from one-way ANOVA; LSD - value for testing intra-group differences at 0.05 level; Y/N - there are/there are not significant LSD differences at 0.05 level between the accessions in the particular heterotic group; H - broad sense heritability; CVg - genotypic coefficient of variation; ΔG - expected genetic gain in the units of measurement (%); ΔG(%) - expected genetic gain in the percent of intragroup mean; AV% - expected average value of protein content after one cycle of intra-group selection for different selection intensities; Ø - overall mean of the parameters for all the heterotic groups.

tude of (0.077; $p < 0.05$) collection site, while medium highly significant positive correlation was found for protein content and altitude of collection site (0.237; $p < 0.01$).

Regarding high protein content, a total of 10 heterotic groups with at least four accessions were identified (Table 1). The largest heterotic group for high protein was L2/B (populations that had a good CA with these two testers) with 19 accessions, followed by L2 group with 18 accessions. Groups L1/L2/ID and L2/B/ID, on the other hand, comprised only four accessions. Average values of protein content among groups ranged from 13.32% (L1/L2 group) to 13.73% (B group). Broad sense heritability showed great variation, from only 5.15% (L1/B group) to 96.84% (L1/L2/ID group). Accordingly, the expected genetic gain, in all three categories, was highest for the latter heterotic group.

High oil core collection included eight heterotic groups (Table 2), with L2 being the largest with 25 accessions. Average values of oil content per group ranged from 7.38% (ID group) to 8.21% (L1/L2 group). Range of intra-group heritability was also extremely high, from 3.00% (B group) to 96.21% (L1/L2 group). L1/L2 group also had the highest expected genetic gain among all high oil heterotic groups.

High starch core collection comprised of 10 heterotic groups (Table 3), with L2 again being the largest (26 accessions). Average starch content per group ranged from 73.78% (B group) to 74.33% (L2 group). Heritability varied from 3.84% (L2 group) to 83.99% (L1/L2/B group), but the expected genetic gain was generally very low for all groups.

Finally, two heterotic groups with five (L2) and four (B) accessions were identified with both high protein and oil content. L2 group had higher protein and oil content compared to B group (14.39% versus 13.38% for protein and 8.56% versus 7.54% for oil content). Heritability for both macronutrients was also much higher for L2 group (Table 4), as well as the expected genetic gain from selection.

Discussion

Since the needs of farmers and industry have changed over time, it is necessary to start looking for new genetic resources that may have desirable characteristics for their fulfilment. Increasing productivity and quality, insect and disease resistance, tolerance to stress conditions and additional traits that add value to the grain (starch, protein, oil, etc.) are characteristics that should be improved in the future.

Traditional food products made from maize in Serbia were: maize bread, hoecake, mush, pastry and pies, corn whiskey, cooked corn, and pickled foods (Bekrić, 1997). All these products were made from landraces traditionally produced by small farmers, in which breeding for grain quality attributes was

Table 2 - High oil core collection including heterotic groups comprised of at least four accessions.

	Heterotic Group								Ø
	ID	L1	L1/L2	L2	L2/ID	L1/L2/B	L2/B	B	
no.of accessions	4	5	7	25	10	4	21	7	10.4
min	7.24	7.39	7.30	7.13	7.12	7.18	7.28	7.31	7.24
max	7.62	8.46	13.00	8.28	11.10	7.88	9.43	8.29	9.26
Range	0.38	1.07	5.70	1.15	3.98	0.70	2.15	0.98	2.01
AV	7.38	7.89	8.21	7.60	7.84	7.50	7.64	7.79	7.73
CVp	3.18	7.48	5.07	6.03	5.33	4.87	5.64	7.48	5.64
SD	0.21	0.59	1.98	0.45	1.19	0.34	0.58	0.56	0.74
MS	0.064ns	0.514ns	8.946***	0.206ns	2.904***	0.175ns	0.455**	0.318ns	1.70
LSD0.05	0.746	1.640	1.018	0.944	0.944	1.161	0.897	1.425	1.10
Y/N	N	N	Y	Y	Y	N	Y	N	
H	7.56	19.12	96.21	0.94	88.69	13.64	42.19	3.00	33.92
CVg	0.91	3.64	25.53	0.59	14.91	1.39	4.81	1.32	6.64
ΔG5	0.04	0.26	4.23	0.009	2.27	0.11	0.49	0.04	0.93
ΔG10	0.03	0.22	3.60	0.008	1.93	0.07	0.42	0.03	0.79
ΔG20	0.03	0.18	2.88	0.006	1.54	0.07	0.33	0.02	0.63
ΔG5(%)	0.51	3.28	51.58	0.12	28.92	1.47	6.44	0.47	11.60
ΔG10(%)	0.44	2.79	43.82	0.10	24.57	1.25	5.47	0.40	9.86
ΔG20(%)	0.35	2.23	35.05	0.08	19.65	1.00	4.38	0.32	7.88
AV5%	7.42	8.15	12.44	7.609	10.11	7.61	8.13	7.83	8.66
AV10%	7.41	8.11	11.81	7.608	9.77	7.59	8.06	7.82	8.52
AV20%	7.41	8.07	11.09	7.606	9.38	7.57	7.97	7.81	8.36

