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Original scientific paper

ASSOCIATION ANALYSIS OF AGRONOMIC TRAITS WITH MICROSATELLITES IN MAIZE INBRED LINES

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Association analysis or linkage disequilibrium mapping is a method for identification of quantitative trait loci (QTLs) in a panel of divergent unrelated individuals based on historical recombinations during a crop's domestication and selection. It should account for the population structure, which can be the result of adaptation to local conditions or selection, to reduce the possibility of declaring false-positive associations. The aim of this study was to determine potentially significant and consistent associations between markers and agronomic important maize (*Zea mays* L.) traits using association analysis in a diverse breeding material that can be ultimately implemented in maize selection. To this end, 96 maize inbred lines were evaluated in field trials at three locations in Serbia for eleven agronomic traits and analysed with microsatellite markers. Twenty five microsatellites were used to assess the population structure using Bayesian model-based clustering method and to test the significance of associations between the markers and the traits with general (GLM) and mixed linear (MLM) models. The cluster analysis divided maize inbred lines in four subpopulations, corresponding to the BSSS (Iowa Stiff Stalk Synthetic), LSC (Lancaster Sure Crop), Iodent heterotic groups and exotic and independent germplasm. The models identified associations between twenty five microsatellite markers and eleven agronomic traits, resulting in 133 and 71 associations across the environments for GLM and MLM, respectively. Some of the identified marker-trait associations were significant and consistent in several environments. The associations stable in several environments were identified between the markers *bnlg1067* and two flowering traits; *nc005* and *bnlg434* and plant height, *bnlg434* and

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ear height; *bnlg1643* and *umc1127* and leaf number, *bnlg1360* and ear diameter; *umc1019* and *umc1506* and number of rows per ear; *bnlg2305* and *bnlg1451* and ear length, and between *bnlg1175* and thousand-kernel weight. The results of this study indicate that these microsatellites could be used in marker-assisted selection of inbred lines, after validation of the marker-trait associations and testing combining abilities of the inbreds during hybrid development.

Key words: association mapping, microsatellites, yield components, *Zea mays* L.

INTRODUCTION

Marker-assisted selection (MAS) implies application of molecular markers in plant breeding in order to increase the selection efficiency of the traits of agronomic and economic interest. It is routinely used while transferring genes from one or more genotypes to another, choosing parents for hybrid combinations in the early stages of selection, determining correlations between traits and markers, and identifying genes and QTLs that control agronomic traits (BOUCHEZ *et al.*, 2002).

Most of the agronomic traits relevant for maize breeders are complex quantitative traits affected by a number of genes and influenced by environmental factors. Moreover, traits such as grain yield are expensive for evaluation as they require multi-location yield trials during several years. Therefore, the contribution of molecular markers to increasing selection efficiency is even more significant, not only for complex traits analysis, but also for facilitating and complementing the conventional maize breeding. A prerequisite for application of molecular markers in maize breeding is to identify the associations between agronomic traits and molecular markers in the vicinity of the genes or QTLs that govern those traits, as well as to provide automated routine analysis of a large number of genotypes (COLLARD *et al.*, 2005).

One of the molecular approaches for identifying genes or QTLs that determine the complex agronomic traits is association analysis. It establishes correlations between phenotypic and genotypic data of a large number of divergent unrelated individuals, on the basis of linkage disequilibrium (YAN *et al.*, 2011). Association analysis is based on a large number of recombinations during maize evolution, domestication and selection, which resulted in breaking the links between genes and markers that are not in physical proximity. Thus, only the markers that are closely linked to QTLs within linkage disequilibrium show significant associations (ZONDERVAN and CARDON, 2004).

Another important aspect in association analysis is to assess the population structure in order to avoid declaring false-positive associations between markers and traits. The population structure is the stratification within a population reflected in differences in allele frequencies due to different ancestry, limited gene flow, genetic drift, and adaptation to different environmental conditions and intensive selection process (CAMUS-KULANDAIVELU *et al*., 2006). The presence of population structure in a population could cause linkage disequilibrium between loci which are not linked, leading to identification of false-positive associations. This issue can be addressed by accounting for population structure in a studied population and integrating it into appropriate statistical models. PRITCHARD *et al.* (2000) developed a method to assess the population structure and kinship between individuals from a molecular data and embed it in a software STRUCTURE reducing the occurrence of false-positive associations of up to 80%.

Due to the complex nature of agronomic important traits, only associations that are stable across environments and different genetic backgrounds may indicate the suitability of a marker for maize breeding. The aim of this study was: 1) to validate population structure of maize inbred lines from the Institute of Field and Vegetable Crops (IFVC) breeding programmes, by comparing it with the population structure previously estimated with a different set of molecular markers and 2) to determine potentially significant and consistent associations between microsatellite markers and agronomic important maize traits using association analysis in a diverse breeding material and tested in several environments that can be further implemented in maize MAS.

MATERIALS AND METHODS

A set of 96 maize inbred lines from the IFVC in Novi Sad, Serbia was chosen for the analyses. The inbred lines belong to Iowa Stiff Stalk Synthetic (BSSS), Lancaster Sure Crop (LSC), Iodent (IDT) and independent (IND) heterotic groups and reflect diversity of the working material in maize breeding programmes at IFVC (MIKIĆ *et al.*, 2016).

The inbred lines were evaluated in field trials at three locations in Serbia (Rimski šančevi, Sombor and Srbobran) in 2011 and 2012. The trials were set in a completely randomised block design with three replications and two-row experimental plots 4 m long, with 0.75 m between rows and 0.22 m within rows and a plant density of 60,600 plants ha^{-1}. The following agronomic traits were evaluated: number of days to anthesis, number of days to silking, plant height (cm), ear height (cm), total number of leaves, number of leaves above the ear, ear length (cm), ear diameter (cm), number of rows, number of kernels per ear row and thousand-kernel weight (g). Flowering traits, namely number of days to anthesis and days to silking, were evaluated in the two locations of Rimski šančevi and Srbobran, while the other traits were evaluated in all three locations (MIKIĆ *et al.*, 2016).

