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Grape Breeding for powdery mildew resistance

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ABSTRACT

Powdery mildew is one of the most common fungal diseases that cause severe damage to grapes. Grape growers spend lot of money for chemical control of this disease, which eventually increases the cost of cultivation. Use of grape varieties resistant to powdery mildew in grape cultivation is cost-effective as well as environmentally friendly. Hence breeding varieties for disease resistance is an important objective of any grape improvement programme. A wild species of grapes *Vitis rotundifolia*, being a good source of resistance to powdery mildew is a natural choice in grape breeding programs all over the world. In the present study, 136 hybrids were developed from a cross of *V. rotundifolia* cv James as a female parent with 6 commercial varieties (Beauty seedless, Gulabi, Sharad seedless, Kali Sahebi, Kishmish Beli and Tas-A-Ganesh) grown in India. They were screened for powdery mildew in epiphytotic conditions in 2014-15 and 2015-16. Out of these hybrids evaluated, eleven hybrids were selected on the basis of powdery mildew disease resistance as well as quality traits. Seven hybrids were rated as resistant and 5 hybrids as moderately resistant on the basis of the severity index. Of these three hybrids identified from a cross of James and Kishmish Beli (H-1301, H-1302, H-1308) showed resistance along with desirable fruit quality characters. These hybrids are being further tested for commercial use and can further be used as genetic resistance source for management of the disease in national grape improvement program.

Key words: *Vitis vinifera*, *Erysiphe necator*, *vitis rotundifolia*, interspecific hybridization.

INTRODUCTION

Maharashtra is one of the major grape producing state in India, in which mostly two types of table grapes, white and coloured are cultivated prominently. In white type, Thompson seedless and its mutants and in coloured type, Kishmish Chernyi and its mutants dominate the cultivation. The commercial table grape varieties belonging to *Vitis vinifera* are highly susceptible to powdery mildew, therefore, breeding for new resistant cultivars has been a common objective around the world. Powdery mildew is caused by a fungus, *Erysiphe necator* (Schw.) Burr. Spores are released when even 2 mm of rain and temperature are above 10°C. These fungal spores are carried away by the wind and cause primary infections. Primary infections develop into lesions which produce conidia resulting in the powdery or dusty appearance of lesions on leaves as well as berries within six to eight days. Infection can occur during temperatures ranging from 10 to 32°C, but temperatures from 20 to 25°C are ideal. High relative humidity is conducive for conidia production. Although the chemical control using fungicides is available as an alternative, concerns about the environmental impact of fungicides overuse have made alternate plant protection strategies compulsory. In addition, use of these chemical fungicides and labour for spraying incur additional cost for grape cultivation. Residue remains on berry due to spraying of fungicides which are health hazardous. Genetic resistance is preferred, because it is a more sustainable means of controlling the disease.

Muscadinia species like *Muscadinia rotundifolia* (Syn. of *Vitis rotundifolia*) and several American and Asian *Vitis* species like *V. riparia*, *V. cinerea*, *V. labrusca*, *V. rupestris*, *V. berlandieri*, and *V. lincecumii* show different levels of resistance. *Vitis rotundifolia* was recognized as early as 1889 as a source of resistance to powdery mildew along with resistance to the insects including root knot nematode (Olmo, 4). The vine produces good yield under highly fluctuating climatic conditions and grows well in poor sandy soils (Patel and Olmo, 5). Earlier, attempts to introgress disease resistance of *Vitis rotundifolia* (as pollen parent) into seedless *Vitis vinifera* as female parent through field crosses and embryo rescue have been made (Goldy *et al.*, 2). Ramming *et al.* (7) investigated and demonstrated embryo culture of seeded and seedless *Vitis vinifera*. The genetic basis of powdery mildew resistance has been evaluated and mapped in several genetic backgrounds including hybrids developed from North American Vitis species, *Muscadinia rotundifolia* accessions and *V. vinifera* cultivar 'Kishmish vatkana' (Kozma *et al.*, 3). Many Chinese Vitis species (*V. amurensis*, *V. romanetii*, *V. piasezkii*, *V. davidii* and *V. liubanensis*) have also

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been used as sources of powdery mildew resistance in breeding efforts (Wan *et al.*, 10, Tian and Wang, 9). The objective of the present investigations was to introgress mildew resistance of *V*. *rotundifolia* to develop disease resistant hybrids.

MATERIALS AND METHODS

The study was carried out during 2010 to 2016 at MACS-Agharkar Research Institute. Targeted hybridization programme was attempted at research farm of this institute situated at Hol, Tal. Baramati using *Vitis rotundifolia* cv James as source of resistance. The cultivars of *Vitis vinifera* (Beauty Seedless, Gulabi, Kishmish Beli, Sharad Seedless and Tas-A-Ganesh were used as male parents and *V. rotundifolia* cv James were used in hybridization as female parent.

