AcademicPres

Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic ISSN 1842-4309

Not. Bot. Hort. Agrobot. Cluj 37 (1) 2009, 261-264

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Genotypic Variability of the Main Apple Cultivars Grown in Transylvania, Romania, Evaluated by Means of RAPD Analysis

Ioana MITRE [1)], Lehel LUKÁCS [1)], Marin ARDELEAN [1)], Viorel MITRE [1)]
Radu SESTRAS [1)], Rodica POP [1)], Mirela CORDEA [1)]

[1)] *University of Agricultural Sciences and Veterinary Medicine, 3-5 Manastur Street, 400372, Cluj-Napoca, Romania; ioanamitre@yahoo.com*

Abstract

Five apple cultivars ('Florina', 'Golden Delicious', 'Idared', 'Jonathan' and Starkrimson), were tested in 2004 and 2005 in five commercial orchards located in different counties of Transylvania, Romania, with quite nonsimilar environments. In June, young shoots were harvested from each cultivar and taken to the laboratory by means of a refrigerating bag. Young leaves were harvested from the shoots and preserved in refrigerator at –70°C until the DNA extraction was performed. Twelve decamer primers were used for DNA amplification out of which ten produced polymorphic bands in each cultivar/location. A dendrograms was constructed based on DNA migration in agarose gel and analysis of formed bands and genetic distances, for revealing the intervarietal polymorphism in the five locations. As it has been expected, at the molecular level there were great differences among the tested cultivars, but the dendrogram also exhibited the existence of obvious differences, at the molecular level, within the same cultivar, depending on location. Such differences should be attributed mainly to the lack of uniformity of initial mother plants used in the process of planting material production for the five cultivars under study. It is concluded that RAPD analysis could successfully be used in checking the authenticity of planting material for apple trees produced in different nurseries, provided there are available standard forms of the interested cultivars from which DNA could be analyzed and used as control.

Keywords: apple cultivars, locations, DNA, RAPD markers

Introduction

There are several molecular markers techniques using on apple for identification cultivars, selecting the most interesting parental forms for crossing or for checking the authenticity of planting material for apple trees produced in different nurseries (Koller *et al*., 1993; Tignon *et al*., 2000; Galli *et al*., 2005).

Random Amplified Polymorphic DNA (RAPD) analysis has become lately a routine procedure for fingerprinting horticultural species including apple cultivars in order to identity intra- and intervarietal diversity at the molecular level (Pop *et al*., 2003; Ardelean *et al*., 2007). This type of analysis has been used in checking the homogeneity, at the molecular level, of planting materials produced for fruit trees by different nurseries. An accurate characterization of the existing cultivars is essential to successful breeding programs, patent protection and nursery control (Tartarini and Sansavini, 2003; Goulao and Oliveira, 2001).

The aim of this research was to reveal the possible differences, at the molecular level, within the planting material of five apple varieties produced in five different nurseries, by means of RAPD analysis. The results of such experiments could be of great help both in apple breeding programs and in the national network of planting material production for apple trees.

Materials and methods

Plant materials

Five apple cultivars ('Florina', 'Golden Delicious', 'Idared', 'Jonathan' and 'Starkrimson'), located in five commercial orchards, in different counties of Transylvania, Romania, with quite nonsimilar environments, were tested in 2004 and 2005. The data regarding sources of plant material, locations and geographical position are presented in Tab. 1. In June, young shoots were harvested from each cultivar and taken to the laboratory by means of a refrigerating bag. Young leaves were harvested from the shoots and preserved in refrigerator at –70°C until the DNA extraction was performed.

DNA extraction

Genomic DNA was extracted according to Lodhi *et al*. (1997) protocol, modified by Pop *et al*. (2003). The concentration and purity of each sample was estimated by means of Eppendorf BioPhotometer.

Tab. 1. Cultivars, locations and their geographical position in Transylvania

Cultivar	Symbol for cultivar	Counties	Geographical position	Location	Symbol for location
'Starkrimson'	STA	Cluj	Central Transylvania	Cluj-Napoca	C
'Florina'	FLO	Bistrita-Nasaud	North-Eastern Transylvania	Mihaesti	M
'Idared'	IDA	Mures	South-Central Transylvania	Reghin	R
'Golden Delicious'	GOL	Timis	South-Western Transylvania	Timisoara	T
'Jonathan'	JON	Maramures	North-Western Transylvania	Seini	S

DNA amplification

The basic method for RAPD was as described by Otoni *et al*. (1995). There were used 12 primers (Mycroshinth) out of which only ten produced polymorphic bands (Tab. 2). The amplified DNA segments were migrated in 1.4% agarose gels prepared in 0.5 x TBE buffer. Electrophoresis was performed at 0.57 V cm-1 for 150 min. Visualization of amplification products was performed in UV light, the image of gels being taken over by means of a video camera Alpha Innotech.

