MOLECULAR DIVERSITY OF AUTUMN GARLIC GENOTYPES USING SSR MARKERS

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Garlic (*Allium sativum* L.) is one of the most important *Allium* species in terms of worldwide production and various usage in human nutrition, medicine, pharmacy and cosmetics. The characterization and preservation of samples in germplasm collections is of crucial importance in plant breeding, as well as availability of information about number and characteristics of samples in gene banks. Since phenotypic traits can vary significantly under the influence of environmental factors, the characterization is more reliable by using DNA markers. The garlic collection of the Institute of Field and Vegetable Crops in Novi Sad (IFVCNS) includes 63 samples of autumn and 67 samples of spring garlic accessions. These genotypes represent a valuable genetic pool for the selection of clones with appropriate characteristics, highly adapted for the production in the agroclimatic conditions of Serbia. Molecular characterization will provide more complete insight into diversity of samples, identification of potential duplicates and enable breeders more efficient selection and development of new cultivars.

Material and Methods

From the IFVCNS garlic collection 52 autumn samples originating from 11 countries, were selected for analysis. Majority of the samples belong to the non-flowering type (*vulgare*), while 12 samples are flowering type (*sagitatum*). The experiment was performed during two years (2017/18-2018/19), on the experimental field on Rimski Šančevi site (45°20' N, 19°51' E, 84 m). DNA extraction from young leaves was performed according to the Somma (2004) protocol. For molecular evaluation 30 SSRs markers were selected, while 10 SSRs were determined to be polymorphic. Separation of amplified PCR products was performed on metaphor agarose gel (3% and 3,5%) by horizontal electrophoresis. The visualization of the product was done under UV light on a Wilber Bio-Print device. All data analyses were performed within the R software environment, version 4.0.5 (R Core Team, 2020).

Results

Several genetic diversity parameters for each SSR locus were estimated (Table 1). A total of 36 alleles were revealed by 10 polymorphic SSR loci. The most informative markers were As 5944 and As 11065. The obtained results enabled the differentiation of majority of genotypes while some of them showed to be potential duplicates. Based on 10 polymorphic loci, 52 garlic genotypes were classified by Principal Coordinate Analysis (PCoA) into 4 groups (Figure 1).

Table 1. Basic indicators of genetic diversity for 10 polymorphic SSR markers

Locus	SSR motif	Primer sequence 5'- 3'	Ta (°C)	Na	Ho	He	PIC
As2655	(AGAAA)5	f:AACTCAATGCATGACAGAAGG r:AGGAGGAGGAGAATGCTGAA	57	4	0.357	0.077	0.331
As 987	(AAT)6	f:GTACCAACTCTTTCCTAACGC r:TCCAATAGTTGTGATGACAGG	57	4	0.195	0.096	0.188
As 614	(AAAT)5	f:AATTCAATGCGCTTCACAGC r:AGCAGGTGCAATCAAACTGG	59	4	0.093	0.019	0.092
As 623	(GCT)6	f:CACAAATTAAAACCCCAATCAAG r:AATGAATCAACATCAAGCGTA	56	2	0.488	0.000	0.369
As 11065	(GA)12	f:AACAGTCGAAAGCGTGGATTG r:TACGGCTTGCTACCAAAGAC	57	5	0.526	0.615	0.485
Asm 78	(GT)12	f:TGTTCCAACCAGATTTAATGC r:AAGTGGCGGTTGTGTCTG	60	3	0.075	0.077	0.073
As 392	(AC)10	f:TTTCAACAGCATCAGTTTGTAGA r:CCTTCACCATCAACCTACATTG	57	3	0.341	0.000	0.295
As 5453	(CAG)11	f:CAGGATGAGGCAAAGGTTTCA r:ACATTTTGGTGTTGCTGTTGG	57	2	0.142	0.000	0.132
ASM 35	(GCC)3, (TCC)3	f:TTGGACTGAATTCTGAATACCT r:GGGTGTGTGGTTCAAGGA	60	2	0.334	0.000	0.278
As 5944	(AC)28	f:AGAGGGTTTTTCGATCTGGA r:AGTGGCATCAAAGCAAGATG	57	7	0.654	0.820	0.610
Mean				3.6	0.321	0.170	0.285

Ta: primer annealing temperature used in PCR amplifications; Na: number of alleles per locus; Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphic information content

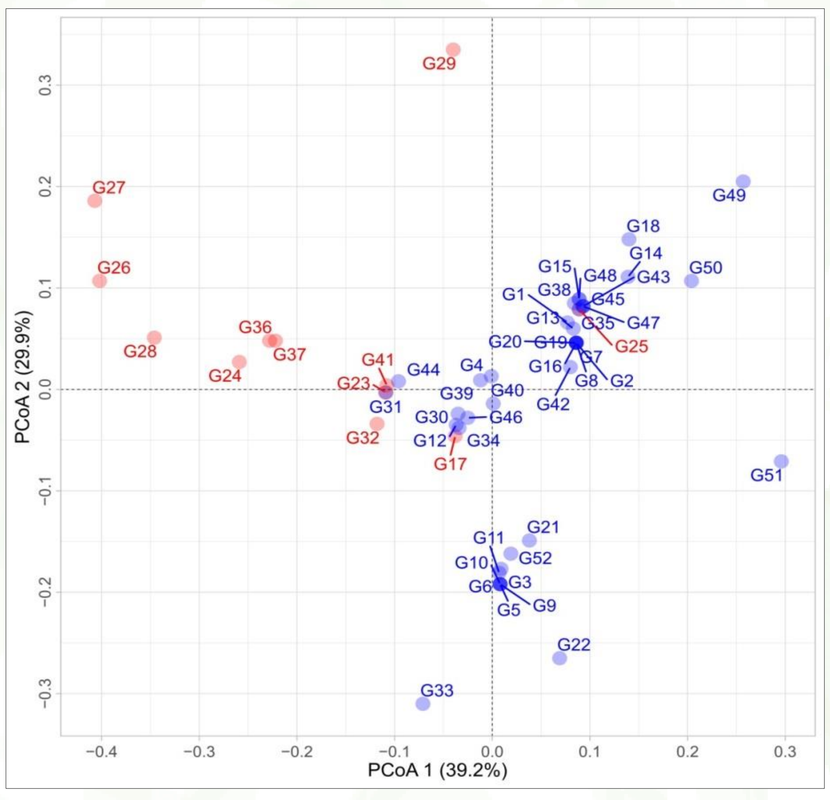

Figure 1. Grouping of genotypes based on molecular data using PCoA
Vulgare genotypes; *Sagitatum* genotypes

The obtained results enabled insight into molecular diversity of collection and easier identification of potential genotypes for selection. Since this is the first research using DNA markers of the IFVCNS garlic collection, and considering the size and complexity of its genome, the obtained results can be considered preliminary. Although the presence of duplicates in the collection was revealed based on 10 SSR loci these results represent guidelines for further research using more DNA markers. Additional testing is needed using a higher number of molecular markers in order to determine, with greater certainty, whether those genotypes are duplicates. In that way, the premature elimination of some accessions that may possess valuable genes for selection of new genotypes and for the conservation of genetic resources would be prevented.

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Somma, M. (2004): Extraction and purification of DNA. In M. Querci, M. Jermini, G. Van den Eede (ed.), The analysis of food samples for the presence of genetically modified organisms (Special Publication No. I.03.114) (Session 4, 13–17). European Commission DG-JRC