After emasculating the flowers at proper stage, pollinations were carried out. Data on berry set, seeds per berry and germination percentage were recorded. To achieve maximum germination, cross seeds were subjected to chilling treatment for 90 days at 4°C; followed by H_2O_2 (0.5M) and GA (1000 ppm) treatments for 24 h. each The seeds were sown after treatment with fungicides in mixture of soil, sand and FYM (1:1:1) in flat trays. Germinated F_1 hybrids were transplanted into polythene bags after two months.

Hybrid seedlings raised in polythene bags were transplanted to the field along with parents after one year for evaluation and further studies. The hybrids and parents were subjected to the same cultural practices. The hybrids started fruiting after 3-4 years and observations on fruit characters and disease incidence were recorded in the field for two consecutive seasons (2014-15 and 2015-16). The bunch and berry characters were also studied. Data on qualitative characters like bunch maturity, berry colour, berry shape, skin thickness, juice colour and quantitative characters like bunch weight, 100-berry weight, berry size, total soluble solids (TSS) and seeds per berry were collected from a random sample of 10 bunches and 50 berries of each hybrid and parents.

Observations for powdery mildew incidence was recorded when disease symptoms were fully developed. Twenty-five leaves were surveyed for each plant. Each leaf was graded as: 0, 1, 2, 3, 4, 5, 6, and 7, based on the estimated percentage of lesions over the whole leaf area: 0, 0.1-5, 5.1-15, 15.1-30, 30.1-45, 45.1-65, 65.1-85.0 and > 85.0 respectively. Results of grading were converted to the severity index (SI) by using following formula as described by Wang *et al.* (11).

$$\text{Severity index (SI)} = \frac{\text{Sum of (Grade value} \times \text{number of leaves in that grade)}}{\text{(Total No. leaf number} \times \text{Highest grade value)}} \times 100$$

The resistance level of each hybrid was rated based on its SI: R, Resistant, SI = 0 -10; MR, Moderately Resistant, SI= 10.1-25; MS, Moderately Susceptible, SI = 25.1-50; S, Susceptible, SI = 51.1-75; HS, Highly susceptible, SI. = 75.1-100.

RESULTS AND DISCUSSION

Details of data recorded on hybridization among the above combinations after October pruning are given in Table 1. Percent berry set on crossing was observed to be maximum in James × Beauty Seedless cross (40.30%), while it was minimum in James × Tas-A-Ganesh (17.46%). Highest seed germination was observed in two hybrids James × Kali Sahebi and James × ×Beauty seedless (86%), whereas it was lowest in James × Sharad Seedless (1.87%). Appearance of both male characters in the hybrid plant confirmed the hybridity. Variations in percent berry set on pollination, seeds per berry and seed germination indicated the genetic effect of male parent. The possible reasons for such differential seed set might be due to (i) genetic behaviour of male parent, (ii) artificial pollination may not be as effective as natural pollination and (iii) relative compatibility among the varieties (Patil *et al*., 6).

Table 1. Hybridization and recovery of seeds in grape using James as seed parent.

Combination	No. of flowers emasculated	No. of flowers pollinated	Berry set (No.)	Berry set (%)	No. of seeds recovered	No. of seeds germinated	Germination (%)
James × Beauty Seedless	80	67	27	40.30	22	19	86.36
James × Gulabi	352	288	94	32.64	98	42	42.86
James × Kali Sahebi	60	50	17	34.00	15	13	86.67
James × Kishmish Beli	320	300	119	39.67	143	43	30.07
James × Sharad Seedless	366	252	56	22.22	107	2	1.87
James × Tas-A-Ganesh	565	481	84	17.46	103	17	16.50

Table 2. Evaluation of James hybrids.

Genotype/ Parentage	Bunch Maturity	Berry colour	Berry shape	Bunch wt. (g)	100- berry wt. (g)	Berry size (cm)		Seed index	T.S.S. (°B)	Skin thickness	Juice colour
						Length	Breadth				
Parents											
P1: James	Uneven	BB	Elliptical	65.1	309*	16.5	16.4*	1.60	20	Thick	Red
P2.1:Beauty Seedless	Even	RB	Globose	113.3	201	16.4	15.5	0.50	21	Thick	Pink
P2.2:Gulabi	Even	RB	Elliptical	158.5	269*	17.0	15.8*	1.50	20	Thick	White
P2.3:Sharad Seedless	Even	RB	Long elliptical	332.3	324*	18.3*	16.3*	0.00	20	Thin	Dull white
P2.4:Kali Sahebi	Uneven	BR	Falcoid	139.8	346*	19.3*	16.5	1.31	22	Thin	Brick red
P2.5:Kishmish Beli	Even	YG	Globose	77.7	199	12.4	12.3	0.00*	22	Thin	White
P2.6:Tas-A-Ganesh	Even	YG	Globose	397.3*	120	12.0	12.0	0.00*	20	Thin	White
Hybrids											
H-80 (James × Beauty Seedless)	Even	RB	Spherical	149.7	170	17.6*	15.6	2.00	23*	Thick	Dull pink
H-265 (James × Gulabi)	Even	RB	Long Conical	114.7	162	15.0	12.9	1.85	21.7	Thick	Pink
H-317 (James × Gulabi)	Even	RB	Elliptical	60.5	174	16.1	14.1	1.56	22.5	Medium	White
H-327 (James × Sharad Seedless)	Even	YG	Spherical	117.9	237	15.2	14.6	1.69	20	Thin	White
H-384 (James × Sharad Seedless)	Even	Green	Elliptical	95.4	158	15.4	13.1	1.00	20	Thick	White
H-387(James × Kali Sahebi)	Even	BR	Spherical	100.4	425*	12.9	11.6	1.46	16.0	Thick	Pink
H-563 (James × Tas-A-Ganesh)	Uneven	RB	Spherical	198	130	12.6	12.0	2.00	21	Thick	Pink
H-1299 (James × Kishmish Beli)	Even	YG	Spherical	87.7	172	13.6	13.0	0.81	21	Thick	White
H-1301 (James × Kishmish Beli)	Even	Green	Spherical	130	183	13.3	12.8	0.00*	24.7*	Medium	White
H-1302(James × Kishmish Beli)	Even	BR	Spherical	398*	208	13.1	12.9	0.00*	22.6	Thick	White
H-1308 (James × Kishmish Beli)	Even	RB	Spherical	150	122	12.5	11.8	1.06	23.2*	Thick	White
Mean	-	-	-	160.35	206.05	14.96	13.84	1.02	21.16	-	-
SD+/-	-	-	-	105.79	15.96	2.25	1.76	0.76	1.87	-	-

