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Full Length Research Paper

Genetic diversity among some blackberry cultivars and their relationship with Boysenberry assessed by AFLP Markers

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Blackberry cultivation has increased its popularity in Turkey due to the use of more blackberries in Turkish cuisine. To provide farmers with well adapted blackberry cultivars, some blackberry cultivars including a Boysenberry genotype from North America has been planted to various geographical regions in Turkey. In this study, genetic diversity among these blackberry cultivars and their genetic relationship with Boysenberry and raspberry were analyzed using AFLP markers. Our results indicated that Blackberry cultivars from North America had narrow genetic background which can pose a problem for future breeding programs. Blackberry genotypes selected from Bursa province of Turkey shared all AFLP markers with the cultivar Chester, which suggests that they were not unique genotypes. Although genetic similarity between Boysenberry and blackberry was low, Boysenberry was genetically related to common blackberry cultivars. On the other hand, AFLP analysis was unable to detect any genetic relationship between Boysenberry and common raspberry cultivars from North America in this study.

Key words: Blackberry, Boysenberry, raspberry, genetic diversity, AFLP markers.

INTRODUCTION

Blackberries are fruiting-bearing species of genus *Rubus* subgenus *Rubus* of Rosaceae family (Clark et al., 2007). Germplasm Resources Information Network describes 13 subgenera for the genus *Rubus* and 12 sections within *Rubus* subgenus *Rubus* (http://www.ars.usda.gov). On the basis of the horticultural traits, the *Rubus* genus was divided into two major groups, blackberries and raspberries (subgenus *Idaeobatus*). While blackberry fruits were picked with receptacle (torus), receptacle remains on plant in raspberries.

In the development of novel blackberry types called "hybridberries", red raspberry (*Rubus idaeus* L.) has played an important role. Hybridberries have been developed by crossing blackberry with raspberry and crossing back to blackberry. 'Nessberry', 'Loganberry', 'Phenomenal' and 'Brazos' are some of these hybridberries of blackberry and raspberry crosses (reviewed in Jennings, 1988; Clark et al., 2007). It has been widely accepted that Boysenberry (*Rubus ursinus* Chamisso and Schlenhtendal) is also a "hybridberry" (Wood et al., 1999). The origin of 'Boysenberry' has been traced back to northern California and Boysenberry was named after its discoverer, Rudolph Boysen. Although convincing evidences for historical background have been presented by Wood et al. (1999), the exact genetic origin of 'Boysenberry' is still not known. It has been hypothesized that *R. idaeus* and *R. ursinus* made significant contribution to development of Boysenberry either through 'Loganberry' or a "Loganberry-like" genotypes (Wood et al., 1999; Hall et al., 2002). Molecular markers can be helpful for explaining the origin of this hybridberry since DNA based markers are found to be a useful tool for plant scientists for establishing phylogenies, tagging desirable genes, determining similarities among in breeding materials, cultivar identification and mapping plant genomes (Li et al., 2001; Graham et al., 2004; Sargent et al., 2007; Lewers et al., 2008; Ercisli et al., 2008). AFLP (amplified fragment length polymorphisms) markers are highly reproducible multi-locus marker system developed by

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Vos et al. (1995). This marker system requires no prior sequence information and is applicable to any plant species (Tohme et al., 1996). High levels of polymorphism and high degrees of discriminative capacity are the other advantages of this marker system.

The Blackberry (*Rubus* subgenus *Rubus* Watson) grows as a perennial shrub in many parts of the world and widespread in wild in Turkey. Although the cultivation of blackberry in Turkey is very recent, the use of soft-bodied blackberries in the desert, jam, jelly and frozen-foods in Turkish cuisine increased its popularity significantly. In addition, phenolic compounds in blackberry fruits due to the possible health benefit with antioxidative properties have promoted its consumption (Barut, 2004). Wild blackberries grow in any available land in road sides, woods and hillside in Turkey and colonize the area rather quickly (Agaoglu, 2003). The fruits of wild blackberries have been collected and consumed locally. Recent increase in its popularity created high consumer demand for blackberry and Turkish farmers have been establishing new blackberry orchards to meet this increasing demand (Barut, 2004).

