Genetic divergence of rapeseed (*Brassica napus* L.) seed yield components

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Abstract

Four morphological (plant height, height to the first branch, branch number, leaf number) and molecular (RAPD) markers were analyzed in order to determine genetic distance between 30 winter rapeseed genotypes. Nine out of the 13 RAPD primers used enabled estimation of DNA polymorphism, while the other primers were monomorphic. Dendrograms were obtained on the basis of the cluster analysis and different methodological procedures for determination of genetic distance were compared. Dendrograms for certain quantitative traits as well as dendrogram obtained with RAPD markers showed partial grouping of genotypes based on their geographical origin. In order to facilitate the choice of genotypes with high values of desired traits or trait combinations, graphical presentation of cluster analysis was analyzed.

Key words: Oilseed rape, *Brassica napus*, genetic divergence, seed yield components

Introduction

Rapeseed (*Brassica napus* L.) is the major oil crop in some thirty countries, becouse large variety of forms and cultivars allow it to be grown under practically all climatic conditions. The plant is grown for various uses, but most frequently for seeds that are further used for oil extraction. The seed contains 40-48% of oil and 18-25% of proteins.

The main objective of rapeseed breeding is the development of cultivars and hybrids possessing high and stable genetic potential for seed yield, high oil content in seed and a low content of glucosinolates. Seed yield is the result of the values and mutual relationships among yield components, which are controlled by a number of genes, whose expression is strongly affected by environmental conditions.

Use of molecular marker techniques should facilitate rapeseed breeding. In the first instance, these techniques should allow rapid detection of genetic divergence. Knowledge of genetic divergence between genotypes in a breeding program facilitates the selection of germplasm sources which are then crossed to make new material for further selection of hybrid combinations capable of providing maximum heterotic effects (Smith et al., 1990). Different molecular methods have been used extensively for determination of genetic distances within the genus *Brassica* (Anthony, 2001; Degani, 2001; Punitha and Raveendran, 2004).

For work with a collection of genotypes, it is necessary to have prior knowledge of their most important morhological traits. A frequently used method for classification of genotypes according to mutual similarities and differences is the cluster analysis. It estimates the differences among the analyzed grous on the basis of Euclidean distances (Veronessi and Falconelli, 1988), providing equal treatment to all populations under study.

The objectives of this study were to determine genetic distances among the tested rapeseed genotypes based on their morphological traits and RAPD analysis and to rank the methodological procedures for determination of genetic distance according to their efficiency.

Material and methods

The thirty rapeseed genotypes tested in this study differed in geographic origin and properties: Sremica, Banaćanka, UM-1 , UM-2, UM-5, UM-6, UM-8, UM-9, UM-10, UM-11, UM-12, UM-13, UM-14 (Institute of Field and Vegetable Crops, Novi Sad, Serbia); Falcon, K-571, K-1550, Alaska, Aligator, H-450, Valeska, Orkan, Pronto, Artus (Germany); Samuray, Jet Neuf, B-009, Duna (France), Oktavia, Jana (Hungary), Casino (Sweden).

Three-year experiments were conducted in a random blocks design with three replications, at Rimski Šančevi Experimental Field of Institute of Field and Vegetable Crops.

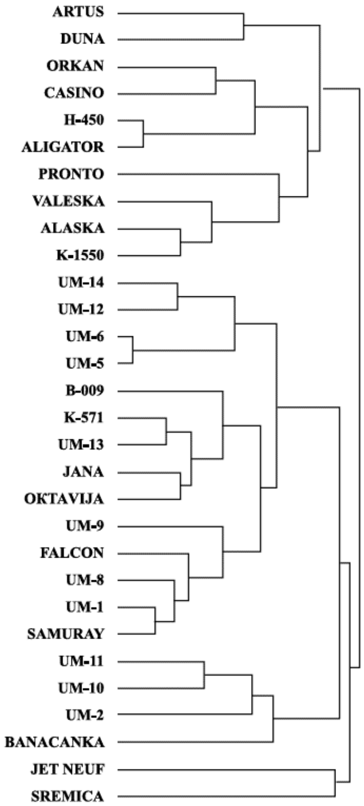
The following traits were analyzed: plant height (cm), height to the first branch (cm), number of lateral branches and leaf number. Measurements and counts were done on samples comprised of 33 plants per genotype.

DNA polymorphism was assessed by the RAPD analysis (Welsh and McClelland, 1990; Williams et al., 1990). DNA polymorphism was estimated on the basis of 13 RAPD primers which were already prooved to be polimorphic in rape seed (Panković et al., 2000). Genetic distance was calculated via simple matching coefficients (Stub et al., 2000).