Data analysis

Total Lab 100 software was used to select only the high light intensity bands and consequently the binary mould was obtained. RAPDistance 1.04 software was used for computing the genetic distances and, on this basis, Jaccard`s coefficient was calculated for each species. Cluster analysis was performed using the Neighbor-Joining method and the results were presented as a dendrogram generated by RAPDistance 1.04 pack of soft.

Results and discussion

The dendrogram constructed based on genetic distances among cultivars in all five location has been thoroughly discussed by Ardelean *et al*. (2007). The analysis of this dendrogram clearly demonstrated that, among the five apple cultivars, in all five locations, there were obvious differences at the molecular level, which meant that each of the five cultivars represented a distinct genetic entity, easily recognizable at the molecular level.

These assumptions would allow one to expect that each tested cultivar showed the same DNA pattern at the molecular level, no matter which nursery the trees were originated in, but the dendrogram presented in Fig. 1 obviously contradicts such expectations. Actually, in all five tested cultivars, there has been noted an obvious polymorphism, at the molecular level, illustrated by more or less large genetic distances within the same cultivar depending on location. This polymorphism was rather low in 'Jonathan' and 'Starkrimson' but high and very high in 'Golden Delicious' an especially in 'Florina'. In 'Florina', the trees analyzed in Reghin and Seini (two counties of Transylvania rather far from each other) were found more closely related at the molecular level with 'Golden Delicious' and 'Idared' than with 'Florina' grown in Timisoara, Mihaesti and Cluj.

The significant variability revealed at the molecular level by RAPD analyses within the same cultivar depending on location of testing can only satisfactorily be explained by an improper management of the planting material production This explanation implies the assumption that, in different nurseries, the mother plants belonged rather to officially released or locally grown clones of the cultivar to be multiplied than to the standard form of the respective cultivar.

It is worth observing that these results do not concord with those obtained by Pop *et al*. (2003) in grapes. Their results showed no variability, at the molecular level, for ten grape cultivars depending on their location of origin. These results are quite normal considering the fact that all grape nurseries in Romania acquired their grafting material from only two or three mother plant vineyards (Valea Calugareasca, Blaj and Murfatlar). For fruit trees, each Fruit Experimental Station had its own mother plant orchards for cultivars to be multiplied and, most probably, the mother plants were rather officially released or local clones of such cultivars.

Tab. 2. Primers used for DNA amplification and number of distinct bands produced, Cluj-Napoca, 2004-2005

Primer	Primer sequence	Polymorphic bands	Primer	Primer sequence	Polymorphic bands
OPAB-18	5`-CTGGCGAATG -3`	9	OPB-10	5`-TGGCGCAGTC -3`	8
OPA-04	5`-AATCGGGCTG -3`	9	OPB-17	5`-TGCGTGCTTG -3`	9
OPA-01	5`-CAGGCCCTTC -3`	7	OPB-08	5`- GACGGATCAG -3`	6
OPA-03	5`- AGTCAGCCAC-3`	9	OPX-03	5`- TGGCGCAGTC -3`	9
OPAL-20	5`-AGGAGTCGGA -3`	9	OPC-14	5`- TGCGTGCTTG -3`	8
	Total	43		Total	36
		Total			79