To provide farmers with well adapted blackberry cultivars for their region, a study to determine performance of 14 blackberry cultivars from North America in 16 regions of Turkey was initiated (Agaoglu, 2003). As a part of this study, 10 blackberry cultivars and a Boysenberry clone have also been planted in a field in the Faculty of Agriculture of Uludag University in Bursa in 2000. This study reports the genetic relationship among these cultivars and Boysenberry using AFLP markers. In addition, three raspberry (*Rubus idaeus* L.) cultivars were also analyzed with AFLP markers to determine possible genetic relationship between boysenberry and raspberry.

MATERIALS AND METHODS

Plant materials

Ten blackberry cultivars (Arapaho, Black Satin, Bursa 1, Bursa 2, Bursa 3, Chester, Dirckson Thornless, Jumbo, Navaho and Loch Ness) and a Boysenberry clone were included in this study for AFLP analysis. While eight of these are known cultivars from North America, remaining three, Bursa 1, Bursa 2 and Bursa 3, are the blackberry genotypes with unknown origin. Three raspberry cultivars (Canby, Heritage II and Tulameen) were also included for AFLP analysis.

Preparation of DNA samples

DNA samples were extracted from lyophilized powdered young leaves of each genotype. For this purpose, 150 mg of powdered leaf samples were used for DNA extraction in micro centrifuge tubes following a modified CTAB protocol described by Fütterer et al. (1995). Phenol chloroform extraction method of DNA was used to increase purity of DNA for AFLP analysis. The concentrations of each DNA samples were measured using Qubit Fluorometer (Invtrogen, Carlsbad, CA, USA) and adjusted to 50 ng/µl for analysis.

AFLP analysis

AFLP procedure was carried out according to methods described previously by Vos et al. (1995) using AFLP system I (Invitrogen). Briefly, after digestion of 300 ng of genomic DNA with *Mse* I and *Eco*R I enzymes, DNA fragments were ligated to *Mse* I and *Eco*R I restriction-site derived adapters using manufacturer protocols (Invitrogen). Pre-amplification were performed using pre-amplification primers (EA+MC) and the PCR product of pre-amplification reactions were diluted 50X to use in selective amplification reactions as a template DNA. Seven selective amplification primer combinations with three selective nucleotides, EAGG/MCAT, EAGC/MCTG, EAGG/MCAT, EACG/MCTC, EAGC/MCTC, EACC/MCTC and EAGG/MCTG were used for selective amplification of AFLP markers according to reaction condition described by the manufacturer (Invitrogen). AFLP selective amplification product was denatured and size fractionated on a 6% polyacrylamide gel by running for 2 h and 30 min at 60 watt. AFLP markers were visualized by silver-staining of DNA fragment using Silver Sequence™ DNA Sequencing System (Promega, Madison, WI, USA) and photographed.

Data analysis

All unambiguous polymorphic AFLP fragments were identified and scored as presence (1) or absence (0). Similarity matrix, generated according to the coefficient of Dice (Dice, 1945) were used for the un-weighted pair-group method with arithmetic averaging (UPGMA) (Sokal and Michener, 1958) cluster analysis with NTSYSpc v. 1.80 program (Rohlf, 1993). A dendrogram indicating the estimated similarity among blackberry genotypes was constructed with TREE program of NTSYSpc. Cophenetic values calculated from the UPGMA dendrogram and compared with Dice similarity matrix by using the Mantel test of significance (Mantel, 1967).

RESULTS AND DISCUSSION

Level of polymorphism and discriminating capacity of the AFLP primer pairs

Seven primer combinations generated a total of 45 polymorphic AFLP markers among 10 blackberry cultivars and a Boysenberry clone with the average of 6.4 polymorphic AFLP markers per primer combination. The number of polymorphic AFLP markers per primer combination ranged from 2 with EACG/MCTC to 10 with EAGG/MCAT. Total scorable AFLP amplified DNA fragments were 29 with EAGG/MCAT primer combination and 10 (34.5%) of these AFLP amplified DNA fragments were polymorphic.

A similarity matrix was prepared using Dice coefficient (Dice, 1945) and similarity among blackberry cultivars ranged from 0.35 to 1.00 (Table 1). On the other hand, similarity between blackberry cultivars and Boysenberry was low, ranging from 0.19 to 0.49. Using this similarity matrix, a UPGMA dendrogram demonstrating the estimated genetic relationship among blackberry cultivars and Boysenberry was developed (Figure 1). To demonstrate how well the Dice similarity matrix was represented by UPGMA dendrogram, the matrix of cophenetic values calculated from UPGMA dendrogram using COPH

Table 1. Similarity matrix among 10 blackberry cultivars and Boysenberry based on Dice (1945) coefficient. Raspberry accessions were not included to table since they did not share any common band with either blackberry or Boysenberry.

