Fragm. Agron. 34(4) 2017, 134–144

ANALYSIS OF MOLECULAR VARIANCE AND GENETIC SIMILARITY BETWEEN SELECTED CULTIVARS OF MAIZE (*ZEA MAYS* L.) REVEALED BY SSR MARKERS

Kamila Nowosad[1], Jan Bocianowski[2], Piotr Szulc[3]

[1]*Department of Genetics, Plant Breeding and Seed Production, Wrocław University of Environmental and Life Sciences, Grunwaldzki 24A, 53-363 Wrocław, Poland*
[2]*Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland*
[3]*Department of Agronomy, Poznań University of Life Sciences, Dojazd 11, 60-623 Poznań, Poland*

Abstract. Genetic diversity was analysed based on 30 SSR primers in a set of thirteen cultivars of maize. A total of 112 SSR markers were detected in these cultivars, which varied in terms of their maturity, grain type, hybrid type and the presence or absence of the stay-green effect. The mean genetic similarity between the cultivars amounted to 0.473, ranging from 0.174 to 0.727. The study showed high diversity in the studied maize cultivars. The UPGMA dendrogram and the PCA plot showed that all the cultivars are divided into four clusters. Analysis of molecular variance indicated a significant genetic differentiation between the inferred groups, accounting for min. 89.43% of the total molecular variance between cultivars within the groups. Microsatellite data used in this study showed that genetic variability was found mainly within the groups rather than among the groups identified by the analysed factors.

Key words: AMOVA, genetic similarity, maize (*Zea mays* L.), microsatellite markers, principal component analysis

INTRODUCTION

Maize (*Zea mays* L.) is one of the worlds' three staple cereals. Thanks to its considerable adaptability it is grown in a greater range of environments than rice or wheat. Maize is a major source of both food and animal, while it is also a valuable raw material for agri-food industries. Moreover, it is an important model plant in research conducted on various biological phenomena and processes such as e.g. hybrid vigor and genome evolution. The maize genome is highly complex and in comparison to other crops and model plants it exhibits exceptional genetic diversity [Fu and Dooner 2002]. While genetic similarity/diversity is a major factor in crop improvement work conducted on maize, modern breeding has drastically limited the variation of important traits, particularly in common maize cultivars widely used in breeding programmes. Investigations of genetic diversity in maize germplasm are based on its assessed usefulness for breeding programmes. Molecular markers provide a promising tool for the evaluation of genetic diversity in plant materials, since phenotype and physiological markers are not sufficiently accurate and are frequently influenced by environmental conditions [Kozak et al. 2011].

Molecular techniques have been extensively applied as powerful tools to investigate germplasm characteristics, to identify cultivars, as well as conduct phylogenetic studies and similarity/diversity analyses in many crop plants. Thanks to their high polymorphism, random distribu-

[1] *Corresponding address* – Adres do korespondencji: kamila.nowosad@upwr.edu.pl

tion and co-dominant Mendelian inheritance microsatellite or simple sequence repeats (SSRs) markers, constitute the most reliable marker system for QTL mapping, cultivar identification and genetic diversity studies [Hokanson et al. 1998, Naghavi et al. 2012, Stępień et al. 2007]. Microsatellites are stretches of DNA comprising tandemly arranged units of 1-6 bp [Gupta et al. 1996], characterised by their co-dominant inheritance, wide genomic distribution, as well as hypervariable and multiallelic nature [Parida et al. 2009, Powell et al. 1996]. The SSR markers are typically markers of choice in diversity studies. Since they are PCR-based, SSRs are technically simple to apply and are amenable to high throughput assays [Mansfield et al. 1994], while they are easy to score and require small amounts of DNA for analysis [Somta et al. 2011]. In recent years early generation selections between breeding populations have been routinely based on the application of SSRs [Gupta and Varshney 2000]. Molecular markers are more powerful tools in the detection of diversity, considerably exceeding traditional methods [Gupta and Varshney 2000]. DNA markers linked to agronomic traits, including SSRs, may increase the efficiency of classical breeding, as they significantly reduce the number of the required backcross generations and limit expensive, phenotypic selection as well as germplasm conservation. Another advantage of DNA markers is also connected with the fact that they may be used efficiently, regardless of the analysed plant developmental phase.

The aim of this study was to analyse molecular variance and genetic similarity between thirteen maize cultivars differing in their maturity, grain type, hybrid type and the stay-green effect using SSR markers.

MATERIAL AND METHODS

The field experiment was carried out at the Department of Agronomy, the Poznań University of Life Sciences, in 2016 in the fields of the Agricultural Experimental Station in Swadzim. Thirteen cultivars (Table 1) of fodder maize, grown for grain and purchased from different

Table 1. Characterization of 13 cultivars of maize (*Zea mays* L.)

