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Acknowledgements

The current issue of the Cruciferae Newsletter (vol. 37) is published online from the Brassica website (http://www.brassica.info/info/publications/cruciferae-newsletter.php). The present issue contains 6 contributions in three different topics: Agronomy and variety trial; Breeding strategies and General information on Brassica. Members of the editing board would like to acknowledge the authors for the quality of their contributions. For future issues, we would be gratefull if all the authors could read and follow carefully the author recommendations before submitting their manuscript, in order to facilitate the editing process. In particular, it is necessary to mention one of the listed topics that is the most relevant to the presented work (see the list at the end of the present issue).

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Evaluation of winter hardiness in some crucifer crops by microsatellite (SSR) markers

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Introduction

In Serbia and other countries of the European Southeast, the autumn-sown grain and forage crucifer crops are traditionally dominant over those that are sown in spring (Marjanović Jeromela et al. 2017). Following the same trend on a global scale, rapeseed (*Brassica napus* L.) is the most important, with about 13,500 ha in 2016 (FAOSTAT 2017) and generally increasing over the years (Marinković et al. 2004). At the same time, fodder kale (*Brassica oleracea* L. var. *viridis* L.) is highly appreciated as the first fresh forage in the spring, with a beneficial effects in the nutrition of milk cows (Mikić et al. 2014). Due to its typical continental climate, with moderately cold winters and often unexpected dry springs, the breeding programme on grain and forage crucifers in the Institute of Field and Vegetable Crops (IFVCNS) in Novi Sad prefers developing the autumn-sown cultivars to the spring-sown ones, especially since the yields of both oil-rich grain and protein-abundant forage is significantly higher in the former (Marjanović Jeromela et al. 2012).

Apart from yield and its quality, one of the most significant goals in breeding autumn-sown crucifers is enhancing winter hardiness, that is, the overall tolerance to the duration and the intensity of low temperatures, which is shared with the research on autumn-sown annual legumes (Mikić et al. 2011). The screening methods allowing accurate and precise assessment of winter survival are critical for winter crop research programs. The most commonly used method is carried out by determining the ratio between the plant number before and after the winter, that is, the so-called winter survival percentage. The inherent difficulties in field trials constantly stimulate defining the improved tests complementing the screening in field conditions in contrasting environments (Rife 1996, Kole et al. 2002, Sun et al. 2007, Waalen et al. 2013).

The genetic variability of current rapeseed breeding material is narrow due to its limited geographic range and intensive breeding for specific oil and seed quality traits (Hasan et al. 2006). Many studies have demonstrated the suitability of molecular marker techniques for evaluation of genetic variation in rapeseed. Some of the breaking-through approaches to investigate the genetic distance in this crop were investigated by random amplification of polymorphic DNA (RAPD, Mailer et al. 1994), restriction fragment length polymorphism (RFLP, Diers et al. 1994) and sequence-related amplified polymorphisms (SRAP) (Riaz et al. 2001). Cluster analysis

using microsatellite or simple sequence repeat (SSR) markers covering the whole rapeseed genome proves as quite suitable and precise to clearly differentiate winter and spring rapeseed from each other (Plieske & Struss 2001).

The goal of our study was to identify the heterotic groups in three crucifer crops for winter survival using SSR molecular markers considered close to quantitative trait loci (QTLs) relatd to this important agronomic characteristic.

Material and methods

The material for this pioneering investigation in our conditions was sampled from the 29 genotypes of three crucifer species, grown in the field conditions at the IFVCNS Experimental Field at Rimski Šančevi, in the vicinity of Novi Sad (Table 1). The genomic DNA was isolated from frozen leaves of each according to the procedure of Permingeat et al. (1998).

Table 1. The three crucifer crop genotypes used for a SSR analysis for winter hardiness and flowering time in the field conditions of Rimski Šančevi

Species/crop	Sowing season	Genotype	Winter survival (%)
Fodder kale	autumn	NS-Bikovo, K-357	
Rapeseed	autumn	JP 26, 57, 81, 149, 152, 232, 238, 303, 352, 373, 410, 412, 446, 449, 468	70-100
		JP 63, 298, 343, 357, 360	50-60
	spring	Galant, Global, Jasna, Pamnik, Ratnik	
Turnip	autumn	B. RAPA	

The investigation of genomic DNA polymorphism was done with SSR markers, positioned in QTL regions for winter survival (Kole et al. 2002), freezing tolerance and flowering time. Three out of nine used primers were unspecific, as shown by smear or superfluous bands, with remaining six primers giving 21 polymorphic fragments. The polymorphic markers were scored as dominant and used to calculate genetic distance between each pair of examined populations. The genetic distance was calculated according to the Jaccard index of genetic similarity (Staub et al. 2000). The pairwise distance matrix of genetic distances for cluster analysis was used by Unweighted Pair Group Method using Arithmetic averages (UPGMA; Statistica for Windows, v.5.0, StatSoft, USA).