,* - statistically significant at 0.01 and 0.001 level, respectively; ns - statistically non-significant; AV - average value per heterotic group; CVp - phenotypic coefficient of variation; SD - standard deviation; MS - mean square of genotypes from one-way ANOVA; LSD - value for testing intra-group differences at 0.05 level; Y/N - there are/there are not significant LSD differences at 0.05 level between the accessions in the particular heterotic group; H - broad sense heritability; CVg - genotypic coefficient of variation; ΔG - expected genetic gain in the units of measurement (%); ΔG(%) - expected genetic gain in the percent of intragroup mean; AV% - expected average value of oil content after one cycle of intra-group selection for different selection intensities; Ø - overall mean of the parameters for all the heterotic groups.

Table 3 - High starch core collection including heterotic groups comprised of at least four accessions.

	Heterotic Group										Ø
	L1	L1/L2	L2	L2/ID	L1/L2/ID	L1/L2/B	L1/L2/B/ID	L2/B	L2/B/ID	B	
no.of accessions	5	15	26	16	12	18	14	18	7	4	13.5
min	73.33	73.29	73.21	73.24	73.22	73.24	73.21	73.24	73.21	73.32	73.25
max	74.65	75.45	77.87	77.02	77.31	78.83	77.05	77.11	74.75	74.19	76.42
Range	1.32	2.16	4.66	3.78	4.09	5.59	3.84	3.87	1.54	0.87	3.17
AV	73.90	74.25	74.33	74.14	74.08	74.10	73.96	74.05	73.89	73.78	74.05
CVp	0.87	1.00	2.16	2.35	0.71	0.72	1.03	1.05	0.63	0.60	1.11
SD	0.73	0.82	1.57	1.64	1.12	1.29	1.10	1.03	0.57	0.58	1.05
MS	0.523ns	0.870ns	2.479ns	2.692ns	2.469***	3.264***	1.955**	1.634**	0.517ns	0.404ns	1.681
LSD0.05	1.780	1.595	3.309	3.709	1.158	1.124	1.645	1.637	1.145	1.402	1.850
Y/N	N	Y	Y	Y	Y	Y	Y	Y	Y	N	
H	11.99	22.28	3.84	5.26	79.83	83.99	54.24	46.15	40.49	35.12	38.32
CVg	0.32	0.54	0.43	0.55	1.41	1.65	1.12	0.97	0.52	0.44	0.80
ΔG5	0.17	0.39	0.13	0.19	1.93	2.30	1.26	1.01	0.51	0.40	0.83
ΔG10	0.14	0.33	0.11	0.16	1.64	1.96	1.07	0.85	0.43	0.34	0.70
ΔG20	0.11	0.26	0.09	0.13	1.31	1.57	0.85	0.68	0.34	0.27	0.56
ΔG5(%)	0.23	0.52	0.17	0.26	2.60	3.11	1.70	1.36	0.68	0.54	1.12
ΔG10(%)	0.19	0.44	0.15	0.22	2.21	2.64	1.44	1.15	0.58	0.46	0.95
ΔG20(%)	0.16	0.35	0.12	0.18	1.77	2.11	1.16	0.92	0.47	0.36	0.76
AV5%	74.07	74.64	74.46	74.33	76.01	76.40	75.22	75.06	74.40	74.18	74.88
AV10%	74.04	74.58	74.44	74.30	75.72	76.06	75.03	74.90	74.32	74.12	74.75
AV20%	74.01	74.51	74.42	74.27	75.39	75.67	74.81	74.73	74.23	74.05	74.61

