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Genetic relationships among turnip (*Brassica rapa* var. *rapa*) genotypes

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ABSTRACT. Turnip (*Brassica rapa* var. *rapa*) is one of the main vegetables consumed by people living in Eastern Anatolia in Turkey. In this region, farmers obtain their own seeds for production, which results in considerable morphological variability. We examined the genetic variation and relationships among 11 turnip genotypes sampled from diverse environments of the Erzurum region located in Eastern Anatolia in Turkey. Thirty-two Operon RAPD primers were screened; among them, 20 gave reproducible and clear DNA fragments after amplification. The average polymorphism ratio was 90.4%. The genetic distance between turnip genotypes were found to range from 0.302 to 0.733, indicating high genetic variability. Eleven genotypes were divided into three main clusters in a dendrogram; ETS2 and ETS8 genotypes were the most distant. We conclude that RAPD analysis would be useful for genotyping turnip genotypes.

Key words: Turnip; *Brassica rapa* var. *rapa*; RAPD; Genetic diversity

INTRODUCTION

Turkey, a bridge between Europe and Asia, is surrounded on 3 sides by seas of different ecological characteristics, and has altitudes ranging from sea level to above 5000 m, resulting in a variety of climatic conditions throughout the country. Thus, Turkey has a biological wealth that is unrivaled by any of the neighboring countries. The ecosystem mosaic of several different ecological characteristics provides nesting and breeding areas for thousands of fauna and flora species and their populations. Another factor that increases this wealth is that, two of the four migratory routes of the West Palaearctic Region pass over Turkey (Akbulut et al., 2009).

It is believed that there are over 50 vegetable taxa successively grown throughout Turkey, and a wide morphological variability is also evident within different taxa (Ercisli, 2004). As well known, almost all morphological characters are controlled by minor polygenic traits and easily affected by environmental factors.

Farming practices and age and developmental stages of plants affect morphological characters as well (Gottlieb, 1984; Khakwani et al., 2005). In addition, more recent intensive plant breeding has resulted in a few dominant cultivars and has narrowed the genetic diversity among cultivars (Reif et al., 2005). These features make the identification of different plants both at the intra- and inter-variety level very difficult. The varietal identification among plants based on biochemical traits such as isozymes and protein analysis is also limited, since protein polymorphism is not so high among cultivars, which makes closely related cultivars indistinguishable (Badenes et al., 1998). More recently, DNA-based markers have gained more popularity because they are unaffected by environment, detectable at all stages of development, and ubiquitous in number, covering the entire genome. They also have the advantage of being abundant, highly polymorphic, and analytically simple. Therefore, they play an increasingly important role in the identification and measurement of genetic diversity of different plants (Benjak et al., 2005; Ercisli et al., 2007; Ilgin et al., 2009).

In Turkey, particularly Eastern Anatolia, *Brassica* crops such as cabbage, cauliflower, radish, broccoli, turnip, etc., have been the main source of vegetables for human consumption because of the cold temperate climate characteristics of the region. In this region, traditional farming practices have been applied on small family farms, and farmers produce *Brasicca* crops, in general, obtaining their own seeds for sowing. This process may lead to a great number of landraces adapted to different conditions and to different uses throughout the region (Padilla et al., 2005). In spite of the wide consumption of *B. rapa* var. *rapa* in this region, little information about genetic resources is available.

Therefore, the aim of this study was to use random amplified polymorphic DNA (RAPD) fingerprinting for characterizing and detecting polymorphisms among turnip genotypes sampled from diverse environments of the Erzurum region in Turkey, and in addition, for investigating the genetic relationships among the genotypes sampled.

MATERIAL AND METHODS

Leaf samples from 11 turnip (*Brassica rapa* var. *rapa*) genotypes were collected in the Erzurum region located in Eastern Anatolia. The leaves were stored immediately at -80°C for later DNA extraction.

Genomic DNA was extracted from powdered (ground in liquid nitrogen) leaf materials using the modified method described by Lin et al. (2001). Approximately 10-15 mg tissue from each plant sample was snap-frozen in liquid nitrogen in 2-mL Eppendorf tubes. A volume of 1000 µL DNA extraction buffer [100 mM Tris-HCl, pH 8.0; 50 mM EDTA, pH 8.0; 500 mM NaCl; 2% SDS (w/v); 2% 2-mercaptoethanol (v/v); 1% PVP (w/v)] was added and the contents well mixed. The mixture was incubated at 65°C in a water bath for 40 min with intermittent shaking at 5-min intervals. The mixture was centrifuged at 12,000 g for 15 min at 4°C.

The supernatant was transferred to a clean 1.5-mL tube, mixed with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1), and then centrifuged. The supernatant was collected and mixed with 1/10 volume 10% CTAB-0.7 M NaCl in a clean tube. After centrifugation, the supernatant was collected and mixed gently with an equal volume of chloroform:isoamyl alcohol (24:1). DNA was precipitated by the addition of 0.6 volume freezer-cold isopropanol, for 10 min at -20°C. DNA was pelleted by centrifugation (12,000 *g*, 10 min) and the isopropanol was poured off; the DNA recovered was allowed to air-dry before being dissolved in 100 µL TE buffer.

