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Genetic Diversity in Commercial Rapeseed (*Brassica napus* L.) Varieties from Turkey as Revealed by RAPD

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Abstract

In cultivated commercial crop species, genetic diversity tends to decrease because of the extensive breeding processes. Therefore, germplasm of commercial crop species, such as *Brassica napus* L. should be evaluated and the genotypes, which have higher genetic diversity index, should be addressed as potential parental cross materials in breeding programs. In this study, the genetic diversity was analysed by using randomly amplified polymorphic DNA analysis (RAPD) technique in nine Turkish commercial rapeseed varieties. The RAPD primers (10-mer oligonucleotides) produced 51 scorable loci, 31 loci of which were polymorphic (60.78%) and 20 loci (39.22%) were monomorphic The RAPD bands were scored as binary matrix data and were analysed using POPGENE version 1.32. At locus level, the values of genetic diversity within population (H_s) and total (H_T) were 0.15 and 0.19 respectively. The genetic differentiation (G_{ST}) and the gene flow (N_m) values between the populations were 0.20 and 2.05 respectively. The mean number of alleles (n_a), the mean number of effective alleles (n_{ae}), and the mean value of genetic diversity (H_e) were 2.00, 1.26, and 0.19 respectively. According to Pearson's correlation, multiple regression and principal component analyses, eco-geographical conditions in combination had significant effect on genetic indices of commercial *B. napus* L. varieties were discussed.

Keywords: *Brassica napus* L. commercial varieties, canola, genetic variation, molecular markers, RAPD polymorphism, vegetable oilseed crop

Introduction

Brassica napus L. (2n = 38, *AACC*) belongs to the genus *Brassica* L. (*Brassicaceae*). It is originated through spontaneous hybridisations between turnip rape (*B. rapa* L., *AA*, 2n = 20) and cabbage (*B. oleracea* L., *CC*, 2n = 18) (Kimber and McGregor, 1995). It is the second important source of vegetable oilseed crop in the worldwide after soybean. Because of intensive breeding processes *Brassica napus* L. has a relatively narrow genetic diversity in current germplasm. The breeders manage plant material originating worldwide to increase the existing genetic diversity, which especially highlights its resistance to agricultural environments (change in climate, biological stress factors, which are new in the area, and market requirements) (Marjanovic *et al*., 2009).

A variety of molecular markers has been used to study the genetic variation among the diverse group of important crops in the genus *Brassica*, such as: Analysis of genetic diversity in the *Brassica napus* L. using SSR (Chen *et al*., 2009; Hasan *et al*., 2006; Qu *et al*., 2012); RAPD markers (Ahmad *et al*., 2007; Fazeli *et al*., 2008; Gečaitė *et al*., 2009; Marjanovic *et al*. 2009; Moghaddam *et al*., 2009; Nisar *et al*., 2007; Shiran *et al*., 2006); Characterisation of *Brassica napus* germplasm based on molecular markers (Shahid *et al*., 2011); Genetic diversity of *Brassica napus* L. varieties estimated by morphological and molecular markers (Fahmi *et al*., 2012).

The aim of this study is to analyse the genetic diversity among nine Turkish commercial rapeseed (*Brassica napus* L.) varieties. Analyses were made to estimate the following genetic parameters: Allele richness (n_a), effective allele number (n_{ae}), genetic diversity (H_e), genetic diversity within (H_S) and total (H_T), genetic differentiation between populations (G_{ST}). In addition, the effects of climate (temperature *T*, humidity *H*, wind *W*, and rainfall *R*) and geography (altitude *AL*, latitude *LT*, and longitude *LN*) on the genetic diversity of five Turkish commercial rapeseed (*Brassica napus* L.) varieties according to RAPD markers.