For molecular analysis, DNA was extracted from young seedlings according to the CTAB protocol (DOYLE and DOYLE, 1990). Twenty five fluorescently labelled microsatellite markers were used for polymerase chain reaction (PCR) and their primer sequences were obtained from the Maize Genetics and Genomics Database (http://www.maizegdb.org). Markers that were reported as polymorphic in previous studies (WILMOT *et al.*, 2006; CHAKRABORTI *et al.*, 2011; TERA *et al.*, 2011; KRISHNA *et al.*, 2012; MLADENOVIĆ-DRINIĆ *et al.*, 2012) were chosen for the analysis (Table 1), taking at least two markers from each maize chromosome and providing similar number of markers per chromosome (2 - 4). The PCR mix contained 25 ng of genomic DNA, 0.2 mM dNTP, 1 × Taq buffer with KCl, 2 mM $MgCl_2$, 1 U Taq polymerase and 0.5 pmol of each primer. The PCR programme was performed at 94 °C for 5 min, following by 38 cycles at 94 °C for 30 s, 53 °C - 60 °C for 45 s, 72 °C for 45 s and at 72 °C for 7 min. PCR reactions with primers *bnlg1067*, *bnlg1360*, *bnlg1451* and *nc005* were performed under the same conditions as the rest, but with the Hot-Start Taq polymerase to avoid non-specific amplifications. For the fragment analysis, 10 μL reaction volumes were prepared with 2 μL mixture of differently labelled PCR products, 0.2 μL GeneScan500 LIZ size-standard and 7.8 μL Hi-Di formamide. The PCR products were separated by capillary electrophoresis on ABI Prism 3130 and visualised with Gene Mapper Software (MIKIĆ *et al.*, 2016).

The basic molecular diversity parameters were calculated using Excel Add-in programme Microsatellite Toolkit. The population structure of maize inbreds was estimated with microsatellite markers using Bayesian model-based clustering method in STRUCTURE software (PRITCHARD *et al.,* 2010). The burn-in period and run length of Markov Chain Monte Carlo algorithm was 100.000 × 100.000. The admixture model for the ancestry of individuals was chosen assuming the possible mixed ancestry of the inbred lines. The program STRUCTURE HARVESTER was used to detect and visualise the number of groups (EARL and VONHOLDT, 2012). The programme performs the Evanno method for detecting the number of K groups that best fit the data (EVANNO *et al.,* 2005).

The significance of associations between the markers and the traits was tested with general linear model (GLM) and mixed linear model (MLM) in TASSEL 2.1 (BRADBURY *et al.,* 2007). Estimation of population structure based on the average value of five iterations of log probability of data from the STRUCTURE were incorporated in GLM, while the matrix for population structure and the kinship matrix for family relatedness correction were implemented in MLM analysis. The marker-trait associations were tested using F-test with multiple degrees of freedom and the percentage of phenotypic variation explained by a marker was determined by coefficient of determination (R^2).

RESULTS AND DISCUSSION

Among the 96 maize inbred lines, 144 polymorphic alleles were detected with 25 microsatellite markers (Table 1). The number of alleles ranged from 3 to 9, with the average number of alleles per locus 5.8. Only 5 alleles were rare, having frequencies less than 5%. The average polymorphism information content (PIC) and gene diversity values were higher, while the average number of alleles was lower than for the same set of inbred lines obtained with different 36 microsatellites (MIKIĆ *et al.,* 2016). This could be due to the presence of more alleles with unequal frequencies and rare alleles identified in the previous study, which contributed to the lower values of genetic diversity parameters estimated with more markers.

According to the output of the STRUCTURE admixture model, the mean likelihood values across K indicated clustering of maize inbred lines in two groups. This clustering corresponds to the general division of maize inbred lines into BSSS and non-BSSS heterotic groups (RASMUSSEN and HALLAUER, 2006). The previous findings indicated no unique approach to determine the number of clusters and recommended that the choice of the optimal number of clusters had its biological justification (PRITCHARD *et al.,* 2007; COULON *et al.,* 2008). It is observed that very often STRUCTURE analysis revealed the presence of two subpopulations (VIGOUROUX *et al.,* 2008; YANG *et al.,* 2010; SEMAGN *et al*., 2012). EVANNO *et al.* (2005) explained this partitioning with a tendency of STRUCTURE to detect the uppermost hierarchical level of structure. Since the STRUCTURE captured the highest level of hierarchy, subsequent independent re-runs of the programme were performed for each cluster to test sub-structuring within clusters and reveal the best assignment of individuals to groups. Both BSSS and non-BSSS groups were further divided in two clusters, resulting in four groups in total (Fig. 1). The clustering corresponded to the BSSS (Iowa Stiff Stalk Synthetic), LSC (Lancaster Sure Crop), Iodent heterotic groups and exotic and independent germplasm. Similarly, SUTEU *et al*. (2014) divided 90 Romanian inbred lines into two clusters, after analysis of the population structure

with 90 microsatellites. After re-run of STRUCTURE, they split two clusters into seven final sub-clusters of inbred lines, which grouped around inbred lines of European flints, LSC and BSSS inbreds.