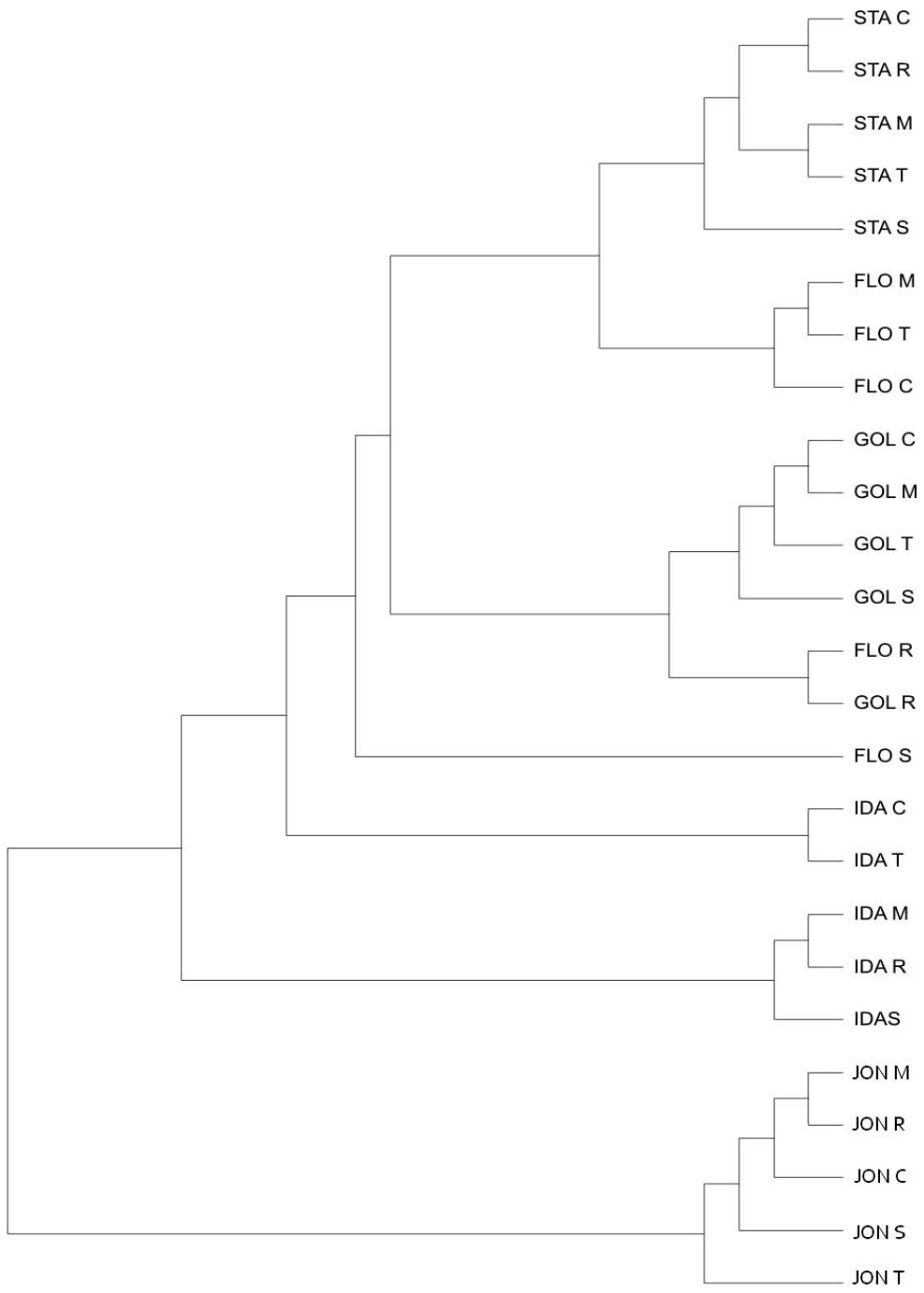

Fig. 1. Dendrogram generated on the basis of genetic distances among cultivars grown in different locations of Transylvania

Conclusions

Based on results discussed in this paper, it can be stated that RAPD analysis fairly illustrated the genetic differences among the five apple cultivars under study emphasizing, at the same time, the phylogenetic relationships existent among them.

There can be admitted that each of the five cultivars represented a distinct genetic entity, easily recognizable at the molecular level.

The dendrogram constructed on the basis of genetic distances among proveniences within the same cultivar revealed a significant variability, at the molecular level, which can only satisfactorily be explained by an improper management of the planting material production. This explanation implies the assumption that, in different nurseries, the mother plants belonged rather to clones of the cultivar to be multiplied than to the standard form of the respective cultivar.

RAPD analysis proved to be a reliable tool for evaluating the authenticity of planting material for apple trees

produced in different nurseries, provided there are available standard forms of the interested cultivars from which DNA could be analyzed and used as control.

References

Ardelean, M., V. Mitre, L. Lukacs, R. Pop, I. Mitre and M. Cordea (2007). Efficiency of nonparametric statistics in evaluating phenotypic variability and of RAPD analysis in evaluating variability at the molecular level, of the main apple varieties grown in Transylvania. Bul.USAMV-CN, 64 (1-2), 29-33.

Galli, Z., G. Halász, E. Kiss, L. Heszky and J. Dobránszki (2005). Molecular identification of commercial apple cultivars with microsatellite markers. HortScience 40(7): 1974-1977.

Goulao, L. and C. M. Oliveira (2001). Molecular characterization of cultivars of apple (*Malus* × *domestica* Borkh.) using microsatellite (SSR and ISSR) markers. Euphytica. 122:81-89.

Koller, B., A. Lehman, J.M. McDermott and C. Gessler (1993). Identification of apple cultivars using RAPD markers. Theor. Appl. Genet. 85:901-904.

Lodhi, M.A., N.F. Weeden and B.I. Reisch (1997). Characterization of RAPD markers in *Vitis*. Vitis 36: 133-140.

Otoni, W.C., N.W. Blackhall, F.B. D`Utra Vaz, V.W. Casali, J.B. Power, M.R. Davey (1995). Somatic hybridization of the *Passiflora species*, *P. edulis* f. Flavicarpa degener and *P. incarnata* L. Journal Exp. Bot. 46:777-785.

Pop, R., M. Ardelean, D. Pamfil and I. Gaboreanu (2003). The efficiency of different DNA isolation and purification protocols in ten cultivars of *Vitis vinifera*. Bul. USAMV-CN, 59, ser. ZB, 259-261.

Tartarini, S. and S. Sansavini. (2003). The use of molecular markers in pome fruit breeding. Acta Hort. 622: 129-140.

Tignon, M., R. Kettmann and B. Watillon (2000). AFLP: Use for the Identification of Apple Cultivars and Mutants. Acta Hort. 521:219-226.