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Marker assisted selection for downy mildew resistance in table grapes

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ABSTRACT

Downy mildew disease is one of the major risk factors for viticulture. It is mainly caused by fungus, affecting grapevine including berries and leaves. It is controlled by the repetitive applications of fungicides leading to pollution, resistant strain development, pesticide residue etc. Therefore, developing resistant grapevine varieties through breeding programs is the most eco-friendly alternative to dealing with such pathogen. Thus, marker-assisted breeding was initiated at ICAR-National Research Centre for Grapes, Pune to introgress the downy mildew resistance using Thompson Seedless as male parent. The grape genome sequence corresponding to downy mildew QTL 'Rpv3' was analyzed and primers were designed for 10 microsatellite regions. Two primers (VVDM2 and VVDM5) showed polymorphism between resistant and susceptible parents and were validated in 133 F1 progenies of Seyve Villard 12375 × Thompson Seedless. F1 progenies were also screened in-field and in-vitro for their response to the downy mildew pathogen. The co-segregation analysis identified a significant association of VVDM2 allele with downy mildew resistance and will be helpful in early screening of resistant progenies.

Keywords: Grape, Marker-assisted breeding, Downy mildew

INTRODUCTION

Downy mildew caused by *Plasmopara viticola* is economically the most important fungal disease for Indian Viticulture. The fungus needs to be controlled by heavy use of pesticides, leading to environmental issues ultimately affecting health of mankind. The development of resistant strains of pathogen against pesticides, challenges the control of devastating pathogens, besides increasing the cost of grape production.

In India, Thompson Seedless is a leading table purpose grape variety, but highly susceptible to downy mildew. Heavy infection, especially during flowering may result in total yield loss. Every year grape growers spend a lot of money for chemical control of this disease, adversely affecting the economy of grape growers. No table variety with downy mildew resistance is commercially available, since they lack in quality traits of Thompson Seedless. In the current study, Seyve Villard (SV)-12375, the source of downy mildew resistance (*Rpv3*) was used to obtained the larger progeny size as a female parent due to the stenospermocarpy nature of the male parent (Thompson Seedless).

In the world, the efforts are being taken to reduce the application of fungicide in grapevine cultivation, with great emphasis on the genetic improvement of grapevine. Some species *viz*., *Muscadinia rotundifolia, V. rupestris, V. riparia*, and *V. berlandieri* (Merdinoglu *et al*., 5; Marguerit *et al*., 4) and *V. amurensis* (Moreira *et al*., 6) have been availed in breeding programs to develop resistant varieties. Downy mildew resistance is a quantitative trait. Conventional breeding to develop a resistant variety is very time consuming. Marker-assisted breeding shortens the time, required to develop a variety considerably. Several studies have identified genomic regions associated with disease resistance. Marker assisted selection (MAS) enables breeders to use the molecular markers flanking the genomic regions giving rise to disease resistance. The genomic regions conferring resistance to downy mildew, which are referred to as "resistance to *Plasmopara viticola* (*Rpv*)", have also been mapped (Bellin, 1) in different resistance sources, and a number of quantitative trait loci (QTLs) have been identified. QTL *Rpv*3 located on chromosome 18 is a major locus conferring significant resistance to downy mildew (Welter *et al*., 14). This locus is responsible for eliciting the hypersensitive response (HR) at infection sites within two days after the inoculation (Casagrande *et al.*, 3). The main objective of the program was to develop the microsatellite markers closely linked with downy mildew resistance (*Rpv3*) in order to utilize them in downy mildew resistance table grape breeding programme.

MATERIALS AND METHODS

A total of 133 F_1 hybrids developed from the cross between Seyve Villard (SV)-12375 and Thompson

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Seedless at the research farm of ICAR-National Research Center for Grapes, Pune were used in this experiment. Among the parental material used, Seyve Villard-12375 was the downy mildew resistance donor (*Rpv3*), while Thompson Seedless is a most popular table grape variety.

The hybrids were screened for downy mildew resistance/ susceptibility by *in vitro* as well as *in field*. *In vitro* screening was carried out by leaf disc method as described by (Staudt and Kassemeyer, 12). In-field, screening was conducted during three consecutive natural epiphytotic conditions from 2016 to 2018. The hybrid progenies were rated according to the sacle 1-9 (1 for highly resistance to 9 for highly susceptible) prescribed by UPOV (Fig. 2).