Table 1. Genetic diversity parameters of maize inbred lines analysed with microsatellites

Locus	Bin	Na	Gene diversity	PIC	Allele size (bp)
bnlg1067	8.03	5	0.738	0.69	116-130
bnlg1175	2.04	8	0.856	0.83	110-154
bnlg1360	10.07	7	0.847	0.82	109-139
bnlg1451	10.02	7	0.824	0.80	163-190
bnlg1643	1.08	4	0.729	0.67	143-155
bnlg1655	10.03	3	0.614	0.53	120-132
bnlg2082	8.03	4	0.618	0.56	220-240
bnlg2248	2.03	5	0.766	0.72	210-260
bnlg2305	5.07	7	0.743	0.71	170-218
bnlg434	7.03	3	0.582	0.49	142-158
dupssr12	1.08	7	0.823	0.80	102-156
nc005	4.05	9	0.860	0.84	121-163
phi022	9.03	9	0.873	0.85	341-367
phi029	3.04	6	0.787	0.75	139-161
phi095	1.03	3	0.558	0.46	183-198
umc1019	5.06	7	0.801	0.77	69-105
umc1101	4.09	6	0.769	0.73	130-154
umc1127	6.07	7	0.775	0.74	151-184
umc1266	3.06	4	0.614	0.54	104-122
umc2093	9.01	5	0.729	0.68	94-102
umc1865	7.03	5	0.748	0.70	117-145
umc1136	3.10	6	0.673	0.64	109-132
umc1506	10.05	7	0.830	0.80	156-192
umc1137	9.08	3	0.633	0.56	139-149
umc1653	6.07	7	0.783	0.75	100-130
Average		5.8	0.743	0.70	-

Na - number of alleles; PIC - polymorphic information content

The LSC group contained 33 inbred lines, the BSSS group encompassed 35 lines, Iodent 7 lines, the group with exotic and independent material consisted of 10 inbreds, while 11 lines had mixed origin. Few BSSS and LSC lines did not group within the BSSS and LSC clusters, respectively. This may be due to the effects of the genetic backgrounds of the other parental genotypes to the BSSS and LSC lines that contributed to and reshaped their genetic make-up. LIU *et al.* (2003) explained the differences in genetic constitution of lines with the same or similar origin by selection and genetic drift in inbreeding during line development, resulting in the

discrepancy between the pedigrees and genetic distances of those lines. The availability of complete data on the pedigrees of the inbred lines used in this study would enable better understanding of their relationships. Nevertheless, the division into four groups was in agreement with the pedigrees of the inbred lines and the main heterotic groups.

The same set of inbreed lines was analysed with different 36 microsatellite markers and clustered in three heterotic groups: BSSS, LSC and Iodent (MIKIĆ *et al.*, 2016). The exotic and independent lines were not assigned into a separate group, but clustered together with Iodent inbred lines. Presumably, the higher values of genetic diversity parameters of the markers used in this study (Table 1) imply higher level of their informativeness and discriminatory power to efficiently distinguish groups of inbred lines.

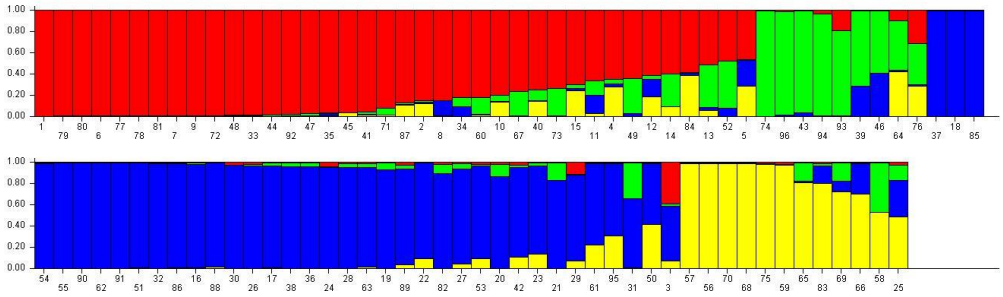

Figure 1. Population structure of maize inbred lines. Each inbred is presented as a bar showing its coefficient membership value Q: LSC (red), Iodent (green), BSSS (blue), European flint, independent and exotic inbred lines (yellow).

The associations between the microsatellite loci and agronomic traits were tested using GLM, taking into account the existence of population structure, and MLM, accounting for both population structure and kinship. Both models identified associations between nineteen microsatellite markers and eleven agronomic traits, resulting in 133 and 71 associations across the environments for GLM and MLM, respectively. Six markers were associated with two flowering traits: number of days from sowing to anthesis and number of days from sowing to silk emergence. Three of them were common for both flowering traits (Table 2). The most consistent associations, detected in all environments with both models, were identified between days to flowering and markers *bnlg1067*, *umc1137* and *bnlg1655*. The percentage of phenotypic variation explained by markers ranged from 12.4% to 20.7% for days to anthesis, and from 14.3% to 25.2% for days to silking. The marker *bnlg1067* was associated with both traits in almost all environments applying GLM and MLM.

The associations between *bnlg1067* and days to anthesis and days to silking are in keeping with the results of ENOKI *et al.* (2006), who found a QTL near *bnlg1067* that affected shortening of flowering time, explaining 18.9% and 36% of phenotypic variations of days to anthesis and days to silk emergence, respectively. This marker is in relative proximity of *early phase change* gene, which in its recessive state causes early flowering (VEGA *et al.*, 2002). Our findings support previously determined associations between the marker *umc1137* and flowering traits (FRASCAROLI *et al.*, 2007; MARINO *et al.*, 2009), and the marker *bnlg1655* and days to

silking (COQUE and GALLAIS, 2006; MU *et al.*, 2009). REMINGTON *et al.* (2001) found that selection for maize adaptability traits, such as flowering time, was reflected in the population structure. The significant associations between microsatellites and flowering time, in their study, depicted differentiation of maize subpopulations and formation of local germplasm within the maize gene pool as a result of adaptation to specific environmental conditions.

