Genetic variability between several Brasicaceae populations of different winter survival ability

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Abstract

Winter survival is an important characteristic for winter rapeseed. It is known that other cold-regulated traits, such as freezing tolerance and flowering time, can modify the ability of winter survival, they all lay in the same QTL regions. Twenty varietal populations of winter rapeseed, with mean winter survival from 50 to 100%, were chosen for the investigation. In order to increase genetic variability for winter survival, 5 spring rapeseed, three *B. oleracea* (winter forage kale), one *B. rapa* populations were also investigated.

The investigation of genomic DNA polymorphism was done with 6 SSR markers, positioned in QTL regions for winter survival, freezing tolerance and flowering time.

The aim of our work was to check the suitability of selected SSR markers for screening of winter survival ability and to identify genetic variability of this genomic region in NS rapeseed breeding material.

Key words: SSR markers, DNA polymorphism, cluster analysis, *B. napus*, *B. oleracea*, *B. rapa*, rapeseed

Introduction

Rapeseed is an important oil crop worldwide. In Serbia growth areas are increasing as well (Marinković et al., 2004). Due to cold winters and severe climate in Serbia both spring and winter types are grown, though winter type is preferred due to its significantly higher yield (Marinković et al., 2004). Sometimes the plants are killed by low temperatures, therefore creating the lines of winter rapeseed with good winter survival is important goal for the breeders.

Screening methods that allow accurate and precise assessment of winter survival are critical for winter crop research programs. Field survival, measured as percentage of plants surviving the winter, is the most commonly used method. The inherent difficulties with field trials have stimulated interest in the development of tests which would complement field screening (Rife, 1996; Kole et al. 2002).

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. Genetic distance in oilseed rape was investigated by RFLPs (Diers et al. 1994) and sequence-related amplified polymorphisms (SRAP) (Riaz et al. 2001). RAPDs have also been used for discrimination among rapeseed cultivars (Mailer et al. 1994). Plieske and Struss (2001) were able to clearly differentiate winter and spring rapeseed in a cluster analysis using simple sequence repeat (SSR) markers covering whole genome.

The goal of our study is the identification of rapeseed heterotic groups for winter survival in our breeding material, using SSR molecular markers close to QTLs for winter survival.

Material and Methods

Twenty-nine populations of rapeseed, used for this investigation, were grown in field at Rimski Šančevi. Twenty varietal populations of winter rapeseed were chosen from NS gene pool: 70-100 % winter survival (JP 26, 57, 81, 149, 152, 232, 238, 303, 352, 373, 410, 412, 446, 449, 468) and 50-60 % winter survival (JP 63, 298, 343, 357, 360). Five spring rapeseed (Pamnik, Ratnik, Jasna, Global and Galant), three *B. oleracea* (NS-Bikovo, K-357, *Brassica oleracea* var. *acephala*) and one genotype from *B. rapa* collection were analysed.

Genomic DNA was isolated from frozen leaves of examined populations according to the procedure of Permingeat et al. (1998). The investigation of genomic DNA polymorphism was done with SSR markers, positioned in QTL regions for winter survival (Kole 2002), freezing tolerance and flowering time. Three out of nine used primers were unspecific, as shown by smear or superfluous bands. Six primers gave 21 polymorphic fragments (Table 1). Polymorphic markers was scored as dominant, and used to calculate genetic distance between each pair of examined populations. Genetic distance is calculated according to Jaccard coefficient of genetic similarity (Staub et al. 2000). The pairwise distance matrix of genetic distances was used for cluster analysis by Unweighted Pair Group Method using Aritmetic averages (UPGMA; Statistica for Windows, v.5.0, StatSoft, USA).

Results

The number of polymorphic fragments per primer varied from two (SSR Ol10 and Ol13) to six (SSR NaRa2 E07), and ranged from 100 to 1000 bp in length (Table 1). Overall, 21 polymorphic fragments were generated and were screened for

presence/absence in each pair of examined populations. Genetic distances among examined populations ranged from 0 to 88% (not presented).

Table 1. **Primer sequence, Linkage Group, map position, number and length of polymorphic fragments**

Primer name	Sequence in 5′-3′ direction	LG	Map position in cM	Number of polymorphic fragments	Length of polymorphic fragments
SSR Ol10	TGCAACAAGGAGACGATGAG TTTGAAATCCGGGACGTAGT	N2	90.6	2	100-1000
SSR Ol11	ATGAAAACCAATCCAGTGCC GATAGCAGATGGAAGAGCCG	N19 N10	2.9 0	5	150-200
SSR Ol13	TTCGCAACTCCTCCTAGAATC AAGGTCTCACCACCGGAGTC	N2	68	2	150-250
SSR Ni2	TGCAACGAAAAAGGATCAGC TGCTAATTGAGCAATAGTGATTCC	N10 N11	46.6 0	2	150-200
SSR Bn Ol10	AATTGGCTTGGTAGCTGTCG ATAGGAATGGGATGCACAGG	N2	91	4	300-800
SSR Ra2 EO7	ATTGCTGAGATTGGCTCAGG CCTACACTTGCGATCTTCACC	N10 N19	46.6 34.6	6	100-200