Cultivar	Maturity (FAO number)	Breeder	Type of grain	Type of hybrid	Stay-green (SG) effect
NK Cooler	medrium-early (240)	Syngenta	flint	tri-hybrid	Yes
Delitop	medrium-early (240)	Syngenta	flint	single-hybrid	Yes
Gazele	early (220-230)	Syngenta	dent	tri-hybrid	Yes
NK Ravello	early (210)	Syngenta	flint	single-hybrid	Yes
ES Palazzo	medrium-early (230-240)	Euralis	flint	single-hybrid	No
ES Paroli	medium-late (250)	Euralis	flint-dent	single-hybrid	Yes
SY Cooky	early (220-230)	Syngenta	flint	single-hybrid	No
Drim	early (220)	Syngenta	flint-dent	tri-hybrid	Yes
Clarica	medium-late (280)	Pioneer	dent	single-hybrid	No
PR 39 G12	medrium-early (230)	Pioneer	flint	single-hybrid	Yes
SY Mascotte	medium-late (260)	Syngenta	flint-dent	single-hybrid	Yes
ES Fortran	early (210-220)	Euralis	flint	single-hybrid	No
PR 39 K 13	early (220)	Pioneer	flint	single-hybrid	No

seed production companies, were compared. The experiment was established in a one-factor randomized block design with four field replications. It was carried out in typical grey-brown podzolic soil composed of coarse sandy soil, shallowly deposited on light loam. The same NPK fertilisation was applied throughout the experimental field: 100 kg N·ha^{-1} in the form of urea, 80 kg P_2O_5·ha^{-1} in the form of NPK-fertilizer (6% N), and 120 kg K_2O·ha^{-1} in the form of 60% potassium salt. All the agricultural and cultivation measures were performed following the guidelines of good agricultural practices. Ten ears were collected from each experimental plot, with ten kernels collected for genetic marker analyses after their manual threshing.

A sample of approx. 0.1 g fresh young tissues was collected from each cultivar and ground into a fine powder using liquid nitrogen in the TissueLyser II system. Total genomic DNA was extracted using a commercially available DNA extraction kit (DNeasy Plant Mini Kit, Qiagen) following the manufacturer's instructions. DNA was quantified using a *Picodrop* Microliter UV/Vis Spectrophotometer (Saffron Walden, United Kingdom).

The polymerase chain reaction was run in a TProfessional thermocycler (Biometra) using a set of 30 SSR primers (Table 2). 100 pair of primers were tested in preliminary experiment. On the basis of results of this experiment we selected 30 SSR primers. Sequences of all tested primers come from database https://www.maizegdb.org. The PCR reaction mix (15 µl) included the following: 70 ng genomic DNA, 100 µM each dNTP, 0.2 µM forward and reverse primers, 1x Taq polymerase buffer and 2 U Tag polymerase (Thermo). Amplifications were carried out for 35 cycles, each consisting of a denaturation step of 40 s at 95ºC, followed by an annealing step of 30 s at 55ºC and an extension step of 1 min at 72ºC, with a final extension at 72ºC for 7 min. The amplified products were analysed on the QIAxcel system with the QIAxcel HighResolution Kit following the AM420 method.

All the cultivars were scored for the presence and absence of the SSR bands. Data were entered into a binary matrix as discrete variables, with "1" for the presence and "0" for the absence of alleles. The polymorphic information content (PIC) for each marker was calculated using the formula described by Wolko et al. [2015]. PIC values ranged from 0 (in the case of fixation of one allele) to 0.5 (when the frequencies of both alleles were equal). Genetic similarity (GS) for each pair of cultivars was estimated based on the coefficient proposed by Nei and Li (1979), defined as $GS=2N_{AB}/(N_A+N_B)$, where N_{AB} is the number of bands shared by cultivars A and B, N_A is the number of bands in cultivar A, and N_B is the number of bands in cultivar B. The cultivars were grouped using the unweighted pair group method with arithmetic mean (UPGMA). Similarities between cultivars were visualised on a dendrogram. On the basis of the same matrix the principal component analysis (PCA) was performed with the use of the GenStat v. 17 statistical package. This multivariate technique allowed us to find and plot the major pattern within the multiple loci data set. The resulting distance matrix was also subjected to the analysis of molecular variance (AMOVA) using GenAlEx 6.5 [Peakall and Smouse 2012]. AMOVA estimated and partitioned the total molecular variance within and between groups of cultivars and then tested the significance of partitioned variance components using non-parametric permutational testing procedures with 999 permutations [Excoffier et al. 1992]. The F_{ST} coefficient was used as a measure of population genetic structure and calculated from the formula: $F_{ST}=(H_T–H_S)/H_T$, where H_T is the probability that two alleles drawn at random (with replacement) from the entire population differ in state (i.e., the probability with no population structure), and H_S is the probability that two alleles drawn at random from a subpopulation differ in state (which, for a two-allele system, will always be $2p_iq_i$, where p_i is the observed allelic frequency in subpopulation i), average over subpopulations. Groups for AMOVA were identified on the basis of the stay-green effect, type of hybrid, type of grain and maturity.

