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MOLECULAR CHARACTERISATION OF SOYBEAN VARIETIES BY SSR MARKERS

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Soybean (*Glycine max* (L.) Merr.) is one of the most economically important legumes. As the source of plant protein and vegetable oil it is used as food and industrial crop in many regions of the world. The genetic base of soybean cultivars is highly narrow, corresponding to the fact that it is largely a self-pollinated species. Twelve soybean varieties were evaluated with SSR (*Simple Sequence Repeat*) markers selected based on their distribution on the 20 genetic linkage groups. Out of 36 SSR markers, 33 markers were found polymorphic among analyzed genotypes. Total number of alleles was 88, ranging between two and four with an average of 2.67 alleles per marker. The polymorphic information content (PIC) ranged from 0.153 (*Satt229, Satt239 and Satt327*) to 0.775 (*Satt276).* Simple matching similarity coefficient was calculated using NTSYSpc2 program package. The average genetic similarity coefficient for all pairwise was 0.57, with highest value (0.84) between *Galina* and *Lela*, while the lowest value (0.46) was found between *Bosa* and *Nena.* Dendrogram by the UPGMA (*Unweighted Pair Group Method with Arithmetic Mean)* method was constructed on the basis of genetic similarity matrix. Genotypes were distributed in two groups and one branch, mostly in accordance with their pedigree.
***Keywords*:** genetic similarity, SSR, *Glycine max*

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the most economically important legumes. As the source of plant protein and vegetable oil it is used as food and industrial crop in many regions of the world. The genetic base of soybean cultivars is highly narrow, corresponding to the fact that it is largely a self-pollinated species with limited out-crossing. Estimation of the genetic diversity of soybean germplasm is imperative to broaden the genetic base and improve breeding programs (NELSON, 2011). Further progress in soybean breeding requires more intensive utilization of existing genetic resources, and that is application of classical breeding and molecular technologies (VRATARIC and SUDARIC, 2008).

Estimation of genetic diversity can be assessed by the differences in morphological and agronomic traits, pedigree information, geographic origins and molecular markers. In the last two decades genetic diversity methods based on phenotypic traits, morphological and pedigree data were improved with the use of DNA markers, with a variety of different techniques (SPOONER *et al*., 2005). Restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) have been used in soybean's molecular characterisation, including both the advantages and disadventages (POWELL *et al*., 1996; THOMPSON *et al*., 1998; NARVEL *et al*., 2000; UDE *et al*., 2003; LI *et al*., 2010).

Simple sequence repeats are the most frequently and successfully used PCR markers in soybean diversity studies due to their high informativeness, high allelic diversity reproducibility, genetically codominant nature and simple application. SSR makers also important in identifying quantitative trait loci (QTL), which can be associated with certain phenotypic traits of individuals, allowing more efficient selection. SNP marker technology based on DNA sequencing can also be used for evaluating genetic diversity, but they have to be adjusted for divergence studies. (YANG *et al*., 2011).

Soybean cultivars grown in Serbia have mainly originated from closely related genotypes, existing in local germplasm collections which are adapted to similar environmental conditions (PERIĆ *et al*., 2014). The objective of this work was to study genetic diversity and relationships of soybean varieties belonging to different breeding programs using molecular (SSR) approache.

MATERIALS AND METHODS

Twelve soybean varieties (Table 1) were evaluated with SSR (Simple Sequence Repeat) markers and each variety was presented with 20 plants.

Table 1. List of 12 soybean varietes used for SSR characterization

Genotype	Pedigree
AFRODITA	S1346/Hodgson
ZPS 015	NBSG1 population (USA)
LAURA	Kunitz/Novka
VOJVOĐANKA	S1346/Hodgson
LANA	Kunitz/Kador
OLGA	OS101/ZPS208 (=Hobbit/Platte)
LIDIJA	(Sibley/A1937)/Kunitz(Sibley=(Evans/Steele)/Hodgson
NENA	OS101/Elf
SELENA	Afrodita/L9073 (Weber/Kador)
BOSA	Weber/Dawson
LELA	Sava/Zen (Sava =(Balkan/Vojvodjanka)x(Afrodita/Gema))
GALINA	Balkan/Ravnica (Balkan=(Evans/Four)/S1346; Ravnica=Hodgson/S1346)