Table 2. Significant associations between microsatellites and flowering traits in maize

				GLM			MLM	
Marker	Chr.	Loc.	Year	F-M	p-M	R^2	F-M	p-M
				Days to anthesis				
bnlg1067	8	RS	2011	3.5083	0.004**	18.8	2.5789	0.024*
	8	RS	2012	3.9397	0.002**	19.3	2.5161	0.027*
	8	SR	2011	2.7526	0.018*	15.9	1.9411	0.083
	8	SR	2012	4.1216	0.001**	19.8	2.7405	0.018*
umc1137	9	RS	2011	2.7341	0.018*	15.4	2.0547	0.067
	9	RS	2012	3.6742	0.003**	18.3	1.5443	0.173
	9	SR	2011	2.3018	0.043*	13.6	1.8149	0.106
	9	SR	2012	3.1709	0.008**	16.2	3.4236	0.008**
bnlg1655	10	RS	2011	2.4165	0.027*	16.0	2.3821	0.029*
	10	RS	2012	3.6517	0.002**	20.7	2.1853	0.044*
	10	SR	2012	2.2614	0.039*	14.1	1.5959	0.148
umc1506	10	RS	2012	2.5209	0.027*	12.5	1.9246	0.076
	10	SR	2011	2.3265	0.039*	12.5	1.1689	0.331
	10	SR	2012	2.5836	0.024*	12.4	1.3532	0.244
				Days to silking				
bnlg1067	8	RS	2011	4.6144	0.001**	25.2	2.6742	0.020*
	8	RS	2012	3.9546	0.002**	20.8	2.4417	0.032*
	8	SR	2011	3.8620	0.002**	21.0	2.4592	0.031*
	8	SR	2012	3.8952	0.002**	22.0	1.9559	0.081
umc1137	9	RS	2011	3.2069	0.007**	19.9	1.9014	0.089
	9	RS	2012	3.4117	0.005**	18.5	1.7397	0.121
	9	SR	2011	3.0529	0.010**	17.5	1.6747	0.137
	9	SR	2012	2.5618	0.026*	15.6	3.0303	0.023*
bnlg1360	10	RS	2011	2.2538	0.047*	14.3	1.4899	0.191
	10	RS	2012	3.1301	0.008**	17.3	1.5310	0.178
	10	SR	2011	2.8611	0.014**	16.6	2.7959	0.045*
bnlg1655	10	RS	2012	2.7821	0.012*	18.0	2.2369	0.040*
	10	SR	2012	2.2173	0.042*	16.1	2.2003	0.041*
phi022	9	RS	2012	2.0664	0.049*	15.9	1.7012	0.110
	9	SR	2011	2.2758	0.031*	17.8	2.1032	0.044*

GLM - general linear model; MLM - mixed linear model; Chr. - chromosome; Loc. - location; F-M - F value from the F test on marker; p-M - P value from the F test on marker; R^2: percentage of phenotypic variation explained by the marker; RS - Rimski šančevi; SR - Srbobran; * - significant at 0.05 probability level; ** - significant at 0.01 probability level

Table 3. Significant associations between microsatellites and plant and ear height in maize

				GLM			MLM	
Marker	Chr.	Loc.	Year	F-M	p-M	R^2	F-M	p-M
				Plant height				
nc005	4	RS	2011	4.3860	0.003**	14.6	3.9322	0.005**
	4	RS	2012	3.8024	0.007**	13.4	3.8023	0.007**
	4	SR	2011	3.3093	0.014*	11.4	2.8683	0.028*
	4	SR	2012	2.8364	0.030*	11.1	2.5187	0.047*
	4	SO	2011	2.5154	0.048*	10.2	2.2049	0.075
bnlg434	7	RS	2011	2.8750	0.013*	14.7	2.2267	0.048*
	7	SR	2011	2.2482	0.047*	10.4	1.6272	0.149
	7	SR	2012	2.4504	0.050*	12.6	2.3167	0.050*
umc1865	7	RS	2011	2.4581	0.029*	13.4	2.3057	0.041*
	7	SR	2012	2.4008	0.029*	13.4	2.2807	0.045*
				Ear height				
bnlg434	7	RS	2011	2.9363	0.012*	16.7	2.6223	0.041*
	7	RS	2012	3.1371	0.008**	18.4	2.5116	0.050*
	7	SR	2011	2.3977	0.035*	14.7	2.4922	0.049*
umc1865	7	RS	2011	2.6022	0.024*	15.3	1.6523	0.122
	7	RS	2012	2.8115	0.016*	16.8	1.9221	0.087
	7	SR	2011	2.4927	0.029*	15.2	1.9137	0.088
	7	SR	2012	3.0133	0.011*	19.2	2.5047	0.028*
phi022	9	RS	2011	2.1737	0.039*	17.1	1.6523	0.122
	9	SR	2011	2.0597	0.049*	15.1	1.7791	0.093
	9	SR	2012	2.2496	0.034*	19.5	1.9124	0.069
	9	SO	2011	3.1351	0.004*	23.0	2.9106	0.007*
bnlg1451	10	RS	2011	2.6487	0.022*	15.5	1.4814	0.194
	10	RS	2012	2.3601	0.038*	14.6	0.9951	0.434
	10	SR	2011	2.4929	0.029*	15.2	1.5183	0.182
	10	SR	2012	2.2518	0.048*	15.1	1.4666	0.200
umc1101	4	RS	2011	3.0629	0.014*	14.9	1.6019	0.168
	4	RS	2012	3.0356	0.015*	15.2	1.8102	0.120
	4	SR	2011	2.5828	0.032*	13.3	1.5000	0.198
	4	SR	2012	2.3397	0.049*	11.8	1.5664	0.179
umc1127	6	RS	2011	3.0530	0.01**	17.4	1.9584	0.081
	6	RS	2012	3.1383	0.008**	18.4	1.8718	0.095
	6	SR	2011	2.5341	0.027*	15.4	1.5183	0.182

GLM - general linear model; MLM - mixed linear model; Chr. - chromosome; Loc. - location; F-M - F value from the F test on marker; p-M - P value from the F test on marker; R^2: percentage of phenotypic variation explained by the marker; RS - Rimski šančevi; SR - Srbobran; SO - Sombor; * - significant at 0.05 probability level; ** - significant at 0.01 probability level

Three markers were associated with maize plant height, while six microsatellites were linked to ear height (Table 3). Significant marker-trait associations for both plant height and ear height, concurrently applying GLM and MLM, were found for only two markers, i.e. *bnlg434* and *umc1865*. The former marker was linked to plant height and ear height in more environments than the latter. The markers that had significant associations with the traits explained 10.2% to 14.7% of plant height phenotypic variation and 11.8% to 23% of ear height phenotypic variation.