	Arapaho	Black Satin	Boysenberry	Bursa 1	Bursa 2	Bursa 3	Chester	Jumbo	Navaho	Loch Ness	D.Thornless
Arapaho	1.00										
Black Satin	0.50	1.00									
Boysenberry	0.20	0.21	1.00								
Bursa 1	0.50	0.93	0.21	1.00							
Bursa 2	0.50	0.93	0.21	1.00	1.00						
Bursa 3	0.50	0.93	0.21	1.00	1.00	1.00					
Chester	0.50	0.93	0.21	1.00	1.00	1.00	1.00				
Jumbo	0.48	0.78	0.49	0.78	0.78	0.78	0.78	1.00			
Navaho	0.55	0.90	0.21	0.97	0.97	0.97	0.97	0.78	1.00		
Loch Ness	0.51	0.85	0.33	0.91	0.91	0.91	0.91	0.87	0.91	1.00	
D. Thornless	0.35	0.60	0.46	0.67	0.67	0.67	0.67	0.72	0.65	0.73	1.00

program in NTSYSpc. Comparison of similarity matrix with the matrix of cophenetic values using Mantel test demonstrated that the correlation between these two matrices was 0.98, suggesting that Dice similarity matrix was represented very well by the UPGMA dendrogram (Rohlf, 1993).

Genetic relationship between the blackberry and Boysenberry accessions

According to dendrogram, three major groups were identified above 65% similarity level. One of the group contained Arapaho, second group composed of Boysenberry and remaining blackberry cultivars were clustered together in a third cluster (Figure 1). Except Arapaho, blackberry cultivars clustered within a group over 65% similarity. These results suggested that blackberry cultivars from North America have narrow genetic background and this can create problem for future breeding effort. Stafne and Clark (2004) also found that genetic base of eastern North American blackberry cultivars is narrow based on their pedigree analysis of North American blackberry cultivars and suggested that introduction of more divers blackberry germplasm to maintain heterogeneity. These results suggest that genetic diversity analysis among the blackberry genotypes available in the USDA germplasm centers using DNA markers is needed for facilitating successful breeding efforts in the future.

The blackberry genotypes, Bursa 1, Bursa 2 and Bursa 3, have been selected from the blackberry plantation in Bursa province of Turkey. AFLP analysis demonstrated that they are same genotypes since they shared 100% of AFLP markers. In addition, these selected genotypes also shared 100% AFLP markers with the cultivar, Chester from North America. Therefore, it is possible that Chester has been brought to Turkey by private collector(s). Since this cultivar showed good adaptation to Bursa province, it was clonally propagated and grown by local farmers.

Genetic relationship of Boysenberry with blackberry and raspberry

With its uncertain origin, the common consensus is that Boysenberry is “hybridberry” and raspberry (*R. idaeus)* and blackberry (*R. ursines)* played important role in the development of Boysenberry (Clark et al., 2007). In this study, along with blackberry cultivars, a Boysenberry clone and three raspberry cultivars (Canby, Heritage II and Tulameen) to represent *R.idaeus* L. subsp. *idaeus*, were included to AFLP analysis (Figure 2). If blackberry contributed to the genome of Boysenberry, one can expected to observe some AFLP markers shared by both Boysenberry and blackberry cultivars. Indeed, there were some AFLP markers present in both Boysenberry and blackberry cultivars (Figure 2). On the other hand, if there was a raspberry genotype in the parentage of boysenberry, there should be some AFLP markers present in both Boysenberry and raspberry cultivars. However, we did not observe any AFLP markers shared by both Boysenberry and raspberry cultivars (Figure 2). Furthermore, Boysenberry had some unique AFLP markers shared by neither blackberry nor raspberry cultivars (Figure 2). These results indicated that Boysenberry is related to North American blackberries. However, presence of AFLP markers unique to Boysenberry suggested that there might be another parent which contributed to the genome of Boysenberry if it is