Table 2. Code and sequences of primers tested, with annealing temperature

Primer code	Left End:	Right End:	Temp (°C)
phi001	TGACGGACGTGGATCGCTTCAC	AGCAGGCAGCAGGTCAGCAGCG	71
phi002	CATGCAATCAATAACGATGGCGAGT	TTAGCGTAACCCTTCTCCAGTCAGC	66
phi008	CGGCTACGGAGGCGGTG	GATGGGCCCACACATCAGTC	65
phi015	GCAACGTACCGTACCTTTCCGA	ACGCTGCATTCAATTACCGGGAAG	67
phi021	TTCCATTCTCGTGTTCTTGGAGTGGTCCA	CTTGATCACCTTTCCTGCTGTCGCCA	63
phi026	TAATTCCTCGCTCCCGGATTCAGC	GTGCATGAGGGAGCAGCAGGTAGTG	70
phi036	CCGTGGAGAGACGTTTGACGT	TCCATCACCACTCAGAATGTCAGTGA	66
phi041	TTGGCTCCCAGCGCCGCAAA	GATCCAGAGCGATTTGACGGCA	64
phi042	ATGTGGCCATCATTCAATGCTGTAGAC	ACACATGCAGGTGCAGCCAGA	68
phi047	GGAGATGCTCGCACTGTTCTC	CTCCACCCTCTTTGACATGGTATG	63
phi049	GATTGCGATAACATTGCGGCAAGTTGT	CTTCTGTTCCGCCATCCAGTATGTT	69
phi054	AGAAAAGAGAGTGTGCAATTGTGATAGAG	AATGGGTGCCTCGCACCAAG	66
phi056	ACTTGCTTGCCTGCCGTTAC	CGCACACCACTTCCCAGAA	63
phi061	GACGTAAGCCTAGCTCTGCCAT	AAACAAGAACGGCGGTGCTGATTC	69
phi064	CCGAATTGAAATAGCTGCGAGAACCT	ACAATGAACGGTGGTTATCAACACGC	68
phi068	GTACACACGCTCCGACGATTAC	TCTTCTCCACCAGAGCCTTGTAAG	62
phi070	GCTGAGCGATCAGTTCATCCAG	CCATGGCAGGGTCTCTCAAG	64
phi072	ACCGTGCATGATTAATTTCTCCAGCCTT	GACAGCGCGCAAATGGATTGAACT	70
phi073	GTGCGAGAGGCTTGACCAA	AAGGGTTGAGGGCGAGGAA	63
phi076	TTCTTCCGCGGCTTCAATTTGACC	GCATCAGGACCCGCAGAGTC	65
phi079	TGGTGCTCGTTGCCAAATCTACGA	GCAGTGGTGGTTTCGAACAGACAA	68
phi080	CACCCGATGCAACTTGCGTAGA	TCGTCACGTTCCACGACATCAC	64
phi085	AGCAGAACGGCAAGGGCTACT	TTTGGCACACCACGACGA	64
phi112	TGCCCTGCAGGTTCACATTGAGT	AGGAGTACGCTTGGATGCTCTTC	66
phi113	GCTCCAGGTCGGAGATGTGA	CACAACACATCCAGTGACCAGAGT	63
phi116	TCCCTGCCGGGACTCCTG	GCATACGGCCATGGATGGGA	68
phi119	GGGCTCCAGTTTTCAGTCATTGG	ATCTTTCGTGCGGAGGAATGGTCA	68
phi120	TGATGTCCCAGCTCTGAACTGAC	GACTCTCACGGCGAGGTATGA	63
phi127	ATATGCATTGCCTGGAACTGGAAGGA	AATTCAAACACGCCTCCCGAGTGT	69
phi129	TCCAGGATGGGTGTCTCATAAAACTC	GTCGCCATACAAGCAGAAGTCCA	65

RESULTS

A total of 112 SSR markers were detected with the set of 30 SSR primers. The average number of alleles per locus was 3.73, ranging from 1 to 17. The set of SSR primers used in this study generated highly informative loci with PIC values ranging from 0 to 0.497, with the mean 0.274. The size of PCR products ranged from 63 to 267 bp.