Results

The number of polymorphic fragments per primer varied from two, in SSR OI10 and OI13, to six, in SSR NaRa2 E07, while its length ranged from 100 to 1000 bp, in SSR OI10 (Table 2). Overall, 21 polymorphic fragments were generated and were screened for presence or absence in each pair of examined populations. The genetic distances among examined populations ranged from 0 to 88% (data not shown).

The genetic distances, analysed by UPMGA and presented in the form of a dendrogram, allowed the evaluation of probable relationships among the examined genotypes (Fig. 1). The accompanying cluster analysis revealed two main clusters, A and B, with a genetic distance of nearly 80%. The genotypes of fodder kale and turnip were placed in the cluster A. The cluster B branched in two subclusters at genetic distance of about 45%. One subcluster consisted of the spring rapeseed cultivars, while another comprised nearly all the autumn-sown rapeseed genotypes. Interestingly, two winter genotypes, namely OZ_GP357 and OZ_GP360,

clustered with the spring ones. Though the genetic distance within the spring subcluster was low (≤ 22%), all the examined spring genotypes were differentiated. Fodder kale clustered with the autumn-sown rapeseed at a genetic distance of 35%. The genetic distance within the autumn-sown rapeseed subcluster was low (< 20%) and some of them could not be differentiated with the set of the used primers covering QTLs for winter survival.

Table 2. The primer name and sequence, linkage groups (LG), map position and number and length of polymorphic fragments used for a SSR analysis for winter hardiness and flowering time in three crucifer crops in the field conditions of Rimski Šančevi

Primer name	Primer sequence in 5'-3' direction	LG	Map position (cM)	Number of polymorphic fragments	Length of polymorphic fragments (bp)
SSR Ol10	TGCAACAAGGAGACGATGAG TTTGAAATCCGGGACGTAGT	N2	90.6	2	100-1000
SSR Ol11	ATGAAAACCAATCCAGTGCC GATAGCAGATGGAAGAGCCG	N19	2.9	5	150-200
		N10	0		
SSR Ol13	TTCGCAACTCCTCCTAGAATC AAGGTCTCACCACCGGAGTC	N2	68	2	150-250
SSR Ni2	TGCAACGAAAAAGGATCAGC TGCTAATTGAGCAATAGTGATTCC	N10	46.6	2	150-200
		N11	0		
SSR Bn Ol10	AATTGGCTTGGTAGCTGTCG ATAGGAATGGGATGCACAGG	N2	91	4	300-800
SSR Ra2 EO7	ATTGCTGAGATTGGCTCAGG CCTACACTTGCGATCTTCACC	N10	46.6	6	100-200
		N19	34,6		

