Indian J. Hort. 67(2), June 2010: 260-263

Short communication

Microsatellite analysis to differentiate clones of Thompson Seedless grapevine

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Grapevine (*Vitis vinifera* L.) is a vegetatively propagated fruit crop and all the vines of a cultivar are genetically identical. However, it is common to observe superior individuals of a cultivar in the vineyards. Such superior individuals, called clonal selections are identified and propagated. Several factors like mutations, pathogen load, epigenetic differences, and a combination of these factors are considered to be responsive for this phenomenon.

Among table grapes, Thompson Seedless is the most prominent variety world over and is also used for raisin, juice and wine production. The variety gets its name from William Thompson who planted a few seedlings of Lady deCoverly in 1878 in California and later renamed as Thompson Seedless (Winkler *et al*., 8). This variety is grown worldwide and is known by different names. Vitis International Variety Catalogue (www.vivc.bafz.de) has listed over 100 synonyms for this variety. The variety has been improved largely by clonal selection and is widely used as a parent for the development of seedless table varieties. It is the most preferred variety in India and is grown over 80% of 65,000 ha grape growing area (Shikhamany, 5). During last six decades of its cultivation in India, several clones have been identified and many of these clones have established and enjoy good consumer acceptance and occupy good share in domestic and export market. Tas-a-Ganesh and Sonaka are the two most popular clones of Thompson Seedless identified in India.

DNA based molecular markers are widely used to differentiate grape species and varieties, parentage analysis and analyzing geographical evolution. RAPD, RFLP, SSRs and AFLP are successfully used for grapevine analysis. However, identification of clonal or somatic mutants is still challenging. RAPD, SSRs and AFLP markers have been used to differentiate clones of single cultivars with varying degree of success.

Microsatellite or simple sequence repeat (SSR) markers are considered to be the most useful marker system for varietal identification and germplasm management. However, contradictory reports are available for the ability of microsatellites for clonal differentiation. Regner *et al.* (4) reported sparse polymorphism for SSR primers in White Riesling genotype. Similarly, limited variability was observed in the clones of Sangiovese by Vignani *et al.* (7). Gonzalez-Techera *et al.* (1) also detected the variation in clones of Tannat with only one of the 89 microsatellite primers. Clones of Cabernet Sauvignon collected from different geographical regions could be distinguished when analyzed with large number of microsatellite primers (Moncada *et al.*, 3). Moncada and Hinrichsen (2) found limited genetic diversity in the clones of Carmenère employing microsatellite primers.

In this paper, we report the detection of variability among the 23 clones of Thomson Seedless using microsatellite markers. Plant material included Thompson Seedless obtained from different countries, its known synonyms and clonal selections. These accessions (Table 1) are maintained in germplasm collection at National Research Centre for Grapes, Pune.

DNA from young leaves was extracted using DNeasy® Plant kit (Qiagen, CA, USA). Ten microsatellite primers were used for the analysis. These primers are VVMD5, VVMD7, VVMD27, VVS2, VrZAG62, VrZAG79, VVMD31, VVMD32, VVMD21 and VVIB01. The PCR amplification reaction mixture (10 µl) contained 10 ng DNA, 0.66 µM forward primer labeled with *FAM, VIC* or *NED*, 0.66 µM reverse primer, 100 µM of each dNTP, 3.0 mM $MgCl_2$ and 1.0 U *Taq* polymerase (Bangalore Genei Pvt. Ltd., India). The PCR was performed either on a PTC 200 gradient thermal cycler (MJ Research, USA) or GeneAmp PCR system 9700 (Applied Biosystems, USA). The temperature profile consisted of the following steps: 10 min. at 94 °C followed by 35 cycles of 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 diluted 50 times and 1 µl (for *FAM* and *VIC* labeled) or 2 µl (for *NED* labeled) of diluted mix was added to a mixture of 10 µl HI-DI formamide and 0.10 µl of GeneScan 500 ROX internal size standard. The mix was denatured at 94°C for 5 min. and analyzed on ABI 3130 genetic analyzer using 36 cm capillary filled with POP7 polymer. GeneMapper ver 4.0 was used to determine the peak size using local Southern method and allele call. PCR reactions and resolution were repeated once again to confirm the results.

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Table 1. Microsatellite profile of Thompson Seedless clones at 10 loci.