Molecular linkage maps and physical map of grape available in public database were used to select the regions around *Rpv3*. A sequence of 3.0 Mb was searched for the presence of microsatellite repeats. Primers were designed in conserved flanking sequences of 10 evenly spaced repeat regions. The designed primers were screened for polymorphism using 10 grape genotypes with differential disease response from highly susceptible to highly resistant (Thompson Seedless, Carolina Blackrose, James, SV-18402, SV-18315, SV-12364, SV-12375, Manjari Naveen, Rizamat and Siebel).

Microsatellite analysis was performed during 2017-2018 using M13 tailed forward primer (Schuelke, 11), and inclusion of fluorescently labelled M13 primer in PCR reaction mix followed by resolution on 3130 genetic analyzer (Thermo Fisher, USA). The PCR reaction mix consisted of 10 ng genomic DNA as template, 1x PCR buffer, 2.5 mM $MgCl_2$, 0.1 mMdNTPs, 2 pmol reverse primer, 0.2 pmol forward primer, 1.8 pmol M13 primer (CACGACGTTGTAAAACGAC) fluorescently labelled with 6-FAM, NED or VIC dyes, 0.5 U *Taq*DNA polymerase in a total volume of 10. The amplification was carried out in a thermocycler (Applied Biosystems) by touchdown method as follows: After 5 min denaturation at 94°C, 30 cycles were performed with 30 s at 94°C, 45 s at 58 or 56°C (depending on the individual microsatellite marker), 45 sec at 72°C followed by 10 cycles with 94°C for 30 Sec, 53°C for 45 Sec, 72°C for and a final elongation of 10 min at 72°C before cooling to 4°C. The PCR products were resolved by capillary electrophoresis on POP filled 36 cm capillary array using 3130 Genetic analyzer (Applied Biosystems) and allele data processed as described by Upadhyay *et al.* (13). The association of phenotype and genotype data was estimated using the ANOVA-GLM function of software SAS 9.3.

RESULTS AND DISCUSSION

Total 133 F_1 hybrid vines were produced from the cross of Seyve Villard-12375 x Thompson Seedless with the intension of developing resistant hybrids contributed with Thompson Seedless genetic background. The resistance to downy mildew has been shown to be quantitatively inherited (Welter *et al*., 14; Bellin *et al*., 1; Marguerit *et al*., 4; Moreira *et al*., 6). The resistance to downy mildew in SV-12375 is govern by *Rpv3* locus (located on linkage group 18) and is known to be involved in hypersensitive response as a defense against downy mildew pathogen attack (Bellin *et al*., 1).

Grape genotype Seyve Villard-12375, an interspecific hybrid and is a known source of RPV3, was used as female parent to cross with Thompson Seedless. The molecular linkage and physical maps available in public domain were used to extract the sequence in and around *Rpv3* locus positioned on contig NW_003724141.1. A total of 27 microsatellite regions were identified in 3.0 Mb region around *RPV3* locus. These microsatellite regions comprised of di- (15), tri- (11) and tetra- (1) nucleotide repeats. Among these, ten evenly spaced repeats were selected for designing the primers in flanking conserved regions. These newly designed primers were tested for polymorphism in the genotypes with various degree of resistance (Sawant *et al*., 10). Among the 10 primers, VVDM2 (F:5'TGGCTTTATCAAAAATCTAACCA3' and R:5'AAAAGTGGAGACAAAAATTTCA3') and VVDM5 (F: 5'ACCATTGTCATACGCTTCTCT3' and R: 5'GCCTTTTGTCCAATGGTATC3') (Table 1) showed the polymorphism among resistant and susceptible genotypes, and thus used for the analysis of segregating population. In the segregating population, VVDM2 detected three alleles of 183, 186

Table 1. Details of primers for SSR Analysis

		SSR Primer Pair Sequences (5'to 3')			
Primer	Chromosome number	Forward Primer	Reverse primer	Annealing temperature (°C)	Product size (bp)
VVDM2	18	5'TGGCTTTATCAAAAATCTAACCA3'	5'AAAAGTGGAGACAAAAATTTCA3'	56	187
VVDM5	18	5'ACCATTGTCATACGCTTCTCT3'	5'GCCTTTTGTCCAATGGTATC3'	58	154

and 195 bp size, whereas VVDM5 detected alleles of 153, 155 and 166 bp size.