Materials and methods

Plant material

In this study, nine different Turkish commercial rapeseed (*Brassica napus* L.) varieties (Tab. 1) were analysed genetically by using 10 RAPD primers, sequence of which were given in Tab. 2. The seeds of five varieties ('Licord', 'Elvis', 'Californium', 'PR46W31', and 'Tarcoola') were harvested from farmers' fields (< 1000 m^2), which are located at Trakya region of Turkey in August of 2010 by Betül Gıdık. Turkey branches of international companies

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Tab. 1. The locations, altitudes (AL), number of sample (N) and variety codes (PC) of the commercial rapeseed (*Brassica napus* L.) varieties used in this study

	PC	Variety	Provinces/Companies	N	AL (m)
Varieties provided by commercial companies	A	'NK Petrol'	SYNGENTA	20	
	B	'Tristan'	KWS	20	
	C	'Triangle'	KWS	20	
	D	'Nelson'	SYNGENTA	20	
Harvested varieties	E	'PR46W31'	Kırklareli-Karıncak Köyü	10	229
	F	'Licord' (K)	Kırklareli-Kofçaz	10	425
	G	'Californium'	Edirne-Kapıkule	10	61
	H	'Elvis'	Edirne-Süloğlu	10	150
	I	'Tarcoola'	Tekirdağ-Karaevli	10	140
	J	'Licord' (T)	Tekirdağ-Muratlı	10	93
			Total:	140	

KWS, and Syngenta kindly provided the seeds of Triangle and Tristan, and NK Petrol and Nelson respectively in the bulk form.

Tab. 1 shows the altitudes of the locations from where the harvested varieties were collected. Also shown are the names of the collection locations and the companies provided canola seeds and the variety codes that were used in this study. Throughout the text, the different varieties were identified by these codes. Ten genotypes per harvested varieties and 20 genotypes per varieties provided by commercial companies were randomly selected in total 140 genotypes for RAPD analysis.

DNA extraction

The genomic DNA was extracted as described by Amani *et al*. (2011) from the leaves of 1-1.5 month old plants, which were grown in the plastic pots.

DNA amplification

For DNA amplification, RAPD assays were carried out in 20 µL reaction mixture containing template 2 µL 10X *Taq* buffer (complete), 0.5 µL dNTP mixture (25 mM each), 0.5 µL of *Taq* DNA polymerase (500 u/µL, Bioron), 0.5 µL primer (100 pmol, Metabion International AG), 5 µL DNA (50-100 ng) and distilled water up to 20 µL. The amplification was performed in a Thermo Scientific thermocycler PCR system. The DNA amplification was obtained as followed: 94°C for 5 min, 40 cycles of 94°C for 1 min, 33°C for 1 min, and 72°C for 2 min, followed by one cycle of 72°C for 10 min. The amplified DNA products were resolved on 1.3% agarose gel, visualized by ethidium bromide (10 mg/µL) staining, and photographed with transmitted UV light (DNR bioimaging System). RAPD bands were scored from photographs and gels into a binary data matrix as 1 (present) or 0 (absent).

Estimation of reproducibility rate of RAPD bands

For estimation of reproducibility rate of RAPD bands, randomly an individual genotype from each harvested variety and two from varieties provided by the commercial companies, in total 14 genotypes were selected and formed a repeat group in this study. Then the same PCR conditions and primers, used in the study were applied for the repeat group to determine the reproducibility rates of the RAPD bands. Finally, the RAPD bands of the repeat group and the first run of the same samples were compared.