Sl. No.	Thompson Seedless clone	Source of collection	Microsatellite marker									
			VVS2	VVMD5	VVMD7	VVMD27	VrZAg62	VrZAG79	VVMD31	VVMD32	VVIB01	VVMD21
1.	2A clone	California	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257
2.	TS	California	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257
3.	TS	France	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257
4.	TS	Italy	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257
5.	Sultana	Australia	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257
6.	H5 clone	Australia	144:151	234	241:254	179:192	188	249:260	212	253	292:299	251:257
7.	Kishmish Belyi	Russia	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257
8.	TS	Tamil Nadu	144:151	234	241:254	179	188	249:260	212	252	292:299	251:257
9.	TS	Abohar	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257
10.	TS	Boregaon	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257
11.	TS mutant 1	Borgaon	144	234	241:254	179:192	188	249:260	212	252	292:299	251:257
12.	TS mutant 2	Borgaon	144	234	241:254	179:192	188	249:260	212	252	292:299	251:257
13.	TAG 1	Borgaon	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257
14.	TAG 2	Borgaon	144:151	234	241:254	179	188	249:260	212	252	292:299	251:257
15.	Manik Chaman	Solapur	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257
16.	Sonaka	Solapur	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257
17.	Sonaka mutant	Sangli	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257
18.	Maruti Seedless	Sangli	144:151	234	241:254	179	188	249:260	212	252	292:299	251:257
19.	Pusa Seedless	IARI, New Delhi	144:151	234	241:254	179:192	188	249:260	212	252	288:292	251:257
20.	TAG mutant 1	Koppergaon	144:151	234	241:254	179	188	249:260	212	252	292:299	251:257
21.	TAG mutant 2	Koppergaon	144:151	234	241:254	179	188	249:260	212	252	292:299	251:257
22.	Vijay Chaman	Nashik	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257
23.	B5 clone	Nashik	144:151	234	241:254	179:192	188	249:260	212	252	292:299	251:257

TS – Thompson Seedless, TAG – Tas-A-Ganesh.

The microsatellite profile of 23 clones with 10 primers is presented in Table 1. As evident no variation among these clones was observed with seven primers and all the clones had identical profiles. However, sufficient polymorphism was observed for the primers VVS2, VVMD27 and VVIB01. While the Thompson Seedless accessions collected from different sources showed same profile for these primers also, variation was detected among clones originating from Thompson Seedless. For primer VVS2 two different genotypes were obtained; allele combination 144:151 was present in 21 (~91%) clones while two clones (~9%) were homozygous at this locus and had 144 bp allele. The VVMD27 locus was polymorphic in 5 (22%) clones, showing a change from heterozygous (179:192 bp) to homozygous (179 bp) state. In case of locus VVIB01, mutational changes in allele size from 292:299 to 288:299 was observed only in one clone, *i.e.* Pusa Seedless. Although microsatellite analysis could differentiate several of the clones of Thompson Seedless, a few clones like 2A, Vijay Chaman and Manik Chaman could not be distinguished and identical profiles were obtained for them. Accessions which are known synonyms of Thompson Seedless like Kishmish Belyi and Sultana also had identical microsatellite profiles.

Ten microsatellite primers used in the present analysis were highly polymorphic and include six primers recommended as standard set for varietal identification (This *et al.*, 6). The probability of identical genotypes estimated in our laboratory for these 10 primers was 8.9×10^{-14}. In spite of high polymorphic nature, these primers together could detect limited genetic variability among the Thompson Seedless clones. In two of the markers, variation was due to occurrence of null alleles. Earlier, Regner *et al.* (4) and Gonzalez-Techera *et al.* (1) also detected limited genetic variation and occurrence of null alleles among clones of White Riesling and Tannant, respectively. Mutation in microsatellites flanking regions and subsequent loss of annealing site is considered to be the reason for null alleles. However, further studies will be needed to confirm this. Only one primer, VVIB01 detected variation in allele size. The difference in allele size was of 11 bases suggesting that insertion/deletion could be the reason of mutation and not slippage which results in variation by a few bases only. In their analysis of clones of Cabernet Sauvignon, Moncada *et al.* (3) detected variation due to appearance of additional alleles in some of the clones. In contrast, no such variation was detected in this study.

Thompson Seedless is the most prominent table purpose variety, where berry appearance and attributes drive the market price. To achieve most optimum parameters, the farmers resort to heavy use of bio-regulators and other agro-chemicals in grape. This contributes to other environmental loads already existing in the field, resulting in frequent appearance of variants in the field. Many of these variants are improvement over the existing one in terms of berry size, shape and appearance which are readily accepted by consumers.

This study showed the limited genetic variation among Thompson Seedless clones with a set of ten microsatellite primers. Analysis with other marker techniques like AFLP which have high polymorphic index might be useful in differentiating different clonal selections and thus it may be possible to develop unique fingerprints for their identification and subsequent variety registration.

ACKNOWLEDGEMENT

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

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Received: November, 2009; Revised: April, 2010;
Accepted : May, 2010