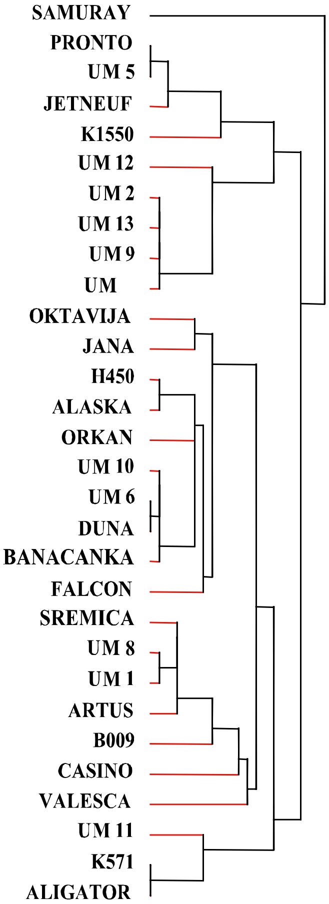
Genotype groups divided by the cluster analysis, according to UPGMA method. This is an additive method, which forms a matrix of Euclidean distances among the mean values of the groups (genotypes) for the construction of a dendrogram (Fox and Rosielle, 1982). In that way, individuals are grouped to the point when all of them belong to a single cluster (Romesburg, 1984).

Results and discussion

DNA POLYMORPHISM. Of the 13 RAPD primers used, nine primers enabled the estimation of DNA polymorphism in the 30 winter rapeseed genotypes, while the other primers were monomorphic. Only clear and reproducible bands served for the analysis of DNA polymorphisms. Of a total of 54 RAPD fragments multiplied, 23 were polymorphic. A dendrogram (Graph 1) showing mutual relationships among the tested rapeseed genotypes was based on the genetic distances obtained by the UPGMA cluster analysis. The dendrogram shows that the genotypes divided into two main groups, designated A and B. Groups A and B were linked at the genetic distance of 67%. Group A comprised 10 genotypes, selected in European breeding centers. The other 20 genotypes were in group B, which was further subdivided into two subgroups and two individual genotypes. All Novi Sad selections were in group B, together with European selections which had served as parent components in development of our genotypes. Using RAPD markers, Yuan et al. (2004) found that rapeseed genotypes grouping depended not only on geogrpahic origin but also on breeding method used for their development.



Graph 1. Dendrogram of relationships among the rapeseed genotypes obtained by the UPGMA analysis of polymorphic RAPD fragments

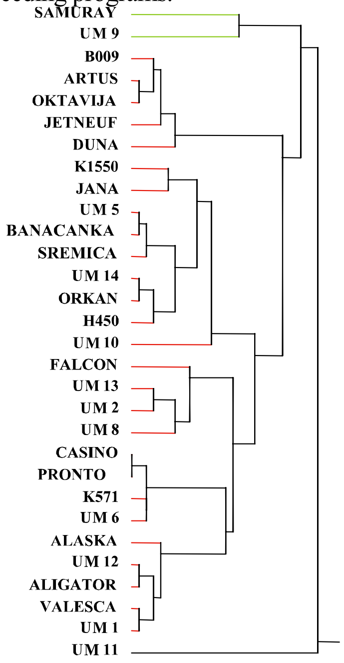


Graph 2. Dendrogram of phenotypic differences among the rapeseed genotypes regarding plant height

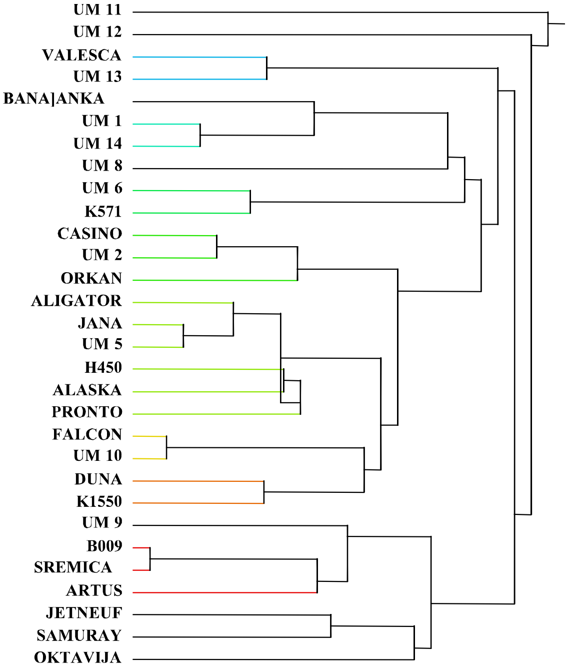
CLUSTER ANALYSIS OF QUANTITATIVE TRAITS. Three-year values of individual quantitative traits were used for cluster analysis. Each dendrogram indicated genetic divergence or relatedness among the genotypes. The dendrograms differed in the number and size of groups and subgroups. According to different level of similarity genotypes were conected on the different hierarhycal levels.