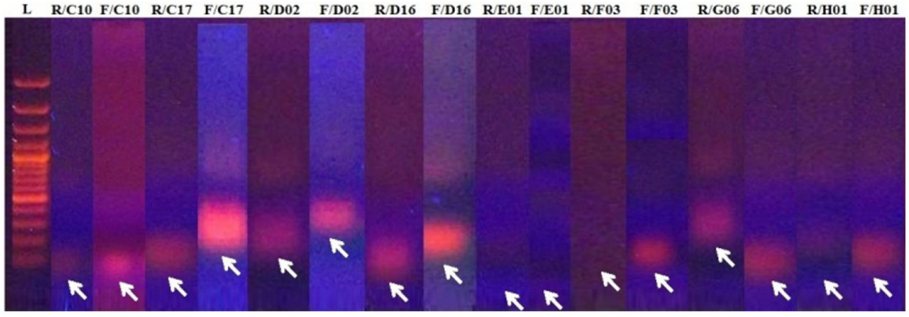

Fig. 1. RAPD-PCR band patterns, which were produced by ABN02 primer, of repeated and first run the same samples. Sample order: Lane 1 molecular weight DNA ladder (100 bp), Lanes 2-5 from variety C, Lanes 6-9 from variety D, Lanes 10-11 from variety E, Lanes 12-13 from variety F, Lanes 14-15 from variety G and Lanes 16-17 from variety H (Abbreviations: DNA Ladder L, first run F and Repeat R)

The results were counted as 184 and 188 RAPD bands in the first run and in the run of the repeat group respectively. Among these bands, 154 were repeated and reproducibility of the RAPD bands was 83.16% in this study (Fig. 1).

Statistical analysis

The RAPD bands are biallelic and dominant markers. The data considered as diploid and dominant were analysed using POPGENE version 1.32 (Yeh *et al*., 1997). The genetic diversity (H_e) based on the allele frequencies at the RAPD loci was calculated according to Nei (1973). The mean number of alleles per locus (n_a) and the mean number of effective alleles per locus (n_{ea}) were also calculated. The genetic differentiation between varieties was measured by G_{ST}. Gene flow (N_m) was calculated from G_{ST} to determine its effect on the gene diversity.

The genetic data of the harvested varieties were correlated with the eco-geographical data to screen the effects of the environmental factors on genetic diversity of varieties by using Pearson's correlation (r_p), multiple regression (MR) and principal component analyses (PCA). Following genetic indices were computed with geographical (*AL*, *LT*, and *LN*) and climatic (*R*, *T*, *H*, and W) variables: n_a, n_{ea}, and H_e. MR employed the genetic variables as dependent and the environmental variables as independent. Pearson's correlation and multiple regression analysis were carried out by using SPSS version 11 for Windows.

Principal component analysis (PCA) was performed using the following genetic indices: n_a, n_{ea}, and H_e as well as geographical (*AL*, *LT*, and *LN*) and climatic (*R*, *T*, *H*, and W) data as variables according to the Pearson's (one-tailed) correlation matrix using XLSTAT (version 2012). For the principal coordinates analysis (PCoA) the dissimilarity matrix values, which were calculated using binary data matrix with Jaccard coefficient, were used to ordinate 140 genotypes representing nine different varieties of canola (*Brassica napus* L.) on a scattered plot using XLSTAT (2012, Addinsoft™).

Results and discussion

In this study, genetic diversity was analysed in the nine different Turkish commercial rapeseeds (*Brassica napus* L.) varieties by RAPD method using 10 primers (10-mer oligonucleotides), which produced 51 scorable loci, 31 loci of which were polymorphic (60.78%) and 20 loci (39.22%) were monomorphic (Tab. 2). The primers ABN13 and AB02/20 produced the highest number of locus (7), while the primer ABN02 produced the lowest number of locus (2). The highest number of polymorphic locus (4) was determined in the RAPD products of AB02/06, AB02/10, and AB02/20 primers, while the lowest polymorphic locus (1) was determined in the RAPD products of ABN02 primer. The molecular weights of RAPD bands ranged between 100 bp and 1200 bp. A representative of RAPD-PCR band patterns was given in Fig. 2. At variety level, the highest number (37) of polymorphic locus (72.55%) was observed in variety I, while the lowest number (21) of polymorphic locus (41.18%) was observed in variety A.

In this study, the RAPD method displayed 83.16% of reproducibility. This is a considerably high amount of reproducibility beside its advantages of being fast, easy, not expensive, and less labour work requiring properties. RAPD analysis showed that the method was successful to differentiate the genotypes, which were from the different varieties in this study.

