Pak. J. Bot., 42(2): 1009-1012, 2010.

GENETIC DIVERSITY AMONG SOME CURRANTS (*RIBES* SPP.) CULTIVARS AS ASSESSED BY AFLP MARKERS

AHMET IPEK*, ERDOGAN BARUT, HATICE GULEN AND MERYEM IPEK

Horticulture Department,
Faculty of Agriculture, Uludag University, Gorukle, Bursa, Turkey.

Abstract

Currants cultivation has increased its popularity in Turkey due to the use of more currants in Turkish cuisine. To provide farmers with well adapted currants cultivars, some currants cultivars have been planted in various geographical regions of Turkey. In this study, genetic diversity among some of these currants cultivars has been analyzed using AFLP markers. Our results indicated that red and black currants genotypes are genetically distinct, sharing very small proportion of AFLP markers. Selected currants genotypes from Turkey shared all AFLP markers suggesting that they might be the same genotype.

Introduction

The berries of wild currants (*Ribes* spp.) are widely consumed locally. Recent increase in its popularity created high consumer demand for currants and Turkish farmers have been establishing new currants orchards to meet this increasing demand. The currants grow as a perennial shrub in many part of the world and grows widespread in Turkey. Although the cultivation of currants in Turkey is very recent, the use of soft-bodied currants in the dessert, jams, jellies and frozen-foods in Turkish cuisine increased its popularity significantly. In addition, ascorbic acid content in currant berries has promoted its consumption (Agaoglu, 1986, Barut, 2004).

DNA based markers are found to be a useful tool for plant scientists for establishing phylogenies, tagging desirable genes, determining similarities among breeding materials, cultivar identification, and mapping plant genomes (Li *et al*., 2001, Graham *et al*., 2004, Sargent *et al*., 2007, Lewers *et al*., 2008, Brennan *et al*., 2008, Mattia *et al*., 2008). AFLP (amplified fragment length polymorphisms) markers are highly reproducible multi-locus marker system developed by Vos *et al*., (1995). High levels of polymorphism and high degrees of discriminative capacity are the main advantages of this marker system.

To provide farmers with well adapted currants cultivars for their region, a study to determine performance of six currants genotypes in different regions of Turkey was initiated (Agaoglu, 2003). As a part of this study, four currants genotypes have been planted in a field in the Faculty of Agriculture of Uludag University in Bursa in 2000. Presented study reports the genetic relationship among these cultivars using AFLP markers.

Materials and Methods

Plant materials: Four currants cultivars (Red Lake, Silvergieters, Tokat 2 and Tokat 3) were included in this study for AFLP analysis. While Red Lake and Silvergieters are common red and black currants cultivars, respectively, Tokat 2 and Tokat 3 are black currants genotypes with unknown origin.

*Communicating author E-mail: maipek@uludag.edu.tr

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 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 (Invitrogen, Carlsbad, CA, USA) and adjusted to 50ng/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 300ng of genomic DNA with *Mse* I and *Eco*R I enzymes, *Mse* I and *Eco*R I restriction-site derived adapters were ligated to DNA fragments 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, EAGC/MCAT, 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 &Michener, 1958) cluster analysis with NTSYSpc v. 1.80 program (Rohlf, 1993). A dendrogram indicating the estimated similarity among currants genotypes was constructed with TREE program of NTSYSpc.

Results and Discussion

There was a great diversity between red currants and black currants (Fig. 1). High degree of this genetic diversity is probably due to the belonging of black and red currants to different species. While black currants belong to *Ribes nigrum*, red currants can be *Ribes sativum, Ribes rubrum, or Ribes petraeum*. However, the genetic diversity within black currants (within a species) was low, suggesting that black currants genotypes analyzed in this study have common genetic background.

A UPGMA dendrogram demonstrating relative similarity among currants genotypes was developed using the similarity matrix based on Dice coefficient (Fig. 2). According this dendrogram, Red Lake (*R. rubrum*) grouped into distinct cluster from black currants genotypes. The similarity between the species was about 5% (Table 1). Although the similarity between currant species analyzed in this study was very low, they have some common AFLP markers suggesting that these species are genetically close species (Fig 1).