,* - statistically significant at 0.01 and 0.001 level, respectively; ns - statistically non-significant; AV - average value per heterotic group; CVp - phenotypic coefficient of variation; SD - standard deviation; MS - mean square of genotypes from one-way ANOVA; LSD - value for testing intra-group differences at 0.05 level; Y/N - there are/there are not significant LSD differences at 0.05 level between the accessions in the particular heterotic group; H - broad sense heritability; CVg - genotypic coefficient of variation; ΔG - expected genetic gain in the units of measurement (%); ΔG(%) - expected genetic gain in the percent of intragroup mean; AV% - expected average value of starch content after one cycle of intra-group selection for different selection intensities; Ø - overall mean of the parameters for all the heterotic groups.

permanently performed by the end users for particular maze products. These traditional landraces were replaced by hybrid maize in 20th century, and most maize products were replaced by products made from wheat. However, landraces from Western Balkan are still preserved at the Maize Research Institute Zemun Polje gene bank and they could serve as an excellent source for pre-breeding specialty maize types with increased grain quality attributes.

According to Eckhoff and Paulsen (1996) maize kernel contains on average 73% starch, 10% protein, and 5% oil, and the rest consists of fibre, vitamins and minerals. In MRI gene bank the average values were slightly different. Namely, overall average was 68.9% starch, 11.5% protein, and 4.2% oil content. However, a huge research of chemical compounds and physical properties of maize grain was done by Narvaez-Gonzales et al (2006). They analysed 71 accessions representing different maize races from Latin America (27,000 accessions from Mexico, Caribbean, Central and South America) for chemical compounds (moisture, total lipids, protein and amylose) and some physical kernel properties (1,000 kernel mass, physical strength and anatomical composition). In their research protein ranged from 6.8 to14.2% and total lipids from 3.8 to 8.4%.

In the search of Radosavljević (1995) chemical and functional starch properties of 10 landraces from Western Balkan and 12 ZP hybrids were examined. The genotypes varied in the endosperm type and colour. Starch content ranged from 63.58 to 70.54%, and on the average it was lower (66.76%) for the landraces than for the hybrids (70.37%).

Berardo et al (2009) used 1,245 maize accessions for NIR spectroscopy for the evaluation of crude protein, lipid and starch content in the kernel - 633 were traditional Italian populations, while 519 accessions were from different countries (Albania, Austria, Canada, Czech Republic, Chile, Cyprus, Spain, Ethiopia, France, Germany, Japan, Morocco, Mexico, Holland, Romania, Turkey, Hungary, USA, and Russia). Protein content in their research varied from 7.39 to 15.42%, lipid content from 2.27 to 7.74% and starch content from 61.18 to 70.07%. The sum of 11 populations was chosen on the basis of favourable chemical composition, with protein content varying from 12.52-15.16% and lipid content from 5.26-7.17%.

The differences among researches of the average macronutrients' contents could be the consequence of different genotypes studied, different environments under evaluation, as well as possible genotype × environment interaction. In different gene banks different genetic accessions are stored (with a possibility of overlapping) and their multiplication is done in different environmental conditions. Significant correlations between macronutrient contents and latitude, and altitude of collection sites for Western Balkan landraces obtained in our research clearly demonstrate the impact of environmental conditions on grain quality of maize populations. Before their collection, selection for grain quality was done almost every year for grain quality attributes. After collection only multiplication (one or several times, depending on the date of collection) was performed, without further selection, but often in drastically different environments (where gene banks are located). All this could have led to

Table 4 - High protein and oil core collection including heterotic groups comprised of at least four accessions.