Analysis of genetic diversity

The mean values of the genetic diversity within population (H_S) and total (H_T) were 0.15 and 0.19 respectively (Tab. 3). The mean values of the genetic differentiation (G_{ST}) and the gene flow (N_m) between the populations were calculated as 0.20, and 2.10 respectively. The mean number of allele (n_a) and the mean value of the genetic diversity (H_e) were 2.00 and 0.15 respectively. The genetic distances (D) between nine different varieties of canola (*Brassica napus* L.) were calculated according to Nei (1973). The highest genetic distance (D = 0.101) was cal-

Tab. 2. The primers, used in the study, total number of locus, which produced by each primer, monomorphic, and polymorphic loci, percentage of polymorphic loci and range of molecular weights produced by 10 RAPD primers in nine different commercial rapeseed (*Brassica napus* L.) varieties and primer sequences (Abbreviations: Number of locus #L, monomorphic locus M, polymorphic locus P, and percentage of polymorphic locus %P, fragment size range FSR)

Primer	#L	M	P	%P	FSR (bp)	Primer Sequence
ABN02	**2**	1	**1**	50	100 bp-500 bp	5- ACCAGGGGCA -3
ABN06	6	3	3	50	100 bp-800 bp	5- GAGACGCACA -3
ABN13	**7**	4	3	42.85	100 bp-1200 bp	5- AGCGTCACTC -3
ABN18	5	3	2	**40**	100 bp-700 bp	5- GCTGAGGTCA -3
AB2/01	3	1	2	66.66	100 bp- 700 bp	5- CCCAAGGTCC -3
AB2/05	5	2	3	60	100 bp-700 bp	5- TCAGGGAGGT -3
AB2/09	4	1	3	75	100 bp-700 bp	5- CTTCACCCGA -3
AB2/06	5	1	**4**	80	100 bp -700 bp	5- AAGACCCATC -3
AB2/10	6	2	**4**	66.66	100 bp-1000 bp	5- CACCAGGTGA -3
AB2/20	**7**	3	**4**	57.14	100 bp-1000 bp	5- AACGGTGACC -3

*The highlighted numbers represent the highest and the lowest values observed in the study

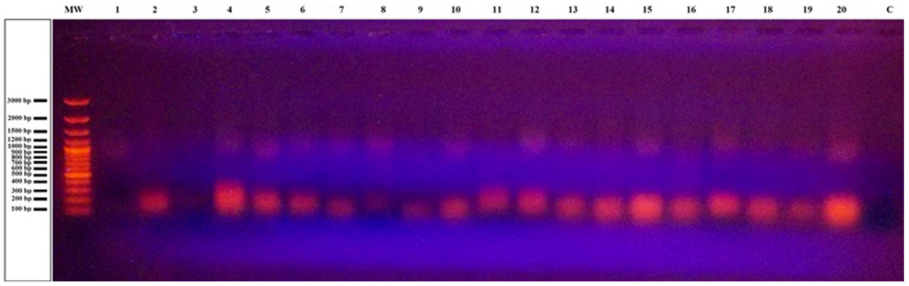

Fig. 2. RAPD band patterns produced by ABN02/09 primer in variety C. Sample order: Lane 1 Molecular weight (MW) DNA ladder (100 bp), Lanes 2-21 samples from population C, Lane 22 negative control (C)

culated between the varieties of H and C, while the lowest genetic distances (D = 0.010) was calculated between the varieties of H and G.

