

Full Length Research Paper

Molecular marker analysis to differentiate a clonal selection of Centennial Seedless grapevine

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Accepted 1 March, 2013

Microsatellite and amplified fragment length polymorphism (AFLP) markers were used to differentiate Manjari Naveen, a clonal selection of Centennial Seedless variety of grape. Twenty one (21) microsatellite primers could not detect variation between parent variety and its clone. AFLP analysis with 40 primer combinations generated 1093 markers. Three AFLP markers were polymorphic; two markers were present only in Centennial Seedless, whereas one was present only in Manjari Naveen. These markers will be useful for establishing genetic identity, variety registration and protection of breeder's right.

Key words: *Vitis vinifera*, AFLP, microsatellites, clonal selection.

INTRODUCTION

Grapevine (*Vitis vinifera* L) is one of the most important fruit crops in India, presently cultivated over an area of about 110 000 ha, and mainly used for table purposes. Development of varieties suitable for different grape growing regions of the country and requiring minimum inputs in term of agrochemicals and labor is the focus of breeding, clonal selection is an important method of cultivar improvement in grapes. Clones with phenotypic variations are observed due to spontaneous somatic mutation in the regenerative cells. The clones with considerable difference in traits are considered as different cultivars, the most important example being the clones of Pinot viz. Pinot Noir, Pinot Blanc, Pinot Gris, each one of which is recognized as a different cultivar. A clonal selection of grape variety Centennial Seedless was identified at National Research Centre for Grapes in 2002 with a balanced canopy, self-thinned bunches and ripening early by about 20 to 25 days at Pune as compared to Thompson Seedless, the predominant table variety. This selection was further evaluated for several years in multi-locational trials and was released as Manjari Naveen in 2008 for commercial cultivation in

India, for table and raisin purpose.

It is important to characterize this selection in order to establish its genetic identity for variety registration and protection. Molecular markers are the most efficient tools for genetic characterization and variety identification. While microsatellite markers have proven to be effective for varietal identification, amplified fragment length polymorphism (AFLP) markers which scan larger proportion of the genome have been more useful for the identification of grape somatic mutants and clonal variants (Blanch et al., 2007; Upadhyay et al., 2011). The aim of this study was therefore, to identify a molecular marker to distinguish Manjari Naveen from its parent Centennial Seedless.

MATERIALS AND METHODS

Six years old vines of *Vitis vinifera* L. var. Centennial Seedless and Manjari Naveen grown at research farms of National Research Centre for Grapes, Pune (latitude 18.5236° N, longitude 73.8478° E) were used for the study. Young fresh leaf samples were collected and DNA was extracted using the DNeasy plant mini kit (Qiagen, Hilden, Germany), following the supplier's instructions. The DNA concentration was determined on nanophotometer (IMPLEN) and quality was tested by agarose gel electrophoresis. Twenty one (21) microsatellite primers used in this study were VMC4f8, VMC7b1, VMC7f2, VMC7g3, VMC8g3.2, VrZAG62, VrZAG79, VVIB23, VVIB63, VVII52, VVIP17A, VVIP22, VVIP25B,

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Table 1. Details of AFLP analysis of Centennial Seedless and Manjari Naveen.

Primer pair record	No.
Total number of selective primer pairs used	40
Number of primer pairs resulting amplification	37
Total number of AFLP markers obtained	1093
Average number of markers per primer pair	29
Number of polymorphic markers	3

VVIP31, VVIP44, VVIV15B, VVIV16, VVIV33, VVMD21 and VVMD27. The microsatellite analysis was performed as follows. The polymerase chain reaction (PCR) reaction mixture (10 µL) contained 20 ng genomic DNA; 1X PCR buffer; 100 µM of each dNTP; 2.5 mM MgCl₂; 0.66 µM forward primer labeled with FAM, VIC or NED, 0.66 µM reverse primer and 1.0 U of Taq-polymerase (Bangalore Genei Pvt. Ltd., India). The temperature profile consisted of the following steps: 10 min at 94°C followed by 35 cycles for 1 min at 94°C, 1 min at 54 or 56°C and 1 min at 72°C and a final extension for 10 min at 72°C. PCR products were analyzed on ABI 3130 genetic analyzer using 36 cm capillary filled with POP7 polymer. GeneMapper Version 4.0 (Applied Biosystems, USA) was used to determine the peak size using local Southern method and allele call.

