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AFLP and SSR marker analysis of grape rootstocks in Indian grape germplasm

Anuradha Upadhyay *, Mamtha D. Saboji, Suvarna Reddy [1], Kiran Deokar [1], G.S. Karibasappa

National Research Centre for Grapes, Manjari Farm, Solapur Road, Pune 412307, Maharashtra, India

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Abstract

Twenty-one rootstock accessions were analyzed with seven grape microsatellite (SSR) primers and seven AFLP primer combinations. SSR primers detected 56 alleles across 21 genotypes and primer heterozygosity varied from 0.617 to 0.856. Similarly 252 AFLP bands were obtained with seven primer pairs. The average similarity index for different rootstocks was relatively low and ranged from 0.068 to 0.36 for AFLP data and from 0.13 to 0.36 for SSR data. A combination of three SSR primers was sufficient to distinguish 21 rootstocks. In cluster analysis majority of rootstocks belonging to same species grouped together. Although the dendrograms obtained with two marker systems were not identical, rootstocks belonging to same species or having common parents were grouped together in both the dendrograms.

Keywords: Molecular markers; Grapevine; Rootstocks; Genetic relationship

1. Introduction

The grapes in India are reported to have been introduced in 620 B.C. (Olmo, 1976). Six species of *Vitis* are found in India, mainly in the foothills of Himalayas. Commercial cultivation of grapes started in the beginning of 20th Century and presently grape (*Vitis vinifera*) is successfully grown in India over an area of 60,000 ha with a production of approximately 1.2 million MT (FAO, 2005), mainly for table purpose. Although mainly grown on own roots, use of rootstocks is gradually increasing to combat the problems of biotic and abiotic stresses. While soil borne nematodes are the major biotic factor of concern in some grape growing regions in India, increased soil and irrigation water salinity and drought are the abiotic stresses which greatly affect grape production and productivity in Maharashtra, the largest grape growing state of India.

The superiority of molecular markers over ampelometry for the characterization of grape cultivars is well established. In grape, molecular markers like RFLP (Bourquin et al., 1993), RAPD (Jean Jaques et al., 1993; Grando et al., 1995; Ye et al., 1998; Vidal et al., 1999; Luo and He, 2001; Tamhankar et al., 2001), microsatellite or SSR (Bowers et al., 1993, 1999; Thomas and Scott, 1993; Cipriani et al., 1994; Scott et al., 2000; Pellerone et al., 2001) and AFLP (Labra et al., 1999; Cervera et al., 2000; Martinez et al., 2003) are widely used for characterization of cultivars, parentage analysis, identification of clones, establishing the genetic relationship and molecular mapping. Microsatellites and AFLP are the two very useful classes of molecular markers. Microsatellites have the advantage of codominant mode of inheritance and high reproducibility whereas AFLP is preferred for its multiplex nature and is found useful for the identification of clonal variation in grape (Cervera et al., 1998).

Commercial varieties of grape are extensively analyzed with molecular markers, however only limited reports are available on molecular characterization of grape rootstocks (Bauer and Zyprian, 1997; This et al., 1997; Lin and Walker, 1998; Sefc et al., 1998; Wolf et al., 1999; Fatahi et al., 2003). This study was undertaken to characterize grape rootstocks available in Indian Grape Germplasm at our Centre, using SSR and AFLP markers.

* Corresponding author. Tel.: +91 20 26914245; fax: +91 20 26914246.
E-mail address: anu_upadhyay@yahoo.com (A. Upadhyay).
[1] The authors have contributed equally.

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2. Materials and methods

2.1. Plant material

Twenty-one rootstock varieties collected from different sources were used for the study. The varieties represent different *Vitis* species or their hybrids and are maintained as active field collection at National Research Centre for Grapes, Pune, India. The young fourth leaf was used for extracting DNA from these rootstocks. The details of rootstocks are given in Table 1.

2.2. DNA extraction

DNA from young leaves was extracted using modified CTAB procedure as described by Lodhi et al. (1994). The DNA was estimated spectrophotometrically and quality was checked by agarose gel electrophoresis. The DNA was diluted to a working concentration of 10 ng/μl.