	Protein			Oil		
Heterotic Group	L2	B	Ø	L2	B	Ø
no.of accessions	5	4	4.5	5	4	4.5
min	13.15	13.20	13.18	7.22	7.18	7.20
max	16.20	13.57	14.89	11.20	7.87	9.54
Range	3.05	0.37	1.71	3.98	0.69	2.34
AV	14.39	13.38	13.89	8.56	7.54	8.05
CV_p	3.09	3.73	3.41	7.44	6.78	7.11
SD	1.11	0.28	0.70	1.49	0.42	1.00
MS	2.871***	0.061ns	1.466	5.083***	0.187ns	2.635
LSD0.05	0.978	1.233	1.106	1.765	1.626	1.700
Y/N	Y	N		Y	N	
H	91.72	22.88	57.30	85.27	12.42	48.85
CV_g	9.46	2.03	5.75	17.88	2.55	10.22
ΔG5	2.31	0.21	1.26	2.91	0.14	1.53
ΔG10	1.96	0.18	1.07	2.47	0.12	1.30
ΔG20	1.57	0.14	0.86	1.98	0.09	1.04
ΔG5(%)	18.66	2.00	10.33	34.02	1.85	17.94
ΔG10(%)	15.86	1.70	8.78	28.90	1.57	15.24
ΔG20(%)	12.68	1.36	7.02	23.12	1.26	12.19
AV5%	14.70	11.59	13.15	11.47	7.68	9.58
AV10%	14.35	11.56	12.96	11.03	7.66	9.35
AV20%	13.96	11.52	12.74	10.54	7.63	9.09

*** - statistically significant at 0.001 level; ns - statistically non-significant; AV - average value per heterotic group; CVp - phenotypic coefficient of variation; SD - standard deviation; MS - mean square of genotypes from one-way ANOVA; LSD - value for testing intra-group differences at 0.05 level; Y/N - there are/there are not significant LSD differences at 0.05 level between the accessions in the particular heterotic group; H - broad sense heritability; CVg - genotypic coefficient of variation; ΔG - expected genetic gain in the units of measurement (%); ΔG(%) - expected genetic gain in the percent of intragroup mean; AV% - expected average value of protein/oil content after one cycle of intra-group selection for different selection intensities; Ø - overall mean of the parameters for all the heterotic groups.

significant differences in the average values of macronutrient contents between accessions from different gene banks (collections).

Camusi et al (1980) have already shown that the variability in maize grain chemical composition could be the consequence of adaptation to specific environments. They found large variability in fatty-acid composition in Italian accessions from different micro-climatic environmental conditions. Namely, accessions from the north of Italy had lower content of saturated fatty acids and higher percentage of linoleic acid than the accessions from the south. The authors concluded that this could also be the consequence of different maize introductions into these regions. Maize has been introduced to the Italian north directly from Spain at the beginning of XVI century, but much later to the south of the country. Based on morphological and cytological data, these two introductions were from completely different sources.

Campbell et al (2010) have used NITS (Near-infrared transmittance) for creating a maize core collection for grain quality from a total of 306 accessions from nine Chilean regions at lower altitudes, representing 17 maize races, as a model system. They used Infratec 1255 grain analyser and made an attempt to link a variation in grain quality with already known data for these accessions - race classification, region of origin and physical kernel characteristics. Core collection obtained in this research only partially fulfilled the goal of maximizing genetic variability for some kernel traits in comparison with random accessions. A higher number of accessions with extreme values was also assigned to this core collection. The authors concluded that the variability in chemical compounds of maize kernel could be the consequence of the adaptation to particular climatic regions. NITS data were partially in correlation with classification of the accessions into races and their region of origin, and in a much lesser extent with kernel colour and texture. These results pointed out that a core collection formed entirely on choosing the representative accessions from every race or region would not satisfactorily remove the repeatability for grain quality. However, some races and regions have shown higher divergence of the searched traits and they require investigation of a higher number of accessions for grain quality analysis.

Maize populations often have twice the lower yield than commercial hybrids and also lower combining ability in comparison with elite inbred lines crossed with the same testers (Gallais and Monod, 1998). Considering these facts, we have chosen a selection criterion of 85% PI and 90% grain yield in comparison with commercial check hybrids for the combining ability of our populations. Our PI favoured populations with low grain moisture at harvest, which is a desirable trait in commercial selection. On the other hand, CA values of our core collection must be taken with a certain precaution. Namely, 2012 (in which test-trials for CA were performed) was the dri-

est year apart from 2015 since meteorological data have been collected in Serbia (www.hidgovnet.rs). Inbred testers L1 and L2 are known for their exquisite drought tolerance, contrary to the testers ID and B. This fact could bias the results of test-trials toward better performance of test-crosses of the accessions with L1 and L2 testers.