In field screening for the disease incidence, distribution of progenies among different rating classes fitted into a double sigmoid (bell) curve, whereas a typical bell shaped curve was obtained for *in-vitro* screening (Fig. 1). Based on field screening, the number of progenies with a rating of 1 and 3 was 5 and 20, respectively. Progenies with these rating were considered as resistant. Similarly, in *in-vitro* screening, 17 progenies with rating score of 1 and 6 progenies with rating score of 3 were found to be resistant (Table 2). The pattern of progeny distribution confirmed the quantitative resistance for the downy mildew. The additive genetic nature of the trait is also demonstrated by number of worker (Brown and Moore, 2; Saifert *et al.*, 9; Revers *et al.*, 8).

Co-segregation analysis of genotyping and phenotyping data revealed the significant association of marker VVMD2_195 with the resistance, based on *in vitro* as well as field rating data (Table 3). Another marker VVDM2_183 was significant only for field screening data. Allele VVDM2_186 showed significant association with susceptibility, based on field data. Among the alleles of VVMD5, though VVDM5_153 showed statistically significant association, however this marker has skewed distribution, which was present in only 4 progenies. Similarly, the skewed distribution was observed for VVDM5_166, which was found to be associated with susceptibility to downy mildew. These two markers (VVDM5_153 and VVDM5_166) though showing statistical significance were not suitable for marker-assisted selection.

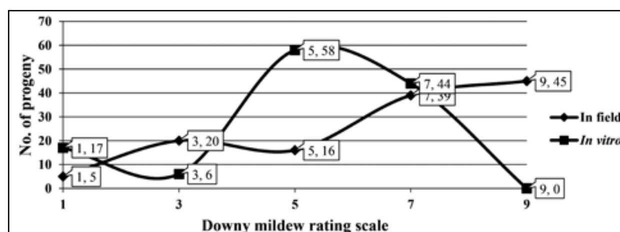

Fig. 1. Distribution pattern of 133 progenies based on UPOV rating

A new closely linked microsatellite marker was developed for the screening of downy mildew resistant progenies of Seyve Villard (SV)-12375 x Thompson Seedless. SV-12375 is an interspecific hybrid carrying *Rpv*3 locus for downy mildew resistance. Several linked microsatellite markers *viz*., UDV108, UDV305, UDV737, VMC7F2, VMC8G9 and VMC1G3.2 (Bellin *et al.*, 1; Marguerit *et al.*, 4; Moroldo *et al.*, 7) have been extensively used for selection of resistant progenies in wine grape breeding. However, when tested in our population, only three reported markers were polymorphic, and only two showed significant association with downy mildew resistance. Hence, new markers were designed to expand the choice of closely linked markers. Among the 10 new markers tested, VVMD2_195 showed significant maker-resistance association both for field and *in-vitro* screening, indicating its close association with the trait and hence usefulness in marker-assisted breeding.