The samples were screened for RAPD variation using the standard 10-base primers supplied by Operon. A 30-µL reaction cocktail was prepared as follows: 10X 3.0 µL buffer, 1.2 µL dNTPs (10 mM), 1.2 µL $MgCl_2$ (25 mM), 2.0 µL primer (5 µM), 0.4 µL *Taq* polymerase (5 unit), 19.2 µL water, and 3.0 µL sample DNA (100 ng/µL). A total of 43 RAPD primers were tested in this study, and the polymorphisms obtained with the primers are shown in Table 1.

The thermocycler (Eppendorf Company) was programmed as follows: 2 min at 95°C; 2 cycles of 30 s at 95°C, 1 min at 37°C, and 2 min at 72°C; 2 cycles of 30 s at 95°C, 1 min at 35°C, and 2 min at 72°C; 41 cycles of 30 s at 94°C, 1 min at 35°C, and 2 min at 72°C, and a final 5-min extension at 72°C, followed by cooling down to 4°C. The markers were checked twice for their reproducibility.

The polymerase chain reaction products (27 µL) were mixed with 6X gel loading buffer (3 µL) and loaded onto an agarose (1.5%, w/v) gel in 0.5X TBE (Tris-borate-EDTA) buffer, and electrophoresis was performed at 70 V for 150 min. The gel was stained in an ethidium bromide solution (2 µL/100 mL 1X TBE buffer) for 40 min, and the bands were visualized under UV in a Bio Doc Image Analysis System with Uvisoft analysis package (Cambridge, UK).

The positions of scorable RAPD bands were transformed into a binary character matrix (1 for the presence and 0 for the absence of a band at a particular position), which were entered in the RAPDistance computer program (Armstrong et al., 1994). These data were used for the calculation of pairwise genetic distances between cultivars using the Jaccard coefficient (JC). The computer program calculated the degree of genetic dissimilarity between each pair of the 21 genotypes using the simple equation: $JC = 1 - a / (a + b + c)$, where a is the number of bands shared by plant x and plant y, b is the number of bands in plant x, and c is the number of bands in plant y. The JC ignores the absence of matches. The distance matrix was used for cluster analysis using the unweighted pair-group method with arithmetic mean (UPGMA).

RESULTS

The total number of DNA bands amplified with the 11 accessions of *Brassica rapa* var. *rapa*, as well as the number of polymorphic and monomorphic bands among these, are presented in Table 1. Banding patterns of the 11 turnip genotypes using the primer OPY-11 are illustrated in Figure 1.

Table 1. List of the selected primers and the degree of polymorphism obtained among the 11 turnip genotypes.

Primer code	Sequence 5'→3'	Polymorphic bands	Monomorphic bands	Percentage of polymorphic markers
OPW-17	GTCCTGGGTT	6	0	100
OPW11	CTGATGCGTG	7	0	100
OPW-13	CACAGCGACA	3	0	100
OPA-13	CAGCACCCAC	2	0	100
OPH-17	CACTCTCCTC	5	0	100
OPA-2	TGCCGAGCTG	6	0	100
OPH-18	GAATCGGCCA	2	1	66.6
OPY-6	AAGGCTCACC	6	1	85.7
OPY-11	AGACGATGGG	9	2	81.8
OPY-1	GTGGCATCTC	9	1	90
OPY-8	AGGCAGAGCA	6	0	100
OPY-15	AGTCGCCCTT	5	2	71.4
OPY-16	GGGCCAATGT	5	0	100
OPW-1	CTCAGTGTCC	7	2	77.7
OPW-6	AGGCCCGATG	7	1	87.5
OPB-8	GTCCACACGG	8	2	80.0
OPW-4	CAGAAGCGGA	9	0	100
OPW-7	CTGGACGTCA	7	0	100
OPB-10	CTGCTGGGAC	8	0	100
OPW-8	GACTGCCTCT	5	1	83.3
Polymorphism (average)				93.4
Total		122	13	

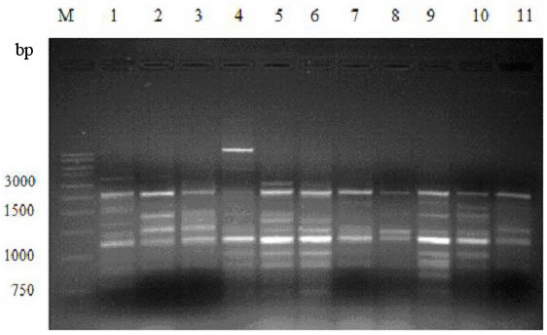

Figure 1. RAPD bands obtained by amplification with primer OPY-11. M = molecular marker. *Lanes 1-11* = ETS1-ETS11 genotypes, respectively.

In order to study the diversity at the DNA level, 32 arbitrary primers were used for RAPD analysis, and 20 primers yielded detectable bands (Table 1, Figure 1). A total of 135 bands were scored for 11 turnip genotypes. Of 135 bands, 122 (90.37%) were found to be polymorphic, while 13 (9.63%) were monomorphic.