Regarding the mentioned unfavourable attributes of maize populations (accessions), the most reliable way of their improvement and incorporation into commercial selection is by crossing with elite materials. Several methods for the introgression of desirable traits from gene bank accessions are proposed. They depend on the heritability of target traits, the mode of their inheritance, heterosis and genotype × environment interaction. The ideal proportion of unselected desirable material into commercial breeding program is yet undefined. According to Bridges and Gardner (1987) this depends on the purpose of selection (short versus long-term programs), as well as the values of the target traits of unselected and elite materials per se. Backcrossing with elite materials seems a good approach for the transfer of the major genes (traits controlled by one or two genes), but it can also be used for larger number of genes, and even for quantitative traits. Another way for incorporation of unselected materials into breeding programs is forming new populations and their successive incorporation in commercial breeding. In the case of our grain quality core collection, the first approach is valid for the accessions with clearly defined CA. On the other hand, 40 accessions showed universal CA (with Lancaster, BSSS and Iodent testers) and could not be properly classified into any already defined heterotic group. For them, the second approach should be more reliable.

For recurrent selection (RS) for increased protein content, composites made of accessions from heterotic groups L1/L2/ID, L1/L2/B, L2/B and B could be formed, since projected protein content value after the first cycle of selection at 20% selection intensity would be over 12% protein. This selection intensity is mostly recommended for unselected or low-selected materials with a number of undesirable traits and selection for one macronutrient at higher selection intensity (for instance 5%) would not diminish genetic variability for improvement of other agronomical important traits.

Regarding oil content, composites from heterotic groups L1, L1/L2 and L2/ID for intrapopulation RS could be formed. L1/L2 heterotic group is especially favourable, which would have 11.09% oil after one cycle of selection at 20% selection intensity (due to the highest oil accession in the research with 13.00% oil content).

Since expected genetic gain is low for starch, what is in accordance with previous research of Vančetović et al (2014) for drought tolerant mini-core MRIZP collection, there would not be any purpose in forming composites for the improvement of this trait. And finally, for simultaneous improvement of protein and oil content, a composite of populations from L2 group with both high protein and oil content could be formed.

The most successful examples of maize pre-breeding programs are Latin American Maize Project (LAMP) and Genetic Enhancement of Maize (GEM) projects (Pollak, 2003). LAMP was the first internationally coordinated project for the evaluation of maize (Salhuana et al, 1998). Twelve countries fulfilled evaluations of their national germplasm maize collections for grain yield, disease and insect resistance, and grain quality, comprising 12,000 accessions. Many LAMP and other accessions and selected materials arising from this project were analysed for oil quality, kernel composition, wet milling traits and starch quality (White et al, 1990; Hameed et al, 1994; Campbell et al, 1995; Dunlap et al, 1995; Ng et al, 1997; Pollak and White, 1997). The results of this project pointed out the enormous possibilities of improving adapted materials for these traits by incorporating exotic germplasm.

Pre-breeding of this material was further done within the GEM project. For the fastest incorporation of favourable LAMP accessions into commercial breeding crossing with adapted inbred lines was chosen. Considering grain quality traits, incorporation of this germplasm into elite inbred lines was expected to gain sources of favourable amino-acid profiles for food and feed, lower protein and higher starch content for wet milling purposes, and genotypes with higher test weight, protein content and harder endosperm for dry milling.

In GEM project one Argentinian accession (AR 16053) was found, whose backcrosses had 16% protein, 7% oil and 75% starch content, even before further selection. During GEM, NIR technology was applied for measuring macronutrients content, as a very valuable tool for non-destructive analysis of a large number of samples (200 per day) (Baye and Becker, 2004). Whole kernel of promising materials was analysed for protein, oil and starch content. Threshold values defined at the beginning of GEM were 13% protein, 6% oil and 75% starch content. In our research a different approach was involved: we used 5% selection intensity for each macronutrient, and finally arrived at a total of 352 accessions (out of initial 3,443 populations) comprising the core collection for increased macronutrients. This represents 10.2% of the whole gene bank collection and it is partially in accordance with Brown (1989a) definition of core collection which should have 10% of accessions. However, we cannot state that this 10% represent a 70% of total genetic variability of the whole collection. This is because our core collection is formed as a tool for practical breeding, with clearly defined selection criteria (increased macronutrients and good combining ability).