A total genomic DNA was isolated from young leaves of each variety using *GeneJET™ Plant Genomic DNA Purification Mini Kit*. SSR characterization was done with 36, but only 33 were chosen for statistical analysis (Table 2). Polymerase chain reaction (PCR) was carried out in 20 µL reaction volume containing: 1 x Buffer (*Fermentas*), 0.8 mM dNTP, 0.5 µM of each primer pair, 1U Taq polymerase (*Fermentas*) and 1µL of DNA sample. The PCRs were performed on thermocycler *Biometra TProfessional Standard 96* using the following programme: an initial denaturation at 94ºC/5min., followed by 36 cycles each of denaturation at 94ºC/30s, annealing at temperatures ranging from 49ºC to 63ºC (Table 2) for 45 seconds and extension at 72ºC /1min. Final elongation was at 72°C for 10 min. Afterwards, PCR fragments were separated on 8% polyacrylamide gel in 0.5xTBE buffer using vertical electrophoresis system (*Mini Protean Tetra-Cell BioRad*) for 50 min, with 20 bp ladder (*Fermentas*) as a marker. Gels were photographed under UV light on *BDA live system Biometra* after staining with 0.5 µg/µL ethidium bromide.

Genetic similarities were calculated on binary data (presence or absence of alleles in each sample) using simple matching coefficient (SOKAL and MICHENER, 1958) in statistical NTSYSpc2 program package (ROHLF F.J., 2000). Cluster analysis was performed by UPGMA (*Unweighted Pair Group Method with Arithmetic Mean)* and relationships among varieties were visualized as dendrogram. Poymorphism information content (PIC) for each marker was calculated by the formula: $1-\sum(Pi^2)$, where Pi is the frequency of i-th allele in a locus (LYNCH and WALSH, 1998). PIC value provides information of the discriminating power of locus by taking into account the number of alleles that are expressed and their relative frequencies.

Table 2. List of 33 SSR primers, with their chromosome position, number of alleles, allele range and PIC

Probe	No. of alleles	Chromosome	Allele size (bp)	PIC
Satt 144	2	13	80-120	0.445
Satt 153	4	10	200-260	0.674
Satt 168	2	14	200-260	0.279
Satt 229	2	19	180-250	0.153
Satt 239	2	20	180-200	0.153
Satt 243	3	9	250-300	0.652
Satt 244	3	16	160-220	0.403
Satt 251	2	11	200-220	0.375
Satt 264	3	9	200-280	0.571
Satt 268	4	15	200-280	0.599
Satt 271	2	2	110-140	0.445
Satt 276	4	5	280-320	0.775

Satt 287	3	16	200-250	0.528
Satt 292	3	20	220-280	0.292
Satt 312	2	6	280-300	0.486
Satt 319	2	6	180-200	0.486
Satt 322	3	6	160-220	0.571
Satt 324	2	18	200-260	0.326
Satt 327	2	8	210-280	0.153
Satt 373	4	19	200-260	0.654
Satt 431	3	16	200-250	0.502
Satt 442	2	12	200-280	0.279
Satt 453	3	11	220-280	0.569
Satt 468	3	1	180-220	0.487
Satt 472	3	18	200-300	0.569
Satt 483	3	15	240-280	0.653
Satt 509	3	11	180-220	0.487
Satt 510	2	13	100-140	0.5
Satt 540	3	7	140-180	0.667
Satt 546	2	2	200-250	0.445
Satt 551	2	7	240-260	0.445
Satt 578	2	4	140-200	0.298
Satt 646	3	4	180-220	0.625
Mean	2.67	-	-	0.471

RESULTS AND DISCUSSION

Genetic relationships among soybean varieties could be relevant for future breeding achievement for yield, quality improvement and pest resistance. Integrated genetic evaluation of soybean varieties could facilitate introgression of diverse germplasm into the commercial soybean genetic base (TARA SATYAVATHI *et al*., 2006).

Genetic characterization of 12 soybean varietes was done using 36 SSR markers distributed across different chromosomes (Table 2). Three primers were monomorphic (identical for all analyzed genotypes) and were not included in further data analysis. Total of 88 alleles among the analysed soybean varietes were identified on polyacrylamide gels. The number of alleles varied from two (Satt 144, Satt 168, Satt 229, Satt 239, Satt 251, Satt 271, Satt 312, Satt 319, Satt 324, Satt 327, Satt 442, Satt 442, Satt 510, Satt 546, Satt 551 and Satt 578) to four (Satt 153, Satt 268, Satt 276 and Satt 373) with the average of 2.67 per locus. Similar mean value of alleles per marker (2.97) was obtained in the work of KUMAWAT *et al*. (2015), while a higer value (4.28) was found in the study of TANTASAWAT et al. (2011).