The genetic distances were analysed by UPMGA and presented in a dendrogram, which allows the evaluation of probable relationships among the examined populations (Fig. 1). Cluster analysis revealed two main clusters, marked A and B, with genetic distance of nearly 80%. Populations of *B. rapa* and *B. oleracea* were in cluster A. Cluster B branched further in two subclusters at genetic distance of about 45%. One subcluster consisted of spring and the other of winter rapeseed types. Interestingly, two winter types, OZ_GP357 and OZ_GP360, clustered with spring types. Though genetic distances between spring types was low (≤ 22%), all examined spring types were differentiated. *B. oleracea* v. *acephala* clustered with winter types of rapeseed at genetic distance of 35%. The genetic distance between winter types was low (< 20%) and several winter type populations could not be differentiated with the set of primers covering QTLs for winter survival.

Discussion

Marinković et al. (2004) have clustered 402 lines, in S6 generation after an initial gene pool cross, in five clusters according to their winter survival. Clusters I-IV and cluster V contained lines with winter survival 70-100 % and 50-60 %, respectively. In our experiment all spring rapeseed populations clustered with two populations of winter rapeseed in one common cluster. These two populations (OZ_GP357 and OZ_GP360) had winter survival 50-60%. As most other populations have winter survival form 70 to 100%, this result is in correlation with the results of winter survival field test. Differentiation between the winter and spring type is has often been revealed (Lombard et al., 2000; Plieske and Struss, 2001; Bond et al., 2004). Plieske and Struss (2001) used 81 microsatellite markers spread over the whole genome, to separate 32 varietal populations of *B. napus* to winter and spring types. We also got this clear differentiation between winter and spring rapeseed with only 21 SSR markers, which indicates their suitability to supplement to winter survival field test data. Genetic distance between winter and spring populations was about 45%, as found previously (Plieske and Struss, 2001).

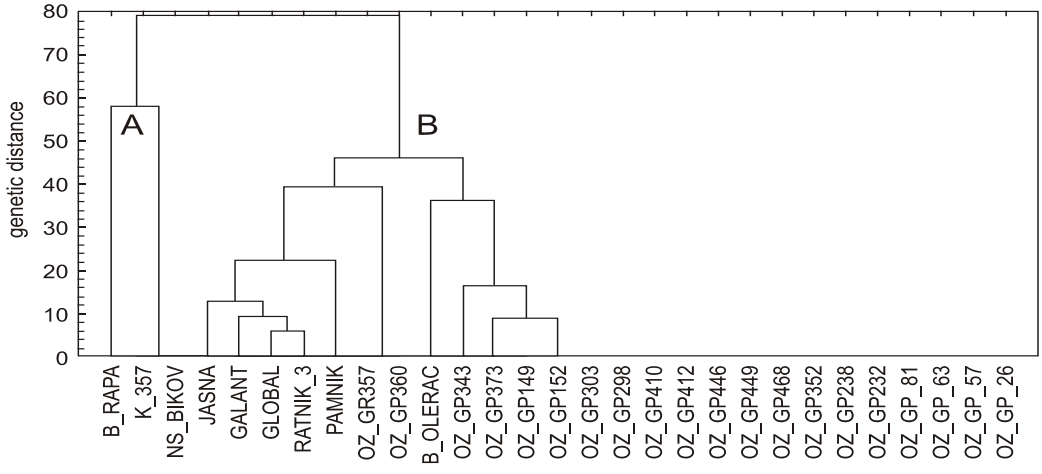

Figure 1. Cluster analysis of 29 *Brasicaceae* populations using 21 polymorphic SSR fragments. Genetic distances between examined populations were calculated via Jaccard coefficient of similarity.

The fact that several winter rapeseed populations could not be differentiated can be explained by their recent common origin. Namely examined lines were in S6 generation after an initial gene pool cross, therefore the genetic distance between them is even lower than the one of cultivated *B. napus* (Seyis et al. 2003).

B. rapa and *B. oleracea* were the most genetically diverse genotypes, with genetic distance of almost 80%. As suggested

by Hasan et al. (2006) the use of such exotic germplasm to improve the heterotic potential of oilseed types could suffer from serious linkage drag for seed yield and quality characters. Therefore the use of diverse spring (PAMNIK) and winter types (OZ_GP357; OZ_GP360), identified in our study, would be more suitable for increasing heterotic potential of spring and winter oilseed rape in our program, respectively.

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