In the last few decades, advancement in biochemistry and genetics allowed manipulations with the components of the grain macronutrients, leading to the creation of grains better adapted to end users. Amino-acid balance, fatty acid composition and physical starch properties are very important traits for selection, influencing the value of maize kernel for feed, human health and industrial use. Developing plants with improved grain quality traits involves the ability to use existing genetic variation and to identify and manipulate commercially important genes. By exploiting genetic variation, the composition of the kernel was altered for both the quantity and quality (structure and chemical diversity) of starch, protein, and oil throughout kernel development.

Beside genetic knowledge and better identification of Quantitative Trait Loci (QTLs) for chemical composition of maize kernel should enable more efficient production of high oil, high protein and high starch maize hybrids. Contents of these macronutrients are quantitative in nature, what was confirmed by the researches of Laurie et al (2004) and Clark et al (2006). They found at least 40 to 50 QTLs for oil, protein and starch content in maize kernel. Currently more than 70% of maize production is used for food and feed; therefore, knowledge of genes involved in protein, starch, and lipids production is relevant for improving the nutritional and food-making properties of maize grains. Thus, the ability of geneticists to discover new genes and to manipulate genetic variation at the level of specific genes offers the potential to use genetic variation for the production of precisely designed specialty maize in the future (Motto et al, 2009).

Accessions from our mini-core collection with universal combining ability (good with one or both Lancaster, BSSS and ID testers) that are comprised of 22 high starch accessions, 10 high protein, five high oil and three both high protein and oil accessions. Eighteen accessions high in protein or/and oil were chosen for forming mini-core collection for grain quality. Starch accessions were excluded, because improvement of starch in our accessions would not be very effective. Also, Pearson's correlation coefficients among macronutrients for the whole collection were strong and highly significant ($p < 0.001$), and amounted 0.418 for protein and oil content, -0.674 for protein and starch content and -0.903 for oil and starch content. This implies that selection using these accessions for high oil would also lead to the high protein content and vice versa. But, since starch is the main component of grain endosperm, and has a great positive impact on grain yield, special care must be taken not to compromise grain yield at the expense of grain quality when using these accessions in selection program.

The mini-core collection for grain quality is planned for further detailed biochemical, phenotypic and genetic characterisation. Biochemical analysis will comprise classical chemical analysis of protein and oil content, estimation of amino-acid and fatty acid composition, as well as contents of micronutrients. Phenotypic characterization will be done in field conditions on several locations, in at least two years for the most important agronomic traits. And finally, genotypic characterisation will involve molecular markers for estimating genetic distance among these populations. In a previous search for drought tolerance in MRIZP gene bank, a total of seven populations with universal combining ability were found, and a diallel cross among six of them served as a guide for heterotic response among them (Vančetović et al, 2015). In the case of grain quality, 18 populations are a too big number to perform a diallel cross, thus more detailed molecular marker data will serve as the basis of dividing the accessions in potential genetic groups. In this way, we hope to form at least two or three composite populations, hopefully for reciprocal recurrent selection for increased grain quality.

Considering that a significant number of metabolic disorders and diseases are caused by malnutrition, and the fact that the majority of the world population consumes maize as the main bread grain, one of the future important breeding objectives in the Maize Research Institute will be development of maize genotypes with the added value traits. The results of the presented research could help in achieving this goal.