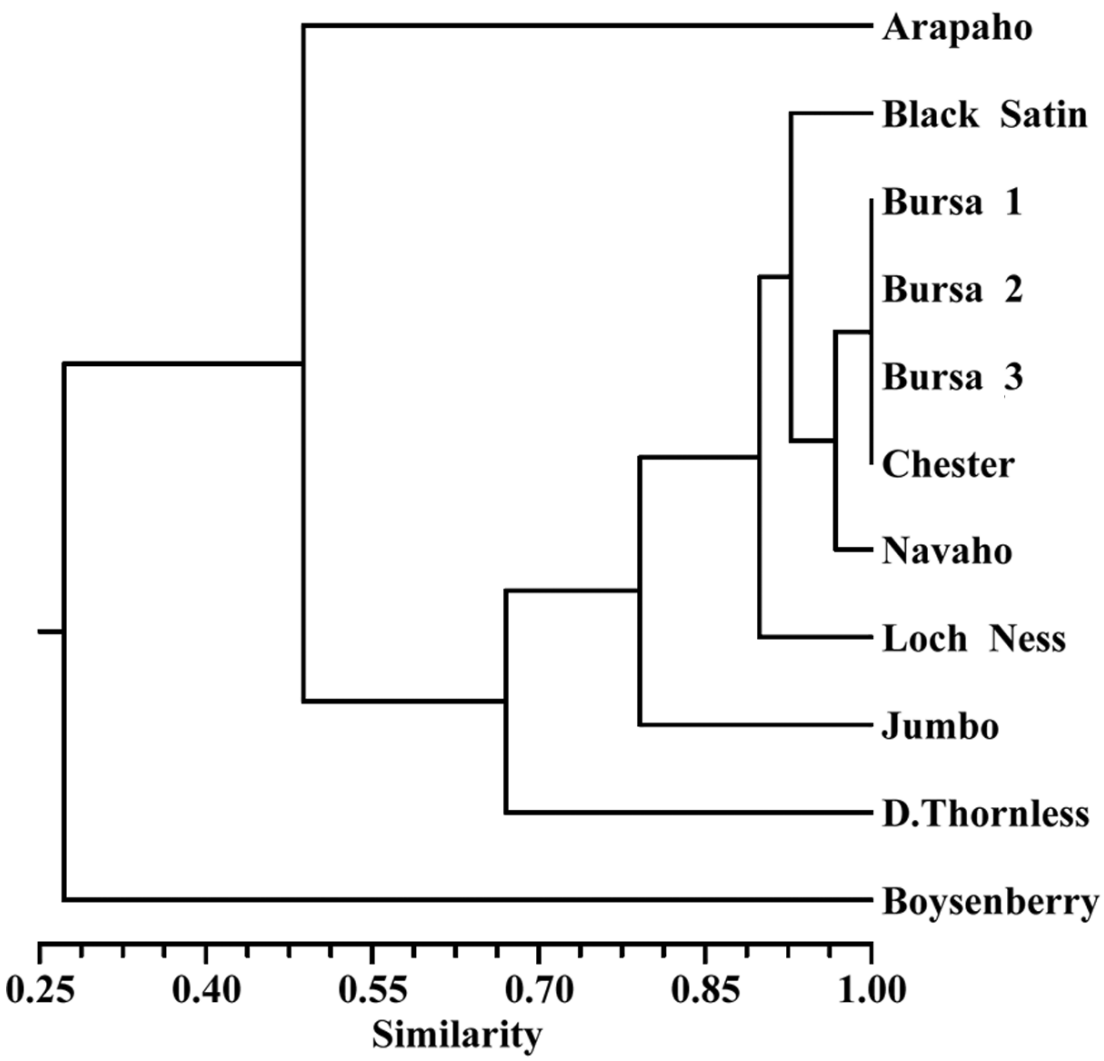

Figure 1. UPGMA dendrogram based on Dice (1945) coefficient illustrating the estimated genetic relationship among blackberry cultivars and Boysenberry. Raspberry accessions were not included to genetic similarity analysis since they did not share any common band with either blackberry or Boysenberry.

"hybridberry". However, this parent may not be related to common raspberry cultivars from North America. There is another theory that Boysenberry is produced from a cross between Loganberry *(R. loganobaccus* Bailey) and a trailing blackberry *(R. baileyanus* Britt.) cultivar such as'Lucretia' or 'Austin Mayes' (Jennings, 1988; Wood et al., 1999). Since there was no Loganberry germplasm available to us, it was impossible to assess genetic relationship of Boysenberry with Loganberry using AFLP in this study.