The data were computed to estimate genetic similarity between studied maize cultivars based on Nei and Li's coefficients. The highest genetic similarity (amounting to 0.727) was found between cultivars Delitop and Gazele, whereas the lowest genetic similarity (0.174) was found for cv. Delitop and PR 39 K 13 (Table 3). The mean value of genetic similarity was 0.473. The SSR markers data were used to group cultivars by the UPGMA method. The relationships between cultivars are presented in the form of a dendrogram (Fig. 1), in which four clusters were clearly distinguished. The first cluster (cluster I) contains cultivars: PR 39 G12, Clarica, SY Mascotte, ES Fortran, SY Cooky and Drim; the second (cluster II): ES Paroli, ES Palazzo, NK Ravello, Delitop and Gazele; the third (cluster III) comprises cv. NK Coller, while the fourth cluster IV – PR 39 K 13. Genetic similarity between cultivars with the stay-green effect and cultivars without the SG effect amounted to 0.945. The genetic similarity between the group of single-hybrids and the group of tri-hybrids was 0.930. Genetic similarity between flint and dent cultivars was 0.895. The flint-dent type cultivars were more similar to the flint type cultivars (0.918) rather than the dent type cultivars (0.875). For the maturity groups the greatest similarity was observed between the group of medium-late cultivars and the group of medium-early cultivars (0.935),

Table 3. Genetic similarity among the studied cultivars of maize based on 112 SSR markers

Cultivar	NK Cooler	Delitop	Gazele	NK Ravello	ES Palazzo	ES Paroli	SY Cooky	Drim	Clarica	PR 39 G12	SY Mascotte	ES Fortran
Delitop	0.478											
Gazele	0.489	0.727										
NK Ravello	0.481	0.719	0.698									
ES Palazzo	0.409	0.556	0.604	0.613								
ES Paroli	0.468	0.491	0.571	0.615	0.509							
SY Cooky	0.388	0.597	0.632	0.612	0.453	0.462						
Drim	0.310	0.441	0.448	0.553	0.394	0.435	0.652					
Clarica	0.440	0.467	0.508	0.529	0.483	0.492	0.494	0.583				
PR 39 G12	0.386	0.478	0.455	0.507	0.431	0.471	0.523	0.481	0.479			
SY Mascotte	0.500	0.515	0.554	0.595	0.469	0.537	0.598	0.590	0.571	0.494		
ES Fortran	0.351	0.388	0.364	0.453	0.400	0.412	0.545	0.633	0.563	0.564	0.675	
PR 39 K 13	0.222	0.174	0.178	0.259	0.182	0.298	0.269	0.276	0.360	0.211	0.321	0.351

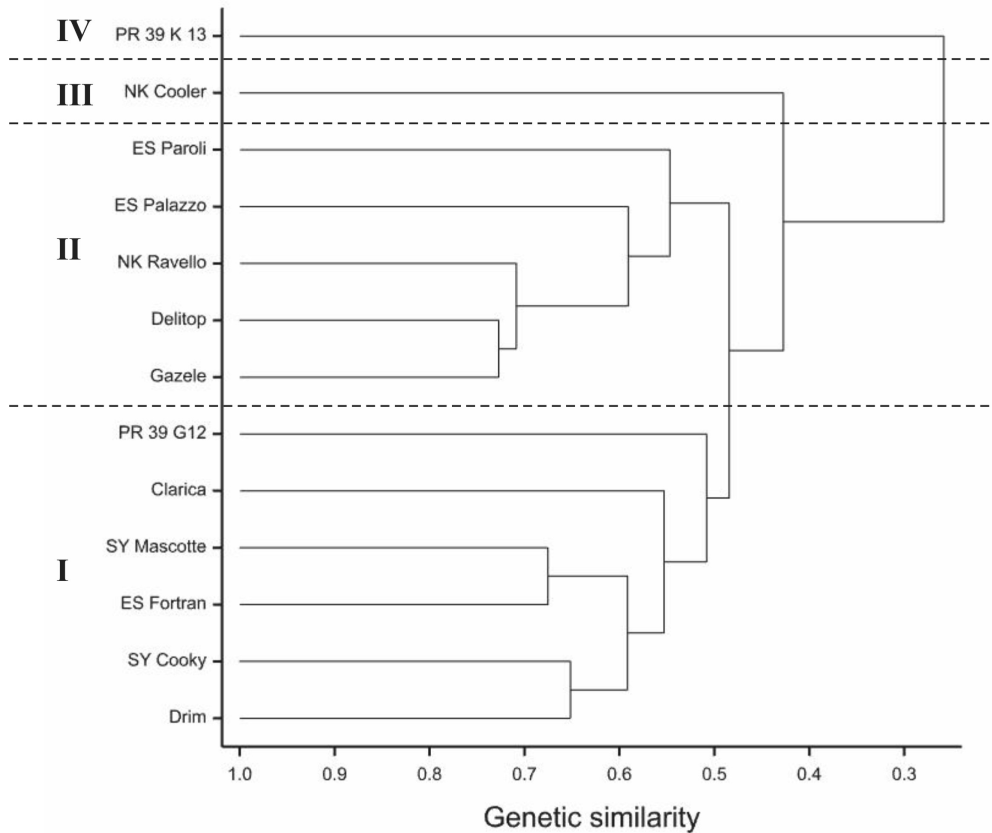

Fig. 1. Dendrogram for 13 cultivars of maize constructed for 112 SSR markers. Cultivars were grouped hierarchically using the unweighted pair group method of arithmetic means (UPGMA)

whereas the lowest genetic similarity was found for the group of early cultivars and the group of medium-late cultivars (0.904). The genetic similarity between the groups of early and medium-early cultivars was 0.917.