The PIC values ranged from 0.153 (Satt 229, Satt 239 and Satt 327) to 0.775 (Satt 276), with the mean value of 0.471. As the PIC values for 26 markers were > 0.3, it can be concluded that most of the markers were highly informative and could therefore

be considered suitable for molecular characterization of soybean genotypes. Diversity scores reported in other studies were in agreement with this result. In the study of SINGH *et al*. (2010) PIC value was 0.5 and slightly lower (0.48) in the work of KUMAWAT *et al*. (2015). On the other hand, PIC values higher than 0.6 were noted in other works (HUDCOVICOVÁ and KRAIC 2003; LI *et al*., 2010; TANTASAWAT *et al*., 2011). The pairwise genetic similarity (GS) values were relatively high and ranged from 0.46 (between Bosa and Nena) to 0.84 (between Lela and Galina).

The mean value of 0.57 is reflecting a slightly higher degree of genetic diversity among soybean cultivars, compared with previous results (0.65) in work of PERIC *et al*. (2014). The average GS observed in this study was lower compared to those described in similar study with 25 soybean genotypes in Thailand (TANTASAWAT *et al*., 2011). On the other hand, PRIOLI *et al*. (2010) reporeted considerable diversity (GS=0.26) among 168 Brazilian soybean cultivars.

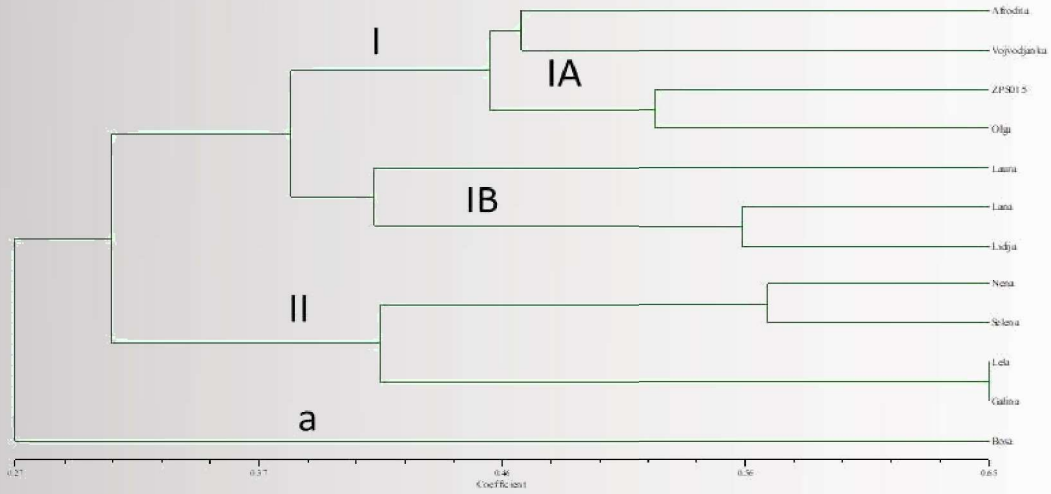

Figure 1. Dendrogram of 12 soybean varieties constructed using UPGMA cluster analysis of simple matching similarity coefficient obtained by SSR data.

The result of cluster analysis based on simple matching genetic similarities was presented in the form of dendrogram (Figure 1). Information about pedigree data of soybean cultivars was crucial for the clarification of the cluster analysis based on SSR data. Two major clusters (I and II) were formed, as well as one attached genotype - branch a. Most of the genotypes (seven) formed cluster I and four formed cluster II. Cluster I was divided into two subclusters. Subcluster IA included genotypes Afrodita and Vojvodjanka, orginating from the same cross, as well as genotypes ZPS015 and Olga, orginating from USA gemplasm. Varietes Laura, Lana and Lidija were grouped together in subcluster IB, originating directly from cultivar Kunitz; therefore, subcluster IB presents a clear example of close positioning of genotypes with the same origin in the

same subcluster. The most heterogeneous was cluster II which included genotypes with different genetic backgrounds, but also some of lines (Selena, Lela and Galina) share a common genetic basis (Hodgson and S1346). Variety Bosa did not group with any of the analyzed genotypes and formed branch *a*. The cluster analysis showed accordance between grouping of genotypes and their pedigree data. Similar results were find in other papers (PERIC *et al*., 2014; BISEN *et al*., 2015; KUJANE *et al*., 2019), comfirming that selection of elite breeding material leading to uniformity.

CONCLUSIONS

The SSR markers used in this work could successfully be applied for genetic diversity researches in soybean cultivars, showing satisfying polymorphism (PIC=0.471). It was found that there was a certain level of agreement between grouping of individual genotypes based on pedigree, but mostly at single pairs and small groups. However, there were also rare cases of individual genotypes founding themselves at greater distances, while according to pedigree data they should share a similar genetic basis.

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