Currants grow in wild in the Black Sea Region of Turkey. Berries of wild currants have been collected from wild and consumed locally. Recently, studies to select currants genotypes with high agronomic value have been initiated. In this respect, selected Tokat-2 and Tokat-3 genotypes are important for development of currants cultivars with good adaptation ability to specific regions of Turkey. In this study, we found that these might be same genotypes since they share 100% AFLP markers (Table 1; Figs. 1 and 2). Therefore, we suggest that selection of genotypes from broader genetic background should be done for the success of such studies.

Fig. 1. Part of polyacrylamide gel picture showing the AFLP banding profile of EAGC/MCAT primer combination.

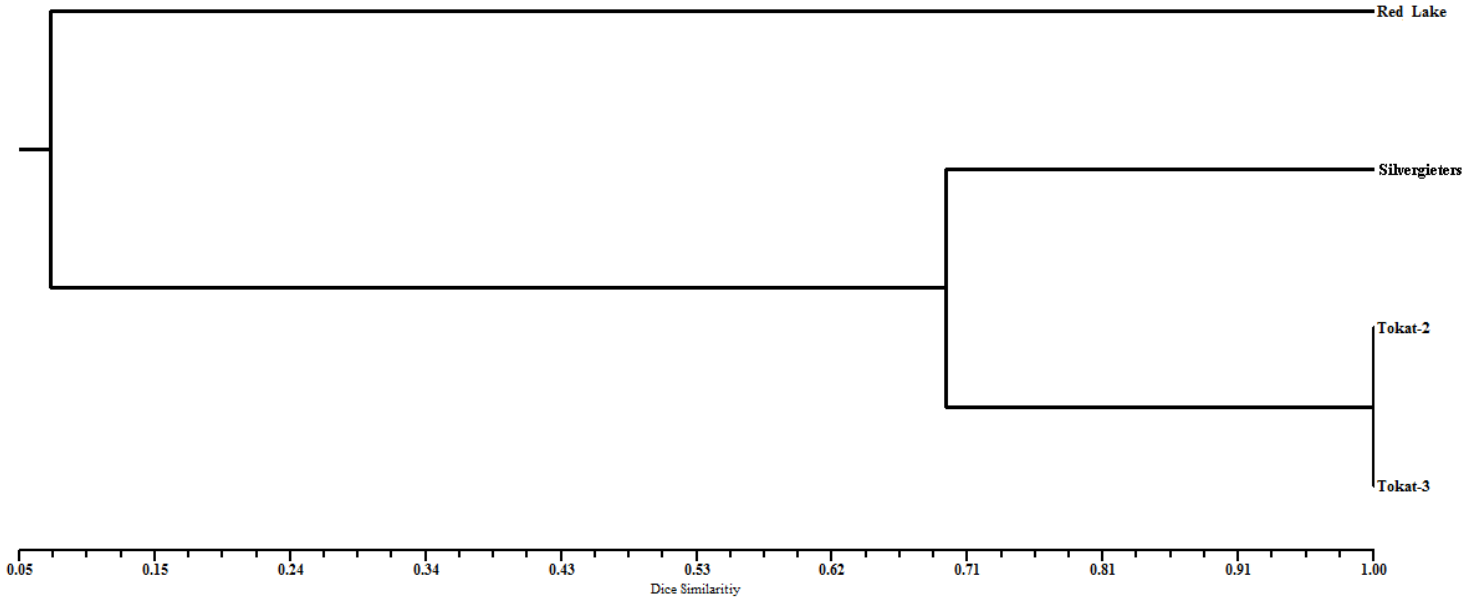

Fig. 2. UPGMA dendrogram based on Dice (1945) coefficient illustrating the estimated genetic relationship among currants genotypes.