The significant differentiation (F_{ST}=0.098; p=0.012) between the cultivars with and without the stay-green effect was further supported by the AMOVA results (Table 4). The intra- and inter-cultivar variabilities were found to be significant, with 9.8% of the genetic variance contributed by the differentiation between the presence and lack of the stay-green effect, whereas 90.2% was partitioned within the groups (Table 4). However, the analysis performed for the groups determined based on the hybrid type detected a small, but statistically significant variation between the groups of single-hybrids and tri-hybrids (F_{ST}=0.103; p=0.040). The percentage of variation between these two groups was 10.3%, whereas 89.7% was partitioned within the groups (Table 4). AMOVA performed for the SSR data showed no significant differences between the type of grain groups (F_{ST}=0.106; p=0.058). However, the AMOVA performed for

Table 4. Summary of analyses of molecular variance (AMOVA) for comparison between and within groups of cultivars for four factors: stay-green effect, type of hybrid, type of grain and FAO number (maturity) by SSR markers

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Estimated variance	Percentage of variation
Stay-green effect					
Among groups	1	26.3	26.3	1.71	9.8
Within groups	11	173	15.7	15.7	90.2
Type of hybrid					
Among groups	1	24.3	24.3	1.82	10.3
Within groups	11	175	15.9	15.9	89.7
Type of grain					
Among groups	2	44.0	22.0	1.83	10.6
Within groups	10	155	15.5	15.5	89.4
FAO number (maturity)					
Among groups	2	43.6	21.8	1.50	8.8
Within groups	10	156	15.6	15.6	91.2
Total	12	199		17.0	100

the groups of FAO number detected a statistically significant variation between the groups (F_{ST}=0.088; p=0.016). The percentage of variation between these groups was 8.8%, whereas 91.2% was partitioned within the groups (Table 4). The largest variation was observed for the early cultivars (FAO 200–220).

The PCA was performed to confirm the structure and to gain insight into the diversity of 13 maize cultivars based on the distance matrix (Fig. 2). The first three PCs explained a total of 45.3% SSR marker variation (18.6, 15.5, and 11.2%, respectively). The first axis, which accounted for 18.6% of the variance, separated the cultivars which were assigned by UPGMA to the first and second clusters. The second axis, accounting for 15.5% of the variance, showed diversity of cultivars NK Coller and PR 39 K 13. It is evident that the results provided by these two methods applied independently, are complementary.

DISCUSSION

Microsatellite markers are some of the most extensively used molecular markers in various research areas, including assessment of genetic diversity, gene mapping and marker assisted selection [Abbasi et al. 2015, Erayman et al. 2014, Ipek et al. 2015, Kumar et al. 2015, Naghavi et al. 2012, Wolko et al. 2010,]. SSR markers exhibit tremendous potential for large-scale DNA fingerprinting of maize genotypes thanks to the high level of polymorphism detected [Smith et al. 1997], which is used to assess genetic relationships between maize inbred lines and other genotypes [Enoki et al. 2002, Li et al. 2002, Pejic et al. 1998, Senior et al. 1998, Smith et al. 1997, Song et al. 2013].