The association between *nc005* and plant height was corroborated in the research of FRASCAROLI *et al.* (2007), who identified a QTL in the vicinity of the marker *nc005* with negative additive effect on the trait, explaining 4.2% of its phenotypic variation. The significant associations found between the marker *bnlg434* and plant and ear height in three environments, were supported by a mapping study that detected a QTL flanked by *bnlg434*, negatively affecting ear height (SIBOV *et al.*, 2003).

For the total leaf number, the most stable associations were found with *bnlg1643*, *bnlg434* and *umc1127*, while the leaf number above the ear formed the most stable associations with the markers *bnlg1643*, *umc1127* and *umc1506* (Table 4). Four markers were linked to the total leaf number and five markers were associated with the number of leaves above the ear. Only two markers, *bnlg1643* and *umc1127*, had significant associations with both traits, in all environments according to GLM model and in three environments according to MLM model. The former marker was also previously linked to a QTL for leaf number that had positive additive effect and explained 6.5% of phenotypic variation (JI-HUA *et al.*, 2007).

Table 4. Significant associations between microsatellites and leaf number in maize

				GLM			MLM	
Marker	Chr.	Loc.	Year	F-M	p-M	R^2	F-M	p-M
				Total leaf number				
bnlg1643	1	RS	2011	3.8890	0.001*	18.3	1.7052	0.119
	1	RS	2012	5.0473	0.000**	20.4	2.9837	0.008**
	1	SR	2011	2.6918	0.015*	17.9	1.9529	0.071
	1	SR	2012	3.6087	0.002**	19.6	2.3809	0.029*
	1	SO	2011	2.9905	0.008**	19.2	2.8424	0.011*
bnlg434	7	RS	2011	7.2061	0.001**	10.5	3.1879	0.046*
	7	RS	2012	5.0005	0.009**	7.4	1.7473	0.180
	7	SR	2011	3.2601	0.043*	6.7	2.0935	0.129
	7	SR	2012	4.4188	0.015*	7.8	2.2523	0.111
	7	SO	2011	3.7335	0.028*	7.5	2.9266	0.050*
umc1127	6	RS	2011	4.0242	0.001**	15.4	2.2172	0.049*
	6	RS	2012	4.0704	0.001**	16.1	2.7366	0.018*
	6	SR	2011	3.4519	0.004**	19.1	2.3890	0.035*
bnlg1451	10	RS	2011	2.7328	0.018*	12.2	1.0426	0.404
	10	RS	2012	3.2004	0.007**	13.4	1.1043	0.367
	10	SR	2012	2.7193	0.019*	13.8	1.1822	0.324

				Number of leaves above the ear				
bnlg1643	1	RS	2011	2.1584	0.047*	13.2	0.9592	0.466
	1	RS	2012	3.5817	0.002**	21.0	2.2887	0.036*
	1	SR	2011	2.2277	0.041*	14.7	1.3918	0.219
	1	SR	2012	3.5299	0.003**	22.0	2.5314	0.022*
	1	SO	2011	2.9018	0.01**	16.3	2.1767	0.046*
umc1127	6	RS	2011	2.3356	0.04*	12.3	0.9153	0.488
	6	RS	2012	4.7048	0.000**	21.8	2.4614	0.032*
	6	SR	2011	2.4678	0.031*	13.9	1.9601	0.080
	6	SR	2012	4.0207	0.002**	21.4	2.8457	0.015*
	6	SO	2011	2.7391	0.019*	13.6	3.6221	0.003**
umc1506	10	RS	2011	3.6887	0.003**	17.8	2.2447	0.048*
	10	SR	2011	3.2389	0.007**	17.4	2.2579	0.046*
	10	SO	2011	4.8445	0.000**	19.5	3.5877	0.003**
bnlg1655	10	RS	2011	9.0054	0.000**	13.3	5.2674	0.007**
	10	SR	2011	4.6103	0.013*	8.8	2.5724	0.082
	10	SO	2011	4.4307	0.015*	7.7	2.2319	0.114
umc2093	9	RS	2011	4.2662	0.004**	15.0	2.3966	0.050*
	9	SR	2011	3.2764	0.016*	13.0	1.7154	0.154
	9	SO	2011	2.5131	0.049*	9.1	1.5171	0.205

GLM - general linear model; MLM - mixed linear model; Chr. - chromosome; Loc. - location; F-M - F value from the F test on marker; p-M - P value from the F test on marker; R^2: percentage of phenotypic variation explained by the marker; RS - Rimski šančevi; SR - Srbobran; SO - Sombor; * - significant at 0.05 probability level; ** - significant at 0.01 probability level

The associations between microsatellites and ear traits were not consistent in all environments and in models tested (Table 5). Significant associations were found for ear diameter and three markers. The associations between ear diameter and *bnlg1360* were stable in most environments. Phenotypic variation of the trait explained by the marker effect ranged from 5.4 to 14%. Similarly, LI *et al.* (2010) identified a QTL for ear diameter flanked by *bnlg1360*, in two locations that explained from 11% to 13% of phenotypic variation.

The associations between the number of rows per ear and three markers, *umc1019*, *umc1506* and *bnlg434*, were identified, but the associations were more stable for GLM model. The effect of these markers on the row number variation varied from 4.8% to 14.8%. The association between *umc1019* and a QTL for ear row number, contributing 15.3% to the phenotypic variation, was also identified in another study (LI *et al.*, 2009). The result of the same study (LI *et al.*, 2009) supported the associations between *umc1506* and the ear row number detected in this research.

Two markers had stable associations with ear length in three out of five environments using either of the models. The phenotypic variation of ear length accounted for by the marker effects was in the range from 8.3% to 13.7%. Both of these markers, *bnlg2305* and *bnlg1451*, were previously associated to QTLs for ear length (MA *et al.*, 2007).

Only one microsatellite, *bnlg2082*, was associated with number of kernels per ear row. This association was in agreement with the previous findings (YAN *et al.*, 2006; LI *et al.*, 2009; TIAN *et al.*, 2014). The associations were significant in only two out of five environments for both models. The inconsistency in detected associations in different environments could be the result of epistasis, effects of the environment and genotype by environment interaction, which is usual for quantitative traits. For this reason, validation of marker-trait associations is recommended before the application of markers in selection processes (BRESEGHELLO and SORRELLS, 2006).