2.3. AFLP analysis

AFLP analysis was performed according to Vos et al. (1995) with slight modifications suitable for silver staining, using AFLP analysis system 1 (Invitrogen Life Technologies, USA). Taq DNA polymerase procured from Bangalore Genei Pvt. Ltd., India was used. Steps till preselective amplification were performed as per the instruction manual. For selective amplification EcoRI primer was diluted 1:3 and 0.5 μl of diluted primer was used per reaction in 20 μl reaction volume. All the 64 primer combinations from the kit were screened with DNA from three genotypes. Seven primer combinations amplifying all the three genotypes were used to analyze 21 rootstocks.

2.4. SSR analysis

A total of seven SSR primers characterized in previous studies were used. The primers were VVS3, VVS4 (Thomas and Scott, 1993), VVMD5, VVMD7 (Bowers et al., 1996), VVMD17, VVMD24 and VVMD31 (Bowers et al., 1999). Primer pairs were synthesized from published sequences (IDT, USA). The PCR reaction in 25 μl volume contained 25 ng DNA, 1.33 μM primer each, 100 μM dNTP each, 3.0 mM $MgCl_2$ and 1.0 U Taq polymerase (Bangalore Genei Pvt. Ltd., India). The PCR was performed on a PTC 200 gradient thermal cycler (MJ Research, USA) using two-step PCR protocol first described by Smith et al. (1995) and successfully used in grape by Sefc et al. (1997) with slight modifications as follows: 5 min at 95 °C followed by 10 cycles of 15 s at 50 °C and 15 s at 94 °C followed by 35 cycles of 15 s at 54 °C and 15 s at 89 °C. The tubes were immediately cooled to 4 °C. A total of 45 cycles of amplification were used to improve intensity of bands in silver staining.

2.5. Electrophoresis and silver staining

Amplified products of AFLP and SSR analysis were mixed with 6× sequencing gel dye (98% formamide, 30 mM EDTA, 0.15% bromophenol blue and 0.15% xylene cyanol), denatured

Table 1
Details of rootstocks used for AFLP and SSR analysis

Sl. no.	Name	Location in NRCG collection	Source of the collection	Species/parent
1	Dogridge	B2/65	MPKV, Rahuri[a]	*V. champinii*
2	1103P	B2/58	MPKV, Rahuri	*V. berlandieri* × *V. rupestris*
3	1616C	B2/61	IIHR, Bangalore[b]	*V. solonis* × *V. riparia*
4	Amtsir	B2/63	RAAS, Russia	Not known
5	DeGrasset	B2/76	ARI, Pune[c]	*V. champinii*
6	161-49	Green house	Champagne Indage Ltd., Narayangaon	*V. berlandieri* × *V. riparia*
7	1613C	B2/72	IIHR, Bangalore	*V. solonis* × *Othello* (*V. vinifera* × (*V. riparia* × *V. labrusca*)
8	Teleki5A	B2/68	IIHR, Bangalore	*V. berlandieri* × *V. riparia*
9	SO4	B2/57	MPKV, Rahuri	*V. berlandieri* × *V. riparia*
10	Freedom	Green house	Champagne Indage Ltd., Narayangaon	*V. champinii* × 1613C
11	41B	Green house	Champagne Indage Ltd., Narayangaon	*V. vinifera* × *V. berlandieri*
12	99R	B2/60	IIHR, Bangalore	*V. berlandieri* × *V. rupestris*
13	St. George	B2/69	IIHR, Bangalore	*V. rupestris*
14	*V. champinii* 1	B2/62	IIHR, Bangalore	*V. champinii*
15	Salt Creek	B2/74	IIHR, Bangalore	*V. champinii*
16	*V. longii*	B2/59-1	MPKV, Rahuri	*V. longii*
17	B2/56	B2/56-1	Farmer's field	Doubtful
18	110R	B2/75-1	ARI, Pune	*V. berlandieri* × *V. rupestris*
19	*V. champinii* 2	B2/40-1	NBPGR, Hyderabad[d]	*V. champinii*
20	SauvisIP	B2/43-1	NBPGR, Hyderabad	*V. champinii*
21	R × R	Green house	IIHR, Bangalore	*V. rupestris* × *V. riparia*

[a] Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India.
[b] Indian Institute of Horticultural Research; Bangalore, Karnataka, India.
[c] Agharkar Research Institute; Pune, Maharashtra, India.
[d] National Bureau of Plant Genetic Resources, Regional Station, Hyderabad, Andhra Pradesh, India.