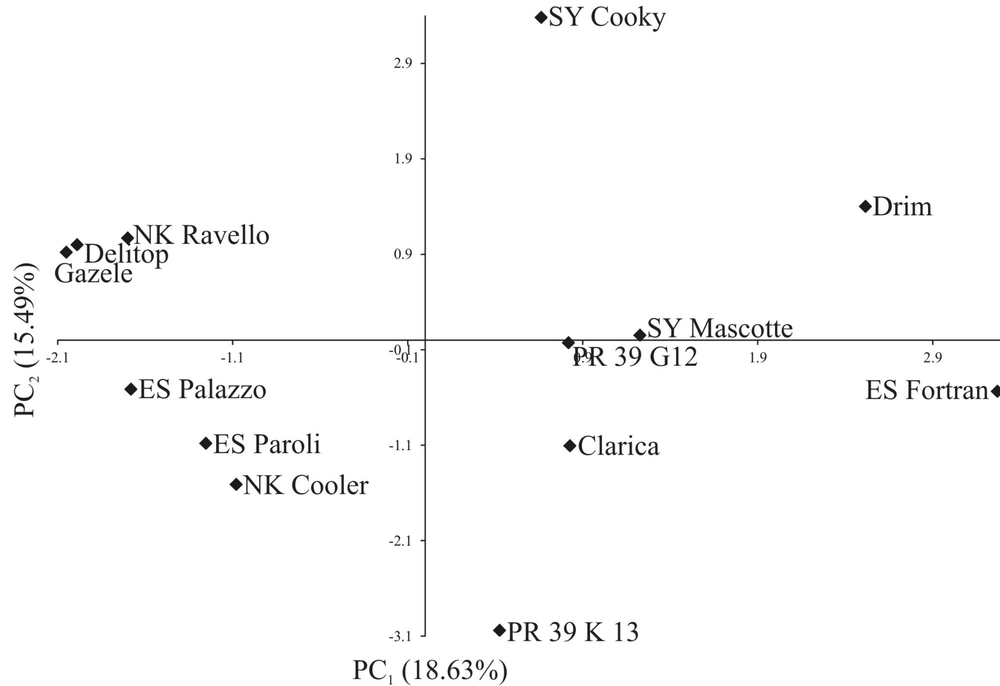

Fig. 2. Principal component analysis of 13 cultivars of maize based on 112 SSR markers

The polymorphic information content defines the applicability of SSR markers to identify genetic similarities and differences between pure lines, in this case it was maize cultivars. The PIC recorded in this study for biallelic markers ranged from 0 to 0.497, with a mean of 0.274. In the case of multiallelic microsatellites other researchers reported greater PIC values: (1) mean PIC of 0.62 was previously provided by 131 SSR markers in a diversity study of 58 maize inbred lines, with the PIC ranging of 0.06 to 0.91 [Smith et al. 1997], (2) PIC values for 70 SSR loci for 94 maize inbreds ranged from 0.17 to 0.92, with an average of 0.59 [Senior et al. 1998], (3) the PIC values of 105 SSR loci for 56 maize inbred lines ranged from 0.31 to 0.71, with a mean of 0.55 [Legesse et al. 2006], (4) PIC ranged from 0.75 to 0.96, with an average of 0.91 for 151 alleles analysed for 63 inbreds [Krishna et al. 2012]. High PIC estimates indicate the strength of the molecular markers to distinguish any variability between cultivars, particularly for SSRs that have the advantage of being co-dominant.

Information on genetic similarity is crucial for crop improvement and it has been successfully applied for efficient germplasm management, fingerprinting and genotype selection. Genetic similarity may be estimated based on phenotypic identification or molecular markers. However, morphological traits have a number of limitations, such as e.g. low heritability and late expression; additionally, they may be controlled by epistatic and pleiotropic gene effects [Bocianowski and Nowosad 2015, Eivazi et al. 2008]. Molecular markers are useful complements supplementing morphological characterisation of genotypes, since they are plentiful, independent of plant tissue

or environmental effects, and provide cultivar identification very early in the course of plant development [Manifesto et al. 2001]. Genetic similarity between the 13 analysed maize cultivars ranged from 0.174 to 0.727. Many researchers characterised genetic diversity in maize using microsatellite markers. The similarity observed in this study is similar to the values obtained in other studies using SSR markers: (1) ranging from 0.16 to 0.87 with an average of 0.62 [Pejic et al. 1998], (2) from 0.66 to 0.97 [Krishna et al. 2012] and (3) from 0.422 to 0.756 [Srdić et al. 2011]. Genotypes exhibiting the greatest genetic diversity (the smallest genetic similarity) may be used to create a gene pool of parental lines applicable in the creation of high yielding maize hybrids.

The 13 cultivars were grouped into four clusters using the model-based analysis, the results of which were consistent with PCA and phylogenic analysis. The difference in genetic diversity between the cultivars is an effective indicator of genetic differentiation. Our results showed variation in the apparent diversity distribution between the cultivars in relation to gene diversity and PIC scores, suggesting distinct differentiation levels. Further, the statistically significant genetic differentiation between the groups was shown by the AMOVA, which confirmed to the differentiation of three (out of four) factors. However, the differences between the groups based on grain type were non-significant. The total variation was connected with the differences between the cultivars within the group, as indicated by the AMOVA. This may have resulted from the acceleration of resource communication between the different groups. The AMOVA has often been used to study many species [Boczkowska and Tarczyk 2013, Contreras-Negrete et al. 2015, Mazid et al. 2013, Naghavi et al. 2012].

The PCA identifies linear combinations of a set of variates (markers), which maximises variation contained within them, thus displaying most of the original variability within a smaller number of dimensions. The total variation of SSR markers explained by the first two PCs exceeded the results recorded in other studies [Mazid et al. 2013].