Table 5. Significant associations between microsatellites and ear traits in maize

				GLM			MLM	
Marker	Chr.	Loc.	Year	F-M	p-M	R^2	F-M	p-M
				Ear diameter				
bnlg1360	10	RS	2011	3.2632	0.007**	14.0	3.2632	0.006**
	10	RS	2012	2.3651	0.039*	10.8	3.5230	0.004*
	10	SR	2011	2.5083	0.029*	9.5	2.2587	0.045*
	10	SR	2012	2.2315	0.048*	11.3	1.5180	0.182
	10	SO	2011	3.4177	0.005**	13.2	1.8588	0.098
bnlg2305	5	SR	2011	3.2313	0.007**	11.6	2.4683	0.031*
	5	SR	2012	2.3948	0.036*	13.1	1.6540	0.143
	5	SO	2011	3.6587	0.003**	13.9	2.6340	0.029*
bnlg2082	8	SR	2011	2.9484	0.037*	5.4	2.1707	0.097
	8	SR	2012	3.7721	0.014*	9.5	2.7672	0.047*
	8	SO	2011	3.4146	0.021*	6.9	2.7108	0.050*
				Number of rows per ear				
umc1019	5	RS	2011	7.4642	0.001**	9.4	6.4089	0.003**
	5	RS	2012	3.0670	0.050*	4.8	2.1090	0.129
	5	SR	2011	7.0803	0.001**	8.4	4.7742	0.011*
	5	SR	2012	3.1496	0.048*	4.9	1.9721	0.145
	5	SO	2011	5.6214	0.005**	7.1	4.1270	0.020*
umc1506	10	RS	2011	2.4905	0.019*	12.6	2.2838	0.030*
	10	RS	2012	2.1439	0.043*	13.1	1.5194	0.167
	10	SR	2011	2.5825	0.015*	12.2	2.3383	0.025*
	10	SO	2011	3.2042	0.004**	14.8	2.5310	0.017*
bnlg434	7	RS	2011	3.6373	0.030*	4.8	2.4100	0.096
	7	RS	2012	4.426	0.016*	7.3	3.1493	0.049*
	7	SR	2011	4.3716	0.010*	5.5	2.6590	0.075
	7	SO	2011	4.2291	0.018*	5.5	2.3759	0.099
				Ear length				
bnlg2305	5	RS	2011	4.8907	0.003**	11.3	3.8930	0.012*
	5	RS	2012	5.9254	0.000**	13.5	4.8511	0.004**
	5	SR	2011	3.5399	0.013*	8.5	3.5389	0.018*
bnlg1451	10	RS	2011	4.0310	0.005**	13.7	3.0211	0.023*

	10	RS	2012	5.4283	0.001**	9.6	3.2607	0.017*
	10	SR	2011	2.4993	0.049*	8.3	2.4992	0.050*
				Number of kernels per ear row				
bnlg2082	8	RS	2011	6.7572	0.002**	13.4	6.1786	0.003**
	8	RS	2012	3.6797	0.031*	8.7	3.7797	0.030*
				Thousand-kernel weight				
bnlg1175	2	RS	2011	6.9909	0.002**	11.3	5.7795	0.004**
	2	SR	2011	4.5575	0.013*	9.2	3.4286	0.037*
	2	SR	2012	3.8003	0.026*	7.7	3.1785	0.047*
phi029	3	SR	2011	2.3307	0.027*	18.2	1.7363	0.102
	3	SO	2011	2.6499	0.013*	20.1	1.8719	0.076

GLM - general linear model; MLM - mixed linear model; Chr. - chromosome; Loc. - location; F-M - F value from the F test on marker; p-M - P value from the F test on marker; R^2: percentage of phenotypic variation explained by the marker; RS - Rimski šančevi; SR - Srbobran; SO - Sombor; * - significant at 0.05 probability level; ** - significant at 0.01 probability level

The associations between thousand-kernel weight and the markers *bnlg1175* were consistent in three environments for both models, while the associations between this trait and the marker *phi029* were less stable. These markers explained 7.7% to 20.1% of the phenotypic variation of the trait (Table 5). Our results were in keeping with the previously determined associations between a QTL for thousand-kernel weight and *bnlg1175* (COQUE and GALLAIS, 2006) and *phi029* (FRASCAROLI *et al.,* 2009; LI *et al.,* 2009).

Significant associations between the markers and the traits consistent in several environments, stable in different genetic backgrounds and confirmed in other studies indicate the presence of genetic factors that control complex biochemical processes with positive effects on favourable agronomic traits and ultimately, the possibility of employing these markers in marker assisted selection.

CONCLUSION

The population structure analysis clustered the maize inbred lines into four groups, which is in accordance with their heterotic responses and pedigrees. This implies the suitability of the microsatellite markers in elucidating lines' origin. The GLM identified twice the number of marker-trait associations across the environments that the MLM. The results of this study indicate that the markers, which had significant and consistent associations with the agronomic traits across the environments, could be useful in selection of inbred lines after the validation of the marker-trait associations.

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REFERENCES

BRADBURY, P.J., Z. ZHANG, D.E. KROON, T.M. CASSTEVENS, Y. RAMDOSS, E.S. BUCKLER (2007): TASSEL: software for association mapping of complex traits in diverse samples. Bioinform., *23*: 2633-2635.

BRESEGHELLO, F., M.E. SORRELLS (2006): Association mapping of kernel size and milling quality in wheat *(Triticum aestivum* L.) cultivars. Genet., *172*(2): 1165-77.

BOUCHEZ, A., F. HOSPITAL, M. CAUSSE, A. GALLAIS, A. CHARCOSSET (2002): Marker-assisted introgression of favorable alleles at quantitative trait loci between maize elite lines. Genet., *162*: 1945-1959.