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References

Abadie T, Magalhaes JR, Parentoni SN, Cordeiro C, Andrade RVD, 1999. The core collection of maize germplasm of Brasil. PGR Newsletter 117: 55-56

Babić V, Vančetović J, Prodanović S, Kravić N, Babić M, Andelković V, 2015. Numerical Classification of Western Balkan Maize Drought Tolerant Landraces. Journal of Agricultural Science and Technology 17: 455-468

Baye T, Becker HC, 2004. Analyzing seed weight, fatty acid composition, oil and protein contents in Veronia galamensis germplasm by near-infrared reflectance spectroscopy. Jour American Oil Chem Soc 81: 641-645

Bekrić V, 1997. Upotreba kukuruza. Str. 38-42. Monografija. Izd. Birografika – Subotica, Srbija

Berardo N, Mazzinelli G, Valoti P, Lagana P, Redaelli R, 2009. Characterization of maize germplasm for the chemical composition of the grain. Jour Agric Food Chem 57: 2378-2384

Bridges Jr WC, Gardner CO, 1987. Foundation populations for adapted by exotic crosses. Crop Sci 27: 501-506

Brown AHD, 1989a. The case for core collections, pp 136-156. In: The use of plant genetic resources. Brown AHD, Frankel OH, Marshal DR, Williams JR eds. Cambridge University Press, Cambridge, UK

Brown AHD, 1989b. Core collections: a practical approach to genetic resources management. Genome 31: 818-824

Campbell MR, White PJ, Pollak LM, 1995. Properties of *sugary-2* maize starch: Influence of exotic background. Cereal Chem 72: 389-392

Campbell MR, Anih E, Conatser C, Grau-Saavedra B, Pollak LM, 2010. Development of a core cubset of Chilean lowland subtropical and temperate maize (*Zea mays* L) populations using near infrared transmittance spectroscopy. PGR Newsletter 148: 1-9

Camussi A, Jellum MD, Ottaviano E, 1980. Numerical taxonomy of Italian maize populations: fatty acid composition and morphological traits. Maydica 25: 149-165

Clark D, Dudley JW, Rocheford TR, LeDeaux JR, 2006. Genetic analysis of corn kernel composition in the random mated 10 generation of the cross of generations 70 of IHO x ILO. Crop Sci 46: 807-819

Coimbra RR, Miranda GV, Cruz CD, Silva DJH, Vilela RA, 2009. Development of a Brazilian maize core collection. Genetics and Molecular Biology 32: 538-545

Crossa J, Taba S, Eberhart SA, Bretting P, Vencovsky R, 1994. Practical considerations for maintaining germplasm in maize. Theor Appl Genet 89: 89-95

Dunlap F, White PJ, Pollak LM, 1995. Evaluation of Latina American Maize Project accessions for fatty acid content. J Amer Oil Chem Soc 72: 989-993

Eckhoff SR, Paulsen MR, 1996. Maize, pp. 77-112. In: Cereal Grain Quality. Henry RJ, Kettlewell PS eds. Chapman and Hall, London, UK

Flint-Garcia SA, Bodnar AL, Scott MP, 2009. Wide variability in kernel composition, seed characteristics, and zein profiles among diverse maize inbreds, landraces, and teosinte. Theor Appl Genet 119: 1129-1142

Frankel OH, Brown AHD, 1984. Plant genetic resources today: a critical appraisal, pp. 149-257. In: Crop genetic resources: conservation and evaluation. Holden JHW, Williams JT eds. Allen and Unwin, London, UK

Gallais A, Monod JP, 1998. La gestion des resources genetiques du mais en France: de leur caracterisation jusqu aux premiers stades de leur valorisation. Comptes Rendus de l Academic d Agriculture de France. 84: 173-181

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

Hameed A, Pollak LM, Hinz PN, 1994. Evaluation of Cateto maize accessions for grain yield and physical grain quality traits. Crop Sci 34: 265-269

Ignjatovic-Micic D, Kostadinovic M, Bozinovic S, Andjelkovic V, Vancetovic J, 2014. High grain quality accessions within a maize drought tolerant core collection. Sci Agr 71: 402-409

Ignjatovic-Micic D, Vancetovic J, Trbovic D, Dumanovic Z, Kostadinovic M, Bozinovic S, 2015. Grain nutrient composition of maize (*Zea mays* L.) drought-tolerant populations. J Agric Food Chem 63: 1251-1260

Laurie CC, Chasalow SD, LeDeaux JR, McCarroll R, Bush D, Hauge B, Lai CQ, Clark D, Rocheford TR, Dudley JW, 2004. The genetic architecture of response to long-term artificial selection for oil concentration in the maize kernel. Genetics 168: 2141–2155

Lambert RJ, 1994. High-oil corn hybrids, pp. 123-145. In: Specialty corns. Hallauer AR eds. CRC Press, London