CONCLUSIONS

The genetic analysis using the SSR markers revealed the extent of similarity and differences between 13 maize cultivars used in this study, which compared favourably with the results reported in similar studies [Somta et al. 2011] using SSRs, including those adopted in this study. In this study, PIC estimates ranged from 0 to 0.497, with a mean of 0.274. The SSR markers highly effectively discriminated between the 13 analysed maize cultivars. 30 SSR primers studied in this paper can be recommended for creation by maize's breeders of separate gene pools.

REFERENCES

Abbasi Z., Majidi M.M., Arzani A., Rajabi A., Mashayekhi P., Bocianowski J. 2015. Association of SSR markers and morpho-physiological traits associated with salinity tolerance in sugar beet (*Beta vulgaris* L.). Euphytica 205: 785–797.

Bocianowski J., Nowosad K. 2015. Mixed linear model approaches in mapping QTLs with epistatic effects by a simulation study. Euphytica 202: 459–467.

Boczkowska M., Tarczyk E. 2013. Genetic diversity among Polish landraces of common oat (*Avena sativa* L.). Genet. Resour. Crop Evol. 60: 2157–2169.

Contreras-Negrete G., Ruíz-Durán M.E., Cabrera-Toledo D., Casas A., Vargas O., Parra F. 2015. Genetic diversity and structure of wild and managed populations of *Polaskia chende* (Cactaceae) in the Tehuaca´n-Cuicatla´n Valley, Central Mexico: insights from SSR and allozyme markers. Genet. Resour. Crop Evol. 62: 85–101.

Eivazi A.R., Naghavi M.R., Hajheidari M., Pirseyedi S.M., Ghaffari M.R., Mohammadi S.A., Majidi I., Salekdeh G.H., Mardi M. 2008. Assessing wheat (*Triticum aestivum* L.) genetic diversity using quality traits, amplified fragment length polymorphisms, simple sequence repeats and proteome analysis. Ann. Appl. Biol. 152: 81–91.

Enoki H., Sato H., Koinuma K. 2002. SSR analysis of genetic diversity among maize inbred lines adapted to cold regions of Japan. Theor. Appl. Genet. 104: 1270–1277.

Erayman M., İlhan E., Guzel Y., Eren A.H. 2014. Transferability of SSR markers from distantly related legumes to *Glycyrrhiza* species. Turk. J. Agric. For. 38: 32–38.

Excoffier L., Smouse P., Quattro J. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

Fu H., Dooner H.K. 2002. Intraspecific violation of genetic colinearity and its implications in maize. Proc. Natl. Acad. Sci. USA 99: 9573–9578.

Gupta P., Varshney R. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113: 163–185.

Gupta P.K., Balyan H.S., Sharma P.C., Ramesh B. 1996. Microsatellites in plants: a new class of molecular markers. Curr, Sci. 70: 45–54.

Hokanson S.C., Mcfadden A.K., Lamboy W.F., Mcferson J.R. 1998. Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus* × *domestica* Borkh. core subset collection. Theor. Appl. Genet. 97: 671–683.

Ipek M., Sahin N., Ipek A., Cansev A., Simon P.W. 2015. Development and validation of new SSR markers from expressed regions in the garlic genome. Sci. Agric. 72: 41–46.

Kozak M., Bocianowski J., Liersch A., Tartanus M., Bartkowiak-Broda I., Piotto F.A., Azevedo R.A. 2011. Genetic divergence is not the same as phenotypic divergence. Mol. Breed. 28: 277–280.

Krishna M.S.R., Reddy S.S., Chinna Babu Naik V. 2012. Assessment of genetic diversity in quality protein maize (QPM) lines using simple sequence repeat (SSR) markers. Afr. J. Biotechnol. 11: 16427–16433.

Kumar V., Kumar S., Chakrabarty S.A., Mohapatra T., Dadlani M. 2015. Molecular characterization of farmers' varieties of rice (*Oryza sativa*). Indian J. Agric. Sci. 85: 118–124.

Legesse B.W., Myburg A.A., Pixley K.V., Botha A.M. 2006. Genetic diversity of African maize inbred lines revealed by SSR markers. Hereditas 144: 10–17.

Li Y., Du J., Wang T., Shi Y., Song Y., Jia J. 2002. Genetic diversity and relationships among Chinese maize inbred lines revealed by SSR markers. Maydica 47: 93–101.

Manifesto M.M., Schlatter A.R., Hopp H.E., Suarez E.Y., Dubcovsky J. 2001. Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. Crop Sci. 41: 682–690.

Mansfield D.C., Brown A.F., Green D.K., Carothers A.D., Morris S.W., Evans H.J., Wright A.F. 1994. Automation of genetic-linkage analysis using fluorescent microsatellite markers. Genomics 24: 225–233.