AFLP analysis was performed using AFLP analysis system I (Invitrogen Life Technologies, USA) following manufacturer's instructions with some modifications as reported earlier in Upadhyay et al. (2011). In brief, 500 ng DNA was used to restrict digestion with *EcoRI-MseI*, followed by adaptor ligation and pre amplification as per the manufacturer's instructions. However, selective amplification fluorescently labeled *EcoRI* primers (Applied biosystems, USA) were used in combination with *MseI* primers. A total of 40 primer combinations were studied. After amplification, 1 µL of amplified product was added to 15 µL formamide containing 0.1 µL ROX internal size standard and denatured at 94°C for 5 min and analyzed on 3130 genetic analyzer (Applied Biosystems, USA) using a 36 cm capillary filled with POP7 polymer. GeneMapper Version 4.0 (Applied Biosystems) was used for data scoring, following the instructions given in the AFLP system analysis getting started guide (Applied Biosystems, USA). All primer combinations that produced polymorphic AFLP markers were repeated to confirm the results.

RESULTS AND DISCUSSION

Twenty one (21) microsatellite primers detected 32 alleles in Centennial Seedless and Manjari Naveen. These two genotypes were heterozygous at 11 loci and two alleles were detected at these 11 loci. However the allele profiles of Centennial Seedless and its clone Manjari Naveen were identical at all the 21 loci. Thus these primers failed to detect any variation between these two genotypes. The probability of identical genotypes for these primers has been estimated to be 7.5×10^{-23} in a separate set of 47 grape genotypes in our laboratory. Thus identical allele profile at 21 loci confirmed the varietal origin of Manjari Naveen. There are contrasting reports on the ability of microsatellite primers to differentiate the grapevine clones.

Microsatellites either fail to differentiate the clones or show limited genetic variability among clones of different varieties. Attempts have also been made to develop highly variable microsatellite loci for clones of a variety (Regner et al., 2006) but the use of microsatellite markers for differentiating the clones of a variety remains limited.

AFLP analysis of Centennial Seedless and its clone Manjari Naveen (Table 1) produced three reproducible AFLP markers that differentiated the mutant clone from its parental genotype. Out of 40 *EcoRI:MseI* AFLP primer pairs used for the selective amplification, 37 primer pairs amplified both the genotypes. A total of 1093 markers were obtained with 37 primer pairs. The number of markers detected by each primer pair ranged from 12 to 55 with an average of 29 markers per primer combination. Thirty four (34) primer pairs resulted in identical AFLP fragment pattern among two genotypes. However, variation between Centennial Seedless and Manjari Naveen was detected by three primer pairs. Primers E-AGG:M-CAC and E-ACC:M-CAG gave AFLP markers of 438 bp and 280 bp size respectively, which were detected only in Centennial Seedless (Figure 1). Another AFLP marker of 408 bp size obtained with primer pair E-AAG:M-CAG was present in Manjari Naveen but absent in Centennial Seedless (Figure 2).

AFLP marker technique has good resolving power, which makes it suitable system for the detection of somatic mutations responsible for clonal variation. High resolution power is due to high polymorphic index and ability to scan wider genome area. The efficiency of AFLP can further be enhanced by using large number of primers and using different restriction enzymes to target the variations arising due to point mutations in restriction sites for different enzymes. We have used a large number of primers in this study and were able to differentiate clone Manjari Naveen from its parent Centennial Seedless though; the percentage of polymorphic bands was little. Several works have reported successful use of AFLP to distinguish clonal selections of a variety (Scott et al., 2000; Upadhyay et al., 2011).

AFLPs have also been used for clonal differentiation of cultivars like Pinot Noir (Blaich et al., 2007), Primitivo (Fanizza et al., 2005), Riesling (Anhalt et al., 2011) and several others. On the other hand, there are reports of inability of AFLP markers to differentiate the clones of a variety (Fanizza et al., 2003). Thus AFLP analysis was successfully used to differentiate Manjari Naveen and Centennial Seedless. The available markers will be useful for identification and variety protection.

ACKNOWLEDGEMENT

The authors acknowledge the financial assistance from the Department of Biotechnology, Government of India, New Delhi.