When the scorable bands were examined for the different primers, OPA-2, OPA-13, OPB-10, OPH-17, OPY-8, OPY-16, OPW-4, OPW-7, OPW-11, OPW-13, and OPW-17 showed the highest polymorphism ratio (100%), while OPH-18 showed the lowest polymorphism ratio (66.6%).

The dendrogram obtained from the RAPD markers grouped the 11 turnip genotypes into 3 main clusters.

The dendrogram results also showed that sample ETS9 is distinct; this is an indication that it has peculiar traits differing from the other genotypes. Generally, many samples were found to be genetically dissimilar, confirming their high genetic diversity.

The first cluster includes ETS8 and the second cluster includes ETS3. The third cluster includes 9 genotypes (ETS6, ETS9, ETS7, ETS10, ETS11, ETS4, ETS5, ETS1, and ETS2) (Figure 2).

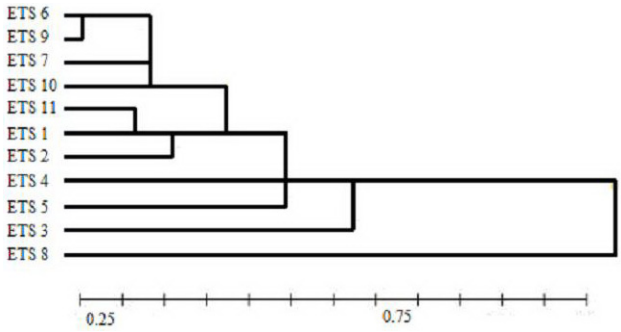

Figure 2. UPGMA dendrogram of the 11 turnip genotypes based on 20 RAPD primers.

The genetic distance between turnip genotypes ranged from 0.302 to 0.733. The genotypes ETS6 and ETS9 were found to be the closest, whereas ETS2 and ETS8 were the most distant (Table 2).

Table 2. Similarity matrix of the 11 turnip genotypes.

	ETS1	ETS2	ETS3	ETS4	ETS5	ETS6	ETS7	ETS8	ETS9	ETS10	ETS11
ETS1	1.000										
ETS2	0.660	1.000									
ETS3	0.535	0.561	1.000								
ETS4	0.598	0.529	0.531	1.000							
ETS5	0.543	0.567	0.510	0.602	1.000						
ETS6	0.563	0.514	0.577	0.608	0.598	1.000					
ETS7	0.610	0.619	0.626	0.592	0.613	0.717	1.000				
ETS8	0.333	0.302	0.398	0.337	0.448	0.359	0.385	1.000			
ETS9	0.582	0.608	0.581	0.648	0.635	0.733	0.673	0.384	1.000		
ETS10	0.602	0.596	0.500	0.565	0.573	0.613	0.612	0.361	0.714	1.000	
ETS11	0.579	0.606	0.560	0.510	0.535	0.641	0.639	0.357	0.667	0.695	1.000

DISCUSSION

We evaluated RAPD markers as tools for assessing genetic variation and determining the relationships among 11 *B. rapa* var. *rapa* genotypes sampled from different areas in the Erzurum region, Turkey.

Our study showed that there was a very high polymorphism ratio among turnip genotypes. The high level of genetic variability detected within the *B. rapa* var. *rapa* germplasm collected in the Erzurum region could be attributed to either the distant geographical or genetic origin.

In recent years, molecular genetic techniques using DNA polymorphism have been increasingly used in *Brasicca* vegetables to characterize and identify germplasm for use in crop breeding (O'Neill et al., 2003; Seyis et al., 2003). A number of previous studies have investigated the genetic diversity of *B. rapa* var. *rapa* through the use of RAPD markers, and it was concluded

that RAPD markers provide a fast and reliable method for analyzing individuals and cultivars of turnip (Tanhuanpaa et al., 1993; Ren et al., 1995). Our results are comparable to those in the cited literature. On the other hand, an average similarity ratio of 0.494 was recorded for the turnip genotypes, suggesting that the genome may differ in turnip genotypes. This result also indicates the potential use of these genotypes, particularly the more distant ones, for future breeding programs.

Comparisons of RAPDs and restriction fragment length polymorphisms in determining genetic similarity among *Brassica oleracea* genotypes revealed that these marker systems provide equivalent levels of resolution for determining genetic relationships (dos Santos et al., 1994). RAPD markers in determining genetic relationships among Cruciferous vegetables have shown RAPDs to be reliable for intraspecific comparisons and among closely related species, but less reliable for higher taxonomic associations (Halldén et al., 1994; Cartea et al., 2005). As mentioned before, the bands ranged in size from 350 to 3000 bp, which is within the range previously reported in *Brassica* germplasm (Kresovich et al., 1992; Mailer et al., 1994; Rabbani et al., 1998; Cassian and Echeverrigaray, 2000).

The RAPD method has previously been found to be applicable for genotype identification in various *Brassica* vegetables (Kresovich et al., 1992; Mailer et al., 1994; Rabbani et al., 1998; Cassian and Echeverrigaray, 2000), and all these studies showed that it involves a very simple technique compared to other molecular techniques.

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