Mazid M., Rafii M.Y., Hanafi M.M., Rahim H.A., Latif M.A. 2013. Genetic variation, heritability, divergence and biomass accumulation of rice genotypes resistant to bacterial blight revealed by quantitative traits and ISSR markers. Physiol. Plant. 149: 432–447.

Naghavi M.R., Monfared S.R., Humberto G. 2012. Genetic diversity in Iranian chickpea (*Cicer arietinum* L.) landraces as revealed by microsatellite markers. Czech J. Genet. Plant Breed. 48: 131–138.

Nei M., Li W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269–5273.

Parida S.K., Kalia S.K., Kaul S., Dalal V., Hemaprabha G., Selvi A., Pandit A., Singh A., Gaikwad K., Sharma T.R., Srivastava P.S., Singh N.K., Mohapatra T. 2009. Informative genomic microsatellite markers for efficient genotyping applications in sugarcane. Theor. Appl. Genet. 118: 327–338.

Peakall R., Smouse P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics 28: 2537–2539.

Pejic I., Ajmone-Marsan P., Morgante M., Kozumplick V., Castiglioni P., Taramino G., Motto M. 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs. Theor. Appl. Genet. 97: 1248–1255.

Powell W., Machray G.C., Provan J. 1996. Polymorphism revealed by simple sequence repeats. Trends Plant Sci. 1: 215–222.

Senior M.L., Murphy J.P., Goodman M.M., Stuber C.W. 1998. Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. Crop Sci. 38: 1088–1098.

Smith J.S.C., Chin E.C.L., Shu H., Smith O.S., Wall S.J., Senior M.L., Mitchell S.E., Kresovich S., Ziegle J. 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.) comparison with data from RFLPs and pedigree. Theor. Appl. Genet. 95: 163–173.

Somta P., Chankaew S., Rungnoi O., Srinives P., Scoles G. 2011. Genetic diversity of the Bambara groundnut (*Vigna subterranea* [L.] Verdc.) as assessed by SSR markers. Genome 54: 898–910.

Song L.Y., Liu X., Chen W.G., Hao Z.F., Bai L., Zhang D.G. 2013. Genetic relationships among Chinese maize OPVs based on SSR markers. J. Integr. Agric. 12: 1130–1137.

Srdić J., Nikolić A., Pajić Z., Mladenović Drinić S., Filipović M. 2011. Genetic similarity of sweet corn inbred lines in correlation with heterosis. Maydica 56: 251–256.

Stępień Ł., Mohler V., Bocianowski J., Koczyk G. 2007. Assessing genetics diversity of Polish wheat (*Triticum aestivum*) varieties using microsatellite markers. Genet. Resour. Crop Evol. 54: 1499–1506.

Wolko Ł., Antkowiak W., Lenartowicz E., Bocianowski J. 2010. Genetic diversity of European pear cultivars (*Pyrus communis* L.) and wild pear (*Pyrus pyraster* (L.) Burgsd.) inferred from microsatellite markers analysis. Genet. Resour. Crop Evol. 57: 801–806.

Wolko Ł., Bocianowski J., Antkowiak W., Słomski R. 2015. Genetic diversity and population structure of wild pear (*Pyrus pyraster* (L.) Burgsd.) in Poland. Open Life Sci. 10: 19–29.

K. Nowosad, J. Bocianowski, P. Szulc

ANALIZA ZMIENNOŚCI MOLEKULARNEJ I PODOBIEŃSTWO GENETYCZNE POMIĘDZY WYBRANYMI ODMIANAMI KUKURYDZY (*ZEA MAYS* L.) NA PODSTAWIE MARKERÓW SSR

Synopsis. Zmienność genetyczną 13 odmian kukurydzy analizowano w oparciu o 30 specyficznych starterów. W sumie 112 markerów SSR zostało wyznaczonych dla tych odmian. Odmiany analizowano ze względu na ich dojrzałość, typ ziarna, typ mieszańca oraz obecność lub brak efektu stay-green. Podobieństwo genetyczne pomiędzy odmianami wynosiło od 0,174 do 0,727, ze średnią wartością równą 0,473. W przeprowadzonym doświadczeniu zaobserwowano dużą zmienność badanych odmian kukurydzy. Wyniki AMOVA wskazują na istotne genetyczne zróżnicowanie pomiędzy rozważanymi grupami, wyjaśniającej co najmniej 89,4% całkowitej zmienności molekularnej pomiędzy odmianami w grupach.

Słowa kluczowe: AMOVA, podobieństwo genetyczne, kukurydza (*Zea mays* L.), markery mikrosatelitarne, analiza składowych głównych

Accepted for print – zaakceptowano do druku: 16.10.2017

For citation – Do cytowania:
Nowosad K., Bocianowski J., Szulc P. 2017. Analysis of molecular variance and genetic similarity between selected cultivars of maize (*Zea mays* L.) revealed by SSR markers. Fragm. Agron. 34(4): 134–144.