In conclusion, AFLP marker system can be used as a powerful tool for molecular markers studies in blackberry. According to our AFLP analysis, blackberry cultivars from North America have narrow genetic background probably due the common parent used during the breeding of these cultivars (Stafne and Clark, 2004). Therefore, we suggest that genetic variation in the blackberry germplasm should be analyzed by including more accessions from the USDA germplasm center for effective utilization in the breeding programs. AFLP analysis demonstrated that Boysenberry is genetically related to common blackberry cultivars but were unable determine any genetic relationship with common raspberry cultivars from North America. To identify the other donor parent crossed with blackberry to produce Boysenberry, Loganberry and Loganberry-like germplasm should be tested using DNA markers. Due to the backcrossing to blackberry during the development of hybrid berries, it is very difficult to estimate what proportion of the Boysenberry genome is from the other parent crossed with blackberry. Hence, we suggest the use of molecular marker systems covering the plant genome very well like AFLP marker system.

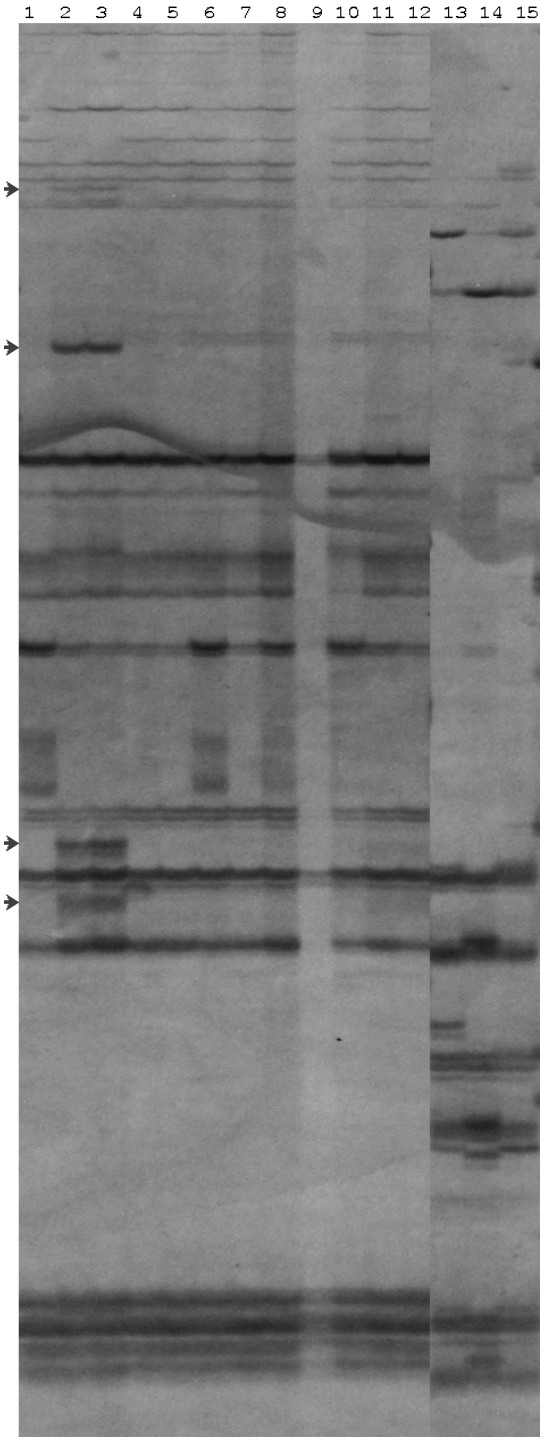

Figure 2. DNA banding profile of AFLP markers in blackberry, Boysenberry and raspberry cultivars. AFLP markers were amplified using EAGC/MCTC selective amplification primers. Lanes 1(Black Satin), 4 (Bursa 1), 5 (Bursa 2), 6 (Bursa-2), 7 (Bursa 3) 8 (Bursa 3), 9 (Chester) 10 (Jumbo) 11 (Navaho) and 12 (Ness) are blackberry cultivars. Lanes 2 and 3 is Boysenberry. Lanes 13 (Canby), 14 (Heritage) and 15 (Tulameen) are raspberry cultivars. Arrow heads point to AFLP markers amplified only in Boysenberry but not blackberry or raspberry.

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