Li Y, Shi Y, Cao Y, Wang T, 2004. Establishment of a core collection for maize germplasm preserved in Chinese National Genebank using geographic distribution and characterization data. Genetic Resources and Crop Evolution 51: 845-852

Malosetti M, Abadie T, 2001. Sampling strategy to develop a core collection of Uruguayan maize landraces based on morphological traits. Gen Res Crop Evol 48: 381-390

Motto M, Balconi C, Hartings H, Lauria M, Rossi V, 2009. Improvement of quality-related traits in maize grain: gene identification and exploitation. Maydica 54: 321-342

Nass LL, Paterniani E, 2000. Pre-breeding: a link between genetic resources and maize breeding. Sci Agr 57: 581-587

Narvaez-Gonzalez ED, Fugueroa-Cardenas J de D, Taba S, Tostado EC, Peniche RAM, Sanchez FR, 2006. Relationships between the microstructure, physical features, and chemical composition of different maize accessions from Latin America. Cereal Chem 83: 595-604

Ng K-Y, Pollak LM, Duvick SA, White PJ, 1997. Thermal properties of sixty-two exotic maize (*Zea mays* L.) lines grown in two locations. Cereal Chem 74: 837-841

Pinto ATB, Pereira J, de Oliveira TR, Prestes RA, Mattielo RR, Demiate IM, 2009. Characterization of corn landraces planted in the campos gerais region (Paraná, Brazil) for industrial utilization. Brazilian Archives of Biology and Technology 52 (Special issue): 17-28

Pollak LM, 2003. The history and success of the public-private project on Germplasm Enhancement of Maize (GEM). Adv Agron 78: 45-87

Pollak LM, White PJ, 1997. Thermal starch properties in corn belt and exotic corn inbred lines and their crosses. J Cereal Chem 74: 412-416

Radosavljević M, Bekrić V, Božović I, Radović G, 1995. Hemijske i funkcionalne osobine skroba

zrna kukuruza. Oplemenjivanje, proizvodnja i iskorišćavanje kukuruza – 50 godina Instituta za kukuruz «Zemun Polje». Str. 425-433. Simpozijum sa međunar. učešćem. 28-29 sept., Beograd – Zemun

Radovic G, Jelovac D, 1995. Identification of the heterotic pattern in Yugoslav maize germplasm. Maydica 40: 223-227

Salhuana W, Pollak LM, Ferrer M, Paratori O, Vivo G, 1998. Agronomic evaluation of maize accessions from Argentina, Chile, the United States, and Uruguay. Crop Sci 38: 866-872

Sing RK, Chaudary BD, 1985. Biometrical methods in quantitative genetic analysis. Kalyani Publ, New Delhi, India

Taba S, Pindea FE, Crossa J, 1994. Forming core subsets from the Tuxpeno race complex, pp. 60-81. In: The CIMMYT maize germplasm bank: genetic resource preservation, regeneration, maintenance, and use. Taba S eds. Mexico (Maize Program Special Report)

Vančetović J, Jankuloski Lj, Božinović S, Dodig D, 2009. The effects of cytoplasmic male sterility and xenia on the chemical composition of maize grain. Genetika 41: 95-106

Vančetović J, Ignjatović-Micić D, Božinović S, Babić M, Filipović M, Grcić N, Andjelković V, 2014. Grain quality of drought tolerant accessions within the MRI Zemun Polje maize germplasm collection. Spanish Jour Agric Res 12: 186-194

Vančetović J, Božinović S, Ignjatović-Micić D, Delić N, Kravić N, Nikolić A, 2015. A diallel cross among drought tolerant maize populations. Euphytica 205: 1-16

Weingatrtner U, Camp KH, Stamp P, 2004. Impact of male sterility and xenia on grain quality traits of maize. Europ J Agronomy 21: 239-247

White PJ, Abbas I, Pollak L, Johnson L, 1990. Intra- and inter-population variability of thermal properties of maize starch. Cereal Chem 67: 70-73

White PJ, Weber EJ, 2003. Lipids of the kernel, pp. 355-405. In: Corn chemistry and technology. 2nd eds. American Association of Cereal Chemists Inc, St Paul