The dendrogram for plant height was divided into four groups with different number of genotypes. The grouping made it easier to see the relatedness among the genotypes. The cultivar Samuray differed from all other groups (Graph 2). The dendrogram for the height of the first lateral branch was divided into four groups. The detached group A contained only two genotypes, Samuray and UM-9 (Graph 3). Due to the positive correlation between both of these traits, differentiation of genotypes in their clusters had very high similarity (Marjanović-Jeromela, 1999). Both traits tended to segregate genotypes in dependence of their geographic origin. Regarding plant height, the genotypes from Germany and Serbia were concentrated in the first and second group. Regarding the height of the first lateral branch, the genotypes from Germany and Serbia were concentrated in the second and third group.The dendrogram for the number of lateral branches, gived a persuasive illustration of the variability of this trait. Of the three groups and one individual genotype, groups one and two included a majority of the genotypes, thirteen and twelve, respectively. It should be noted that the second group, in addition to the genotypes developed at the Novi Sad Institute, included the cultivars Samuray and Falcon which had been used as parent components in the Novi Sad breeding program (Graph 4).

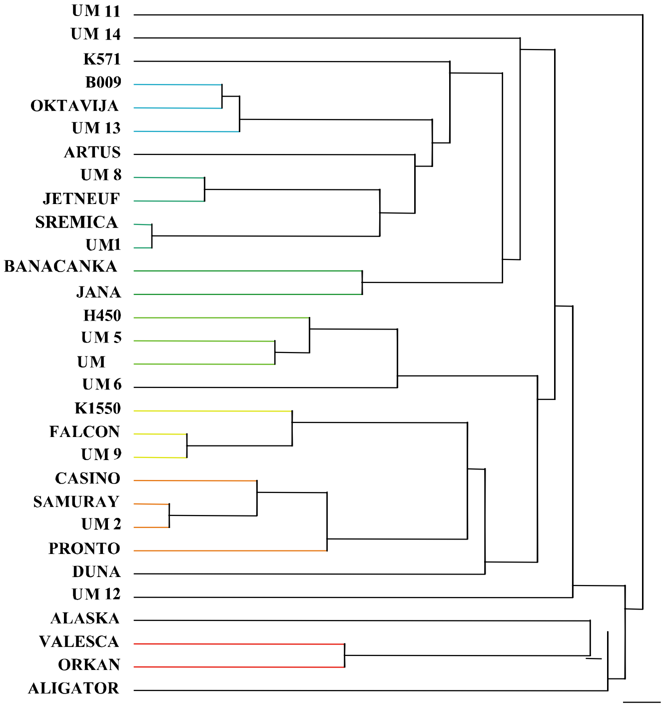
The dendrogram for the number of leaves consisted of two uneven groups and two individual genotypes. Line UM-11 had the highest mean value, as in the dendrogram for the number of branches (Graph 5).Lines UM-11 and UM-12 formed a separate group in the dendrogram for the height of the first lateral branch. Because of a high position of the first branch and large numbers of densely arranged lateral branches and leaves, the architecture of these lines is considered an acceptable ideotype in current breeding programs.



Graph 3. Dendrogram of phenotypic differences among the rapeseed genotypes regarding the height of the first branch



Graph 4. Dendrogram of phenotypic differences among the rapeseed genotypes regarding the number of lateral branches



Graph 5 Dendrogram of phenotypic differences among the rapeseed genotypes regarding the number of leaves

A certain regularity could be discerned when comparing the dendrogram obtained on the basis of RAPD markers with the dendrogram made on the basis of the analysis of the five individual traits. The dendrograms for the number of lateral branches, leaf number and pod number divided the genotypes according to their geographic origin, i.e., according to genetic distances among them.

Conclusion

Based on the results of RAPD and cluster analyses applied in the three-year study of 30 rapeseed genotypes, it was concluded that the tested material was significantly variable regarding all traits under study. In the dendrograms made on the basis of genetic distances assessed by RAPD markers, the genotypes were grouped according to geographic origin. Use of RAPD markers allows to differentiate rapeseed genotypes that differ slightly regarding their morphological traits. The dendrograms constructed on the basis of individual quantitative traits showed regularities in the geographic and genetic origins and partially in genotype pedigree. Dendrogram analysis is a rapid and simple method for selecting individual genotypes or their groups for further breeding.

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