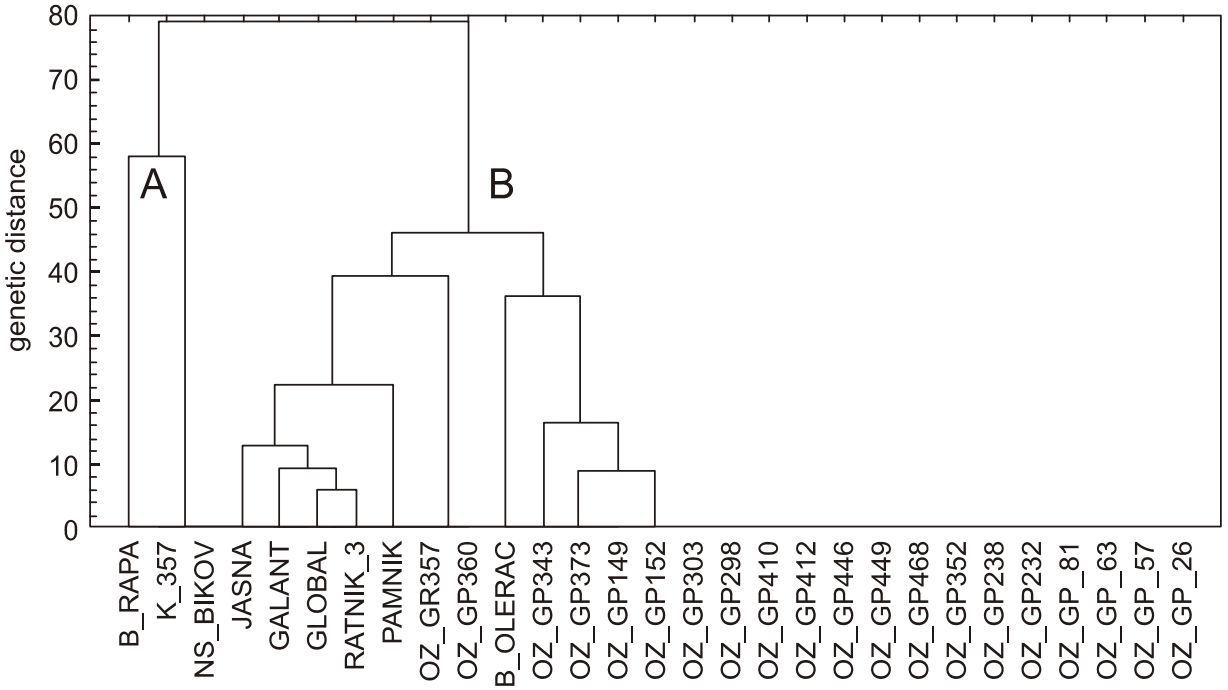

Figure 1. Cluster analysis of 29 genotypes of three crucifer crops using 21 polymorphic SSR fragments; the genetic distances between examined populations were calculated via Jaccard similarity coefficient.

Discussion

In one of the previous researches (Marinković et al. 2004),there have been clustered 402 lines, in S6 generation after an initial gene pool cross, in five clusters according to their winter survival. The clusters I-IV and the cluster V contained lines with the winter survival 70% - 100 % and 50% - 60 %, respectively. In our experiment, all the spring rapeseed cultivars clustered together, with addition of two autumn-sown rapeseed genotypes. These two, OZ_GP357 and OZ_GP360, had a value of winter survival of 50% - 60%. Since most of remaining genotypes had a winter survival values from 70% to 100%, this result is in correlation with the results of winter survival field test.

The differentiation between the winter and spring rapeseed genotypes was often revealed (Lombard et al, 2000, Plieske & Struss 2001, Bond et al., 2004). Plieske & Struss (2001) used 81 microsatellite markers spread over the whole genome, to separate 32 varietal rapeseed populations into winter and spring types. We have achieved such clear differentiation with significantly lower number of SSR markers, which indicates their suitability to supplement the winter survival field test data. The genetic distance between the winter and spring populations was about 45%, as found previously in a similar analysis with different genetic material (Plieske & Struss, 2001).

The fact that several autumn-sown rapeseed genotypes could not be differentiated can be explained by their recent common origin. In other words, they were in the S6 generation after an initial gene pool crossing, resulting in the fact that the genetic distance between them was even lower than the one in cultivated rapeseed (Seyis et al. 2003). Numerous cluster analysis show that cultivars bred by the same institute have the highest level of genetic similarity (Xu et al. 2008).

Both fodder kale and turnip were the most genetically diverse genotypes, with a genetic distance of almost 80%.

The use of novel genetic diversity for maximization of heterosis in hybrid may improve the heterotic potential of the rapeseed cultivars, but it could also lead to suffering from serious linkage drag for grain yield and quality traits (Basunanda et al. 2007). Therefore, as identified in our study, the use of diverse spring, such as PAMNIK, and winter genotypes, like OZ_GP357 or OZ_GP360, could be quite beneficial for increasing the heterotic potential in the future spring and autumn-sown rapeseed programmes.

In conclusion, the first steps in using SSR markers for developing new germplasm of rapeseed and other crucifer crops in the Southeast Europe may be rather encouraged to be carried on not solely in breeding, but also in various agronomic and phytopathological researches, such as nitrogen use efficiency and yield stability (Bouchet et al. 2016) and resistance to prevailing diseases, such as blackleg, caused by fungus *Leptosphaeria maculans* (Sowerby) P.Karst. (Fredua-Agyeman et al. 2014), in our region as well.

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Dedication

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