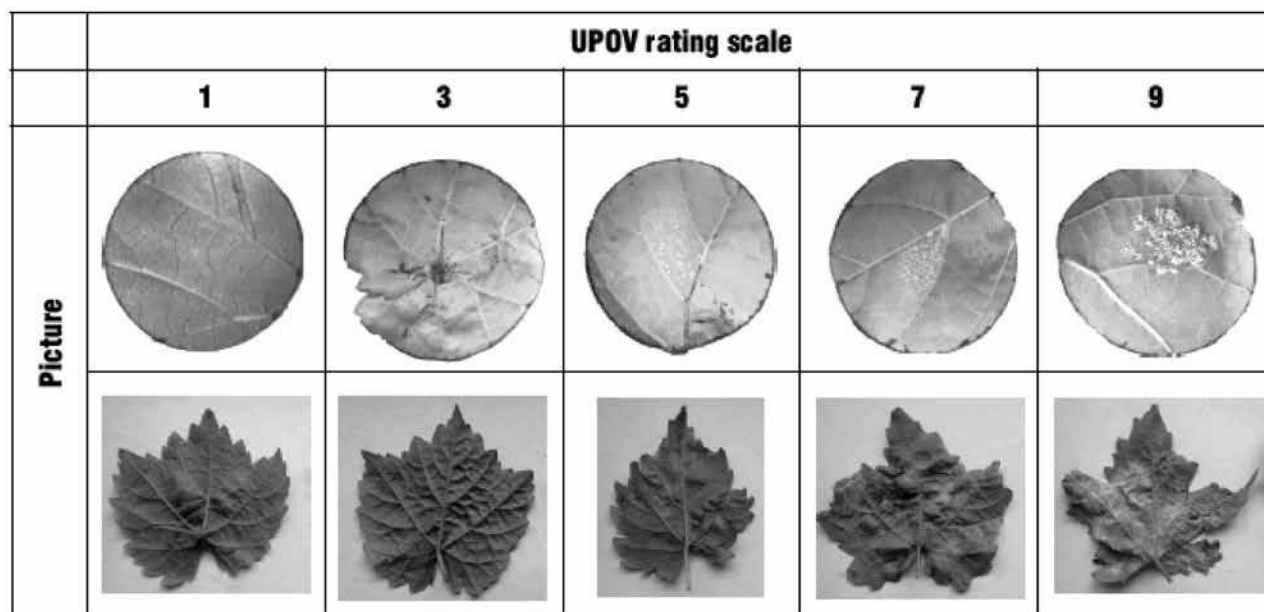

Fig. 2. Downy mildew reaction as per UPOV rating scale

Table 2. Marker phenotype analysis with *in-vitro* and in field screening

	In field screening (UPOV rating 1-9 scale)						
Progeny	1	3	5	7	9	Total Progeny screened	Progeny available
SV-12375 X TS	5	20	16	39	45	125	141
	In-vitro screening (UPOV rating 1-9 scale)						
	1	3	5	7	9	Total Progeny screened	Progeny available
SV-12375 X TS	17	6	58	44	0	125	141

SV: Seyve Villard, TS: Thompson Seedless

Table 3. Marker phenotype co-segregation analysis for downy mildew

Marker	Genotype	Mean rating (field)	p value	Mean rating (*in-vitro*)	p value
VVDM2_195	0	6.93	0.0007	6.05	0.0023
	1	5.14		5.00	
VVDM2_186	0	5.32	0.017	5.50	NS
	1	6.65		5.57	
VVDM2_183	0	6.75	0.019	5.21	NS
	1	5.76		5.70	
VVDM5_166	0 (2)	2.00	0.031	2.00	0.006
	1 (116)	6.21		5.64	
VVDM5_155	0	5.99	NS	5.63	NS
	1	6.89		5.32	
VVDM5_153	0 (114)	6.23	0.0184	5.58	NS
	1 (4)	3.50		5.50	

The efforts were taken to develop the closely linked microsatellite markers with downy mildew resistance in table grapes. Ten new microsatellite primers were designed using the sequence corresponding to *Rpv3* locus located on chromosome 18. Among these, only two markers were found polymorphic and both generated three different band size. Both these markers were validated in 133 F_1 hybrids for co-segregation with downy mildew reaction. During the marker trait association analysis, VVMD2_195 showed significant association. This new marker will be useful in downy mildew resistance breeding programme in table grapes.

AUTHORS' CONTRIBUTION

Conceptualization of research (Roshni Samarth, Anuradha Upadhyay), Designing of the experiments (Roshni Samarth, Anuradha Upadhyay, Indu Sawant), Contribution of experimental materials (Roshni Samarth, Anuradha Upadhyay), Execution of field/ lab experiments and data collection (Roshni Samarth, Anuradha Upadhyay, Pushpa Deore, Vidya Mane), Analysis of data and interpretation (Roshni Samarth, Anuradha Upadhyay, Indu Sawant), Preparation of the manuscript (Roshni Samarth, Pushpa Deore, Anuradha Upadhyay)

DECLARATION

The authors declare no conflict of interest.

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