Table 1. Similarity matrix among four currants cultivars based on Dice (1945) coefficient.

	Red lake	Silvergieters	Tokat-2	Tokat-3
Red lake	1.00			
Silvergieters	0.05	1.00		
Tokat-2	0.08	0.70	1.00	
Tokat-3	0.08	0.70	1.00	1.00

In conclusion, genetic diversity studies with more currants genotypes could be helpful to assess the genetic relationship between and within currant species which can help to improve the efficiency of future breeding efforts and other genetic studies.

Acknowledgment

Authors wish to thank to Uludag University Commission of Scientific Research Projects for their financial support for this study through project, Z-2006/57.

References

Agaoglu, Y.S. 1986. Üzümsü Meyveler. Ankara Üniversitesi Ziraat Fakültesi Yayınları. No: 984, pp 377.

Agaoglu, Y.S. 2003. Past, Present and Future of Small Fruits in Turkey. I. National Kiwifruit and Small Fruits Symposium, Ordu. pp. 1-13.

Barut, E. 2004. Dünya ve Türkiye'de Üzümsü Meyve Yetiştiriciliği ve Ticareti. Türktarım Dergisi, 156: 60-67

Brennan, R., J. Jorgensen, H. Christine, S. Gordon, M. Woodhead and R. Joanne. 2008. The development of a genetic linkage map of blackcurrant (*Ribes nigrum* L.) and the identification of regions associated with key fruit quality and agronomic traits. *Euphytica*, 161: 19-34.

Dice, L.R. 1945. Measures of the amount of ecological association between species. *Ecology*, 26: 297-302.

Fütterer, J., A. Gisel, V. Iglesias, A. Kloti, B. Kost, O. Mittelsten-Scheid, G. Neuhaus, G. Neuhaus-Url, M. Schrott, R. Shillito, G. Spangenberg and Z.Y. Wang. 1995. Standard molecular techniques for the analysis of transgenic plants. In: *Gene Transfer to Plants*. (Eds.): I. Potrykus, G. Spangenberg. Springer-Verlag, New York, pp. 215-218.

Graham, J., K. Smith, K. MacKenzie, L. Jorgenson, C. Hackett and W. Powell. 2004. The construction of a genetic linkage map of red raspberry (*Rubus idaeus* subsp. *idaeus*) based on AFLPs, genomic-SSR and EST-SSR markers. *Theor. Appl. Genet*., 109: 740-749.

Lewers, K.S., C.A. Saski, B.J. Cuthbertson, D.C. Henry, M.E. Staton, D.S. Main, A.L. Dhanaraj, L.J. Rowland and J.P. Tomkins. 2008. A blackberry (*Rubus* L.) expressed sequence tag library for the development of simple sequence repeat markers. *BMC Plant Biol*., 8: 69-76.

Li, C.D., C.A. Fatokun, B. Ubi, B.B. Singh and G.J. Scoles. 2001. Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellite markers. *Crop Sci*., 41: 189-197.

Mattia, F.D., F. Grassi, S. Imazi1 and M. Labra. 2008. Chloroplast and nuclear DNA markers to characterize cultivatedand spontaneous *Ribes. Plant Biosystems*, 142: 204-212.

Rohlf, F.J. 1993. NTSYS-pc, Numerical Taxanomy and Multivariate Analysis System. Exeter Publ., Setauket, NY.

Sargent, D.J., F. Fernández-Fernández, A. Rys, V.H. Knight, D.W. Simpson and K.R. Tobutt. 2007. Mapping of *A*1 conferring resistance to the aphid *Amphorophora idaei* and *dw* (dwarfing habit) in red raspberry (*Rubus idaeus* L.) using AFLP and microsatellite markers. *BMC Plant Biol*., 7: 15.

Sokal, R.R. and C.D. Michener. 1958. A statistical method for evaluating systematic relationships. *Univ. Kansas Sci. Bull*., 38: 1409-1438.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res*., 23: 4407-4414.

(Received for publication 19 February 2009)