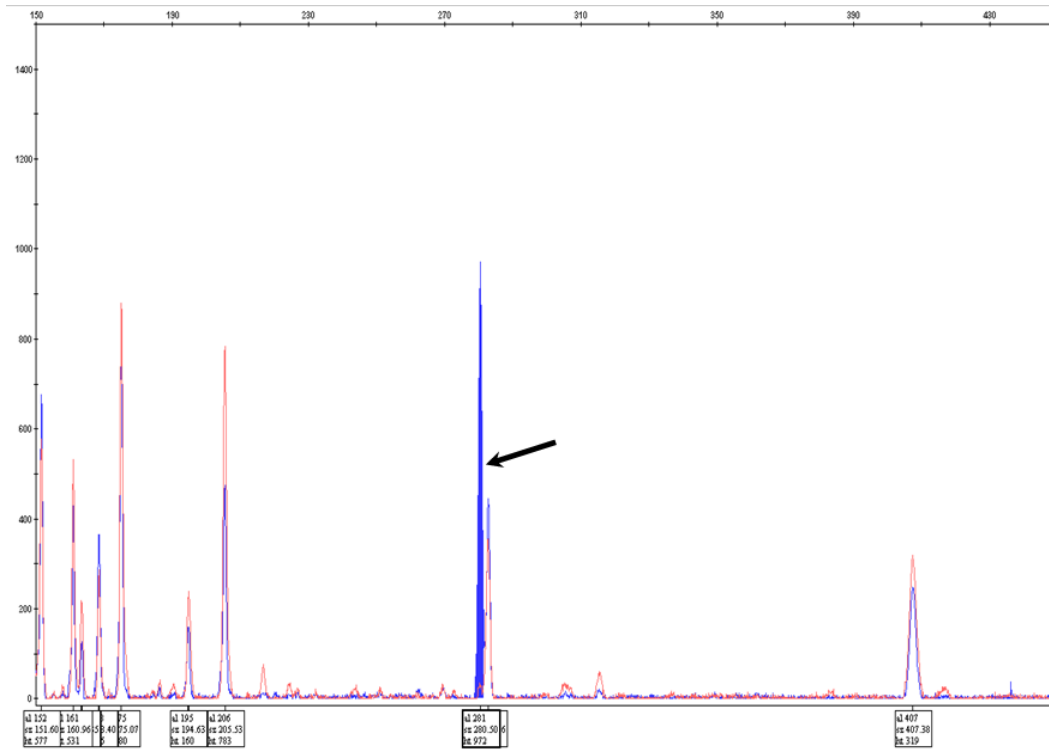


Figure 1. Polymorphic AFLP marker present in Centennial Seedless.

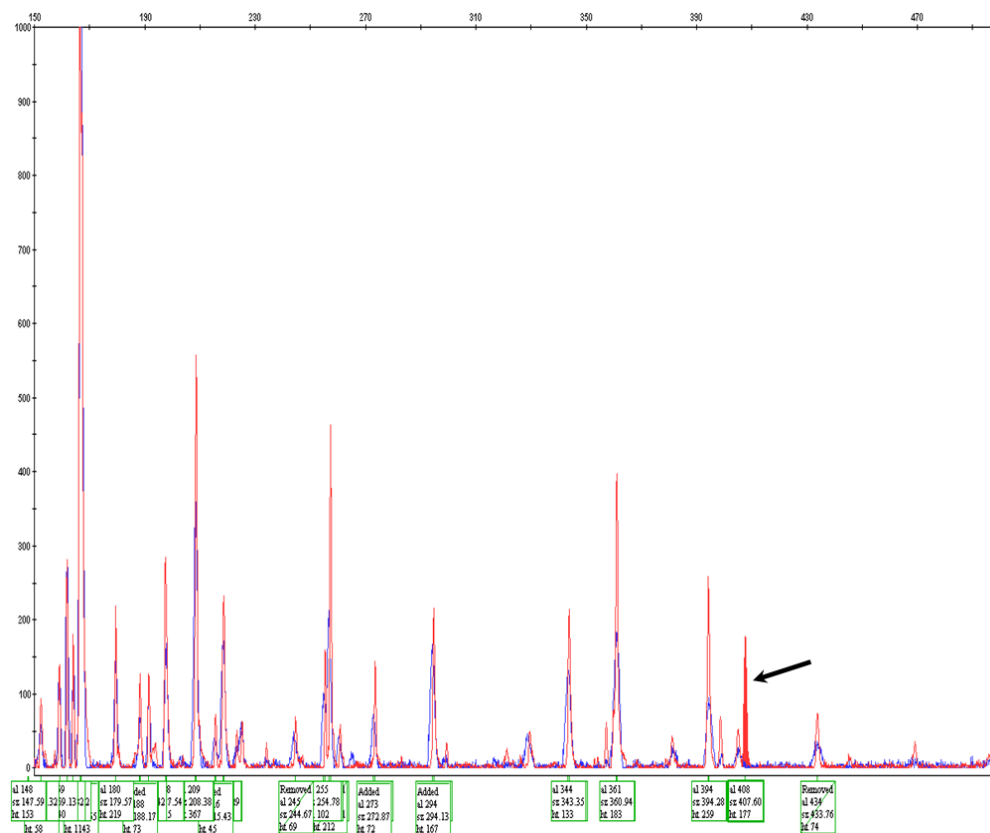


Figure 2. Polymorphic AFLP marker present in Manjri Naveen.

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