Genetic diversity estimates were considerably high for *B. napus* L. as it was observed in previous studies (Fahmi *et al*., 2012; Fazeli *et al*., 2008; Hasan *et al*., 2006; Marjanovic *et al*., 2009; Moghaddam *et al*., 2009; Qu *et al*., 2012). In addition, slightly higher genetic diversity was observed in the harvested varieties than the varieties provided by the commercial companies. A higher-level genetic variation was observed in this study, might be related with hybridization process. In *Brassicaceae* family, hybridization, introgression and hybrid speciation were reported as significant evolutionary forces. Interspecific gene flow and hybridization contributed to evolution and species diversity of some genera and causes genetic variation (Marhold and Lihova, 2006). The outcrossing mating system, associated with the perennial habit and vegetative reproduction are significant factors for hybridization, constitution, and emplacement of the polyploids (Ančev, 2006).

Molecular tools indicated that other mechanisms are also included in genetic variations; such as: rapid sequence elimination in synthetic hybrids and allopolyploids (Shaked *et al*., 2001; Song *et al*., 1995); genomic recombinations in the hybrids; extensive shift in DNA methylation patterns (Liu *et al*., 2004; Natali *et al*., 1998; Wang *et al*., 2005) or activation of mobile genetic elements (transposons and retro transposons; Liu and Wendel, 2000; Shan *et al*., 2005; Wang *et al*., 2005) (cited in Tu *et al*., 2009).

The hybridisation process might also cause to change in the constituent of the canola oil, especially the level of erucic acid. It contains 30-60% of the total fatty acids of rapeseed, mustard seed, and wallflower seed and it represents up to 80% of the fatty acids of nasturtium seeds (Food Standards Australia New Zealand, 2000). It was reported that rapeseed oils have toxic effect causing myocarditis in rats due to high level of erucic acid (Roine *et al*., 1960). Therefore, the low-level erucic acid trait was transferred in selective breeding programs and agronomically important *Brassica napus* and *Brassica compestris* cultivars adapted. The term "canola" is used to refer seeds from the varieties of *Brassica napus* and *Brassica campestris* that constitute less than 2% erucic acid in the oil. These canola varieties comprise almost the entire rapeseed crop produced in the world today (Food Standards Australia New Zealand, 2000).

Tab. 3. The alleles (n_a), the effective alleles (n_{ae}) and the gene diversity (H_e) at variety level for nine different commercial rapeseed (*Brassica napus* L.) varieties (Abbreviations: Polymorphic locus PL, percentage of polymorphic locus PL (%)

Variety	N	n_a	n_{ea}	H_e	PL	PL (%)
A	20	**1.41**	1.20	**0.11**	**21**	**41.18**
B	20	1.71	**1.27**	0.17	36	70.59
C	20	1.71	1.24	0.16	36	70.59
D	20	1.45	**1.17**	**0.11**	23	45.10
E	10	1.71	1.24	0.17	36	70.59
F	10	1.69	1.19	0.14	35	68.63
G	10	1.71	1.24	0.16	36	70.59
H	10	1.69	1.26	**0.18**	35	68.63
I	10	**1.73**	1.24	0.17	**37**	**72.55**
J	10	1.51	1.19	0.13	26	50.98

*The highlighted numbers represent the highest and the lowest values observed in the study

Pearson's correlation and multiple regression analyses

According to Pearson's correlation (2-tailed) analyses, wind had negative correlation with the mean number of allele and the mean value of the genetic diversity in the varieties displaying negative correlation; the correlation values were r_p = -0.883 (p = 0.020 at $p < 0.05$ significant level) and r_p = -0.858 (p = 0.029 at $p < 0.05$ significant level) respectively. The results of MR analysis also showed that wind had effect on the variance of the mean number of allele (88.3%) and of the mean value of genetic diversity (85.8%).

The Pearson's correlation analysis indicated that wind had substantial negative effect on the genetic indices of the harvested varieties of *B. napus* L. PCA and MR analyses displayed that eco-geographical factors had substantial effect on genetic indices of harvested varieties of *B. napus* L. Because, extensive breeding programs for some characters might increase their vulnerability to environmental factors, in which they were grown.