CAMUS-KULANDAIVELU, L., J.B. VEYRIERAS, D. MADUR, V. COMBES, M. FOURMANN, S. BARRAUD, P. DUBREUIL, B. GOUESNARD, D. MANICACCI, A. CHARCOSSET (2006): Maize adaptation to temperate climate: relationship with population structure and polymorphism in the Dwarf8 gene. Genet., *172*(4): 2449-2463.

CHAKRABORTI, M., B.M. PRASANNA, F. HOSSAIN, S. MAZUMDAR, A.M. SINGH, S. GULERIA, H.S. GUPTA (2011): Identification of kernel iron-and zinc-rich maize inbreds and analysis of genetic diversity using microsatellite markers. J. Plant Biochem. Biotechol., *20(*2): 224-233.

COLLARD, B.C.Y, M.Z.Z. JAHUFER, J.B. BROUWER, E.C.K. PANG (2005): An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica, *142*: 169-196.

COQUE, M., A. GALLAIS (2006): Genomic regions involved in response to grain yield selection at high and low nitrogen fertilization in maize. TAG, *112*: 1205-1220.

COULON, A., J.W. FITZPATRICK, R. BOWMAN, B.M. STITH, C.A. MAKAREWICH, L.M. STENZLER, I.J. LOVETTE (2008): Congruent population structure inferred from dispersal behaviour and intensive genetic surveys of the threatened Florida scrub-jay (*Aphelocoma cœrulescens*). Mol. Ecol., *17*: 1685-1701.

DOYLE, J.J., J.L. DOYLE (1990): Isolation of plant DNA from fresh tissue. Focus, *12*:13-15.

EARL, D.A., B.M. VONHOLDT (2012): STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour., *4*(2): 359-361.

ENOKI, H., K. MIKI, K. KOINUMA (2006): Mapping of quantitative trait loci associated with early flowering of a northern flint maize (*Zea mays* L.) inbred line. Maydica, *51*(3): 515-523.

EVANNO, G., S. REGNAUT, J. GOUDET (2005): Detecting the number of clusters of individuals using the software structure: a simulation study. Mol. Ecol., *14*: 2611-2620.

FRASCAROLI, E., M.A. CANE, P. LANDI, G. PEA, L. GIANFRANCESCHI, M., VILLA, M. MORGANTE, M.E. PE (2007): Classical genetic and quantitative trait loci analyses of heterosis in a maize hybrid between two elite inbred lines. Genet., *176*: 625-644.

FRASCAROLI, E., M.A. CANE, M.E. PE, G. PEA, M. MORGANTE, P. LANDI (2009): QTL detection in maize testcross progenies as affected by related and unrelated testers. TAG, *118*: 993-1006.

JI-HUA, T., T. WEN-TAO, Y. JIAN-BING, M. XI-QING, M. YI-JIANG, D. JIN-RUI, L. JIAN-SHENG (2007): Genetic dissection of plant height by molecular markers using a population of recombinant inbred lines in maize. Euphytica, *155*(1-2):117-124.

KRISHNA, M.S.R., S.S. REDDY, V.C.B. NAIK (2012): Assessment of genetic diversity in quality protein maize (QPM) lines using simple sequence repeat (SSR) markers. Afr. J. Biotechnol., *11*(98): 16427-16433.

LI, M., X. GUO, M. ZHANG, X. WANG, G. ZHANG, Y. TIAN, Z. WANG (2010): Mapping QTLs for grain yield and yield components under high and low phosphorus treatments in maize (*Zea mays* L.). Plant Sci., *178*(5): 454-462.

LI, Y.L., X.H. LI, J.Z. LI, J.F. FU, Y.Z. WANG, M.G. WEI (2009): Dent corn genetic background influences QTL detection for grain yield and yield components in high-oil maize. Euphytica, *169*(2): 273-284.

LIU, K., M. GOODMAN, S. MUSE, J.S. SMITH, E. BUCKLER, J. DOEBLEY (2003): Genetic structure and diversity among maize inbred line as inferred from DNA microsatellites. Genet., *165*: 2117-2128.

MA, X.Q., J.H. TANG, W.T. TENG, J.B. YAN, Y.J. MENG, J.S. LI (2007): Epistatic interaction is an important genetic basis of grain yield and its components in maize. Mol. Breed., *20*: 41-51.

MARINO, R., M. PONNAIAH, P. KRAJEWSKI, C. FROVA, L. GIANFRANCESCHI, M.E. PE, M. SARI-GORLA (2009): Addressing drought tolerance in maize by transcriptional profiling and mapping. Mol. Genet. Genomics, *281*(2): 163-179.

MIKIĆ, S., A. KONDIĆ-ŠPIKA, L. BRBAKLIĆ, D. STANISAVLJEVIĆ, D. TRKULJA, M. TOMIČIĆ, A. NASTASIĆ, B. KOBILJSKI, S. PRODANOVIĆ, G. ŠURLAN-MOMIROVIĆ (2016): Multiple marker-traits associations for maize agronomic traits. Chil. J. Agric. Res., *76*(3): 300-306.

MLADENOVIĆ-DRINIĆ, S., M. KOSTADINOVIĆ, D. RISTIĆ, M. STEVANOVIĆ, Z. ČAMDŽIJA, M. FILIPOVIĆ, D. KOVAČEVIĆ (2012): Correlation of yield and heterosis of maize hybrids and their parental lines with genetic distance based on SSR markers. Genetika, *44*(2): 399-408.

MU, G., Y. LIANG, Z. ZHANG, Y. WU, S. LIU, H. PENG, S. ZHANG, G. PAN (2009): Mapping quantitative trait loci associated with photoperiod sensitivity in maize (*Zea mays* L.) Agric. Sci. China, *8*(1): 24-30.

PRITCHARD, J.K., M. STEPHENS, P.J. DONNELLY (2000): Inference of population structure using multilocus genotype data. Genet., *155*: 945-959.

PRITCHARD, J.K., W. XIAOQUAN, D. FALUSH (2010): Documentation for structure software: Version 2.3. University of Chicago, Chicago, IL

RASMUSSEN, C.C., A.R. HALLAUER (2006): Evaluation of heterotic patterns of Iowa Stiff Stalk Synthetic and Non-Stiff Stalk Synthetic maize populations. Maydica, *51*: 177-186.

REMINGTON, D.L., J.M. THORNSBERRY, Y. MATSUOKA, L.M. WILSON, S.R. WHITT, J. DOEBLEY, S. KRESOVICH, M.M. GOODMAN, E.S. BUCKLER (2001): Structure of linkage disequilibrium and phenotypic associations in the maize genome. Proc. Natl. Acad. Sci. U.S.A., *98*(20): 11479-11484.

SEMAGN, K., C. MAGOROKOSHO, V.S. BINDIGANAVILE, D. MAKUMBI, Y. BEYENE, S. MUGO, B.M. PRASANNA, M.L. WARBURTON (2012): Molecular characterization of diverse CIMMYT maize inbred lines from eastern and southern Africa using single nucleotide polymorphic markers. BMC Genomics, *13*: 113.

SIBOV, S.T., J.R. DE SOUZA, C. LOPES, A.A. GARCIA, A.R. SILVA, A. GARCIA, C.A. MANGOLIN, L.L. BENCHIMOL, A.P. DE SOUZA (2003): Molecular mapping in tropical maize (*Zea mays* L.) using microsatellite markers. 2. Quantitative trait loci (QTL) for grain yield, plant height, ear height and grain moisture. Hereditas, *139*(2): 107-115.

SUTEU, D., I. BACILA, V. HAS, I. HAS, M. MICLAUS (2014): Romanian Maize (*Zea mays*) inbred lines as a source of genetic diversity in SE Europe, and their potential in future breeding efforts. PloS one, *8*(12): p.e85501.

TERRA, T.D.F., P. WIETHÖLTER, C.C.D.S. ALMEIDA, F. BERED, M. SERENO, J.C.D.M., J.F. BARBOSA NETO (2011): Genetic variability in maize and teosinte populations estimated by microsatellites markers. Ciência Rural, *41*(2): 205-211.

TIAN, B., J. WANG, G. WANG (2014): Confirmation of a major QTL on chromosome 10 for maize kernel row number in different environments. Plant Breed., *133*(2): 184-188.

VIGOUROUX, Y., J.C. GLAUBITZ, Y. MATSUOKA, M.M. GOODMAN, J.G. SANCHEZ, J. DOEBLEY (2008): Population structure and genetic diversity of new world maize races assessed by DNA microsatellites. Am. J. Bot., *95*(10): 1240-1253.

WILLMOT, D.B., J.W., DUDLEY, T.R. ROCHEFORD, A.L. BARI (2006): Effect of random mating on marker-QTL associations for grain quality traits in the cross of Illinois High Oil x Illinois Low Oil. Maydica, *51*(2): 187-199.

YAN, J., H. TANG, Y. HUANG, Y. ZHENG, C. SUBHASH, J. LI (2006): A genome scan for quantitative trait loci affecting grain yield and its components of maize both in single-and two-locus levels. Chin. Sci. Bull., *51*(12): 1452-1461.

YAN, J., M. WARBURTON, J. CROUCH (2011): Association mapping for enhancing maize (*Zea mays* L.) genetic improvement. Crop Sci., *51*: 433-449.

YANG, X., J. YAN, T. SHAH, M.L. WARBURTON, Q. LI, L. LI, Y. GAO, Y. CHAI, Z. FU, Y. ZHOU, S. XU, G. BAI, Y. MENG, Y. ZHENG, J. LI (2010): Genetic analysis and characterization of a new maize association mapping panel for quantitative trait loci dissection. TAG, *121*: 417-431.

VEGA, S.H., M. SAUER, J.A.J. ORKWISZEWSKI, R.S. POETHIG (2002): The early phase change gene in maize. Plant Cell, 14: 133-147.

ZONDERVAN, K.T., L.R. CARDON (2004): The complex interplay among factors that influence allelic association. Nat. Rev. Genet., *5*(2): 89-100.

ASOCIJATIVNA ANALIZA AGRONOMSKIH OSOBINA INBRED LINIJA KUKURUZA MIKROSATELITIMA

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Izvod

Asocijativna analiza je metoda mapiranja lokusa za kvantitativne osobine među nesrodnim individuama na osnovu velikog broja rekombinacija tokom domestikacije i selekcije gajenih vrsta. Ukoliko se u analizu uključi struktura populacije, koja nastaje usled adaptacije na lokalne uslove sredine ili selekcije, može se značajno smanjiti broj lažno pozitivnih veza. Cilj rada bio je da se utvrde značajne i stabilne veze između markera i agronomskih osobina primenom asocijativne analize u divergentnom oplemenjivačkom materijalu u nekoliko sredina, koje bi se primenile u selekciji kukuruza. Devedeset šest inbred linija kukuruza je ocenjeno u poljskim ogledima na tri lokaliteta za 11 osobina i analizirano mikrosatelitskim markerima. Dvadeset i pet mikrosatelita je primenjeno za ocenu strukture populacije pomoću Bejzovog modela i za testiranje veza između markera i osobina pomoću opšeg i mešovitog linearnog modela. Klaster analizom, inbred linije su podeljene u četiri grupe: BSSS, LSC, Iodent i nezavisni materijal. Opšti model je utvrdio 133 veze, a mešoviti 71 vezu između markera i osobina. Stabilne veze u više sredina pronađenje su između markera *bnlg1067* i vremena cvetanja; *nc005* i *bnlg434* i visine biljke; *bnlg434* i visine klipa; *bnlg1643* i *umc1127* i broja listova; *bnlg1360* i prečnika klipa; *umc1019* i *umc1506* i broja redova zrna; *bnlg2305* i *bnlg1451* i dužine klipa; *bnlg1175* i mase hiljadu zrna. Rezultati ukazuju da se ovi mikrosatelitski markeri mogu koristiti u selekciji inbred linija, nakon potvrde veza marker-osobina i testiranja kombinacionih sposobnosti linija pri stvaranju hibrida.

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