Principal component analysis (PCA)

The PCA (one-tailed) accounted for 96.74% of the genetic variation based on three components. The first component, which explained 61.09% of the total variance, was formed by the variables of n_a, n_{ea}, H_e, *W, H, T, RA,* and *LO*. The second component, representing 19.85% of the total variance, was formed by the variable *LA*, while the third component, which explained 15.80% of the total variance, was composed of the variable *AL*.

The mean values of genetic differentiation (G_{ST}) indicated that 80% of variation was within varieties and 20% of variation was between varieties. This amount of genetic differentiation is great amount especially for outcrossing plant species. On the other hand, there is no any knowledge about these varieties, which are the products of a selective breeding program for which characters. These characters might be high yield, biodiesel usage, or for some other characters. RAPD analysis indicated that the genetic distances among the nine Turkish commercial rapeseed varieties studied were very low.

Principal coordinates analyses (PCoA)

According to the PCoA results, the codes (the letter and the number represent the variety code and genotype number respectively) of the genotypes from each variety were plotted on the two axes of a scattered plot graph (Fig. 3). In the scattered plot graph, the genotypes from the harvested varieties E, F, G, H, I, and J separated clearly from the genotypes representing varieties A, B, C and D provided by the commercial companies. The genotypes from varieties A and C were grouped together, while the genotypes from variety B separated away from all other varieties. Some of the genotypes (D6, D7, D8, D10, D11, D14, and D20) from variety D were grouped closer to the harvested varieties, while some of the genotypes (D2, D3, D4, D5, D12, D13, D13, D15, D16, D17, D18, and D19) from variety D were grouped with varieties of A and C.

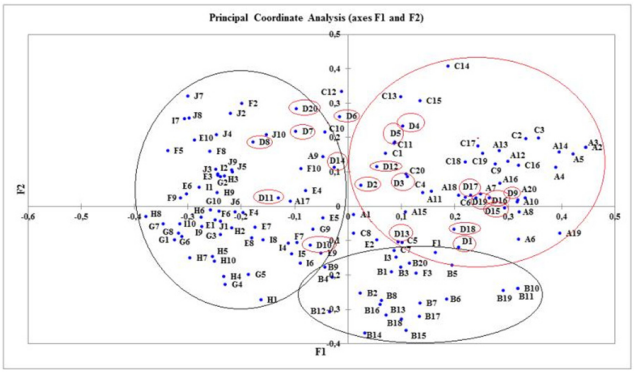

Fig. 3. The scattered ordination plot of the first and second principal coordinates of nine different commercial rapeseed (*Brassica napus* L.) varieties based on dissimilarity matrix obtained by using 10 RAPD primers

Rapeseeds are important crop plants for food industry, livestock, beekeeping, and biodiesel production etc. They are quite adaptable to the different habitats, except habitats, which have soil with intensive salt stress. It is reported that canola is one of the most profitable crops available to grain growers in southern Australia and rotations have been adapted to accommodate it. Canola increases the yields to benefits of subsequent cereal crops by facilitating an effective disease break. Usually, canola was often planted as the first crop after pasture but now canola is often grown more intensively in rotations, posing problems of herbicide use, disease carryover and increasing potential of blackleg in existing varieties. The sulphur, gypsum, and lime required to grow canola in many regions are beneficial to crops and pastures grown in rotation (Norton *et al*., 1999). Smith *et al*. (2008) reported that in their study they rotated canola with field pea, wheat, and flax in different time intervals and determined net canola yield. Canola has high oilseed prices, cereals have low prices, and canola yields are usually 55-60% of wheat yields when grown under similar situations (Norton *et al*., 1999). Therefore, canola is the most profitable crop for rotated farming.

Conclusions

Extensive breeding process is causing to decrease the genetic diversity in germplasm of crop species. Therefore, germplasm of crop species, such as *Brassica napus* L. should be examined and the genotypes, which have higher genetic diversity index, should be determined and used as potential parental cross material in breeding programs.

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