In this experiment 136 seedlings were raised from 488 cross seeds. After planting these seedlings in field, they started fruiting after 3-4 years. Hybrids were evaluated for ten different fruit characters. Performance of selected eleven hybrids based on resistance and quality of fruits are shown in Table 2. The results did not indicate any set pattern of inheritance of characters in most of the cases, including vegetative characters that are in consonance with inheritance pattern of vegetative characters observed by Choudhari (1). A total of 459 hybrids studied by Sharma & Uppal, (8) showed significant positive heterosis for berry weight manifested in 39 hybrids over better parent in five inter-varietal crosses. Of these, 22 showed heterosis for berry length and 17 for berry diameter. In present study, James × Kishmish Beli hybrids (H-1299, H-1301, H-1302 and H-1308), James × Gulabi (H-265) and James × Beauty Seedless hybrid (H-80) showed significant positive heterosis for bunch weight. James × Kishmish Beli hybrids showed significant heterosis with respect to TSS also.

Hybrid H-1302 showed the highest bunch weight, while James × Kali Sahebi recorded the highest 100 berry weight, *i.e.* 425 g. Hybrids H-1301 and H-1302 James × Kishmish Beli showed good fruit quality as well as moderate powdery mildew resistance. Both these hybrids matured in 120-130 days. Bunches were conical with medium sized berries. The difference between these hybrids is that hybrid H-1301 had green coloured berries, while H-1302 had brick red colour berries at maturity. Both hybrids have seedless berries with sweet taste (TSS 24 and 22°B, respectively, hence making them suitable for table purpose. According to Olmo (4), reciprocal approach to introduce the better fruit quality of *vinifera* has been hindered by the lack of cross compatibility when *rotundifolia* is used as the female parent. In our experiment cross compatibility was observed when we used *rotundifolia* as female parent. This was may be due to the use of different cultivar of *Vitis rotundifolia* on present study.

James × Sharad seedless hybrids (H-327 and H-384) produced yellowish-green and green coloured berries respectively though both the parents from which there were derived have bluish black and reddish black berries. This might be due to the heterozygous nature of parents. Similar observations were recorded by Sharma and Uppal (8) while studying the inheritance of berry colour in *vinifera* grapes. Berry colour segregated in six cross combinations involving seven parents. In Foster's seedling × Cardinal hybrid 45.8 per cent white and 54.2 per cent red-fruited hybrids were obtained.

Hybrids along with the parents were evaluated for powdery mildew resistance in epiphytotic conditions (Table 3). Generally, in Maharashtra under natural

Table 3. Severity index and resistance to powdery mildew during 2014-15.

Genotype/ Hybrid	2014	2015	Mean SI	Deviation (+/-)	Rating
James	8.32	6.34	7.33	1.40	R
Beauty Seedless	64.31	71.78	68.05	5.28	S
Gulabi	74.84	59.88	67.36	10.58	S
Sharad Seedless	18.71	15.33	17.02	2.39	MR
Kali Sahebi	59.11	53.69	56.4	3.83	S
Kishmish Beli	45.58	36.56	41.07	6.38	MS
Tas-A-Ganesh	24.98	26.69	25.84	1.21	MS
H-80 (James × Beauty Seedless)	14.44	8.82	11.63	3.97	MR
H-265 (James × Gulabi)	10.28	3.86	7.07	4.54	R
H-317 (James × Gulabi)	11.85	9.43	10.64	1.71	MR
H-327 (James × Sharad Seedless)	19.1	15.34	17.22	2.66	MR
H-384 (James × Sharad Seedless)	7.4	2.67	5.04	3.34	R
H-387 (James × Kali Sahebi)	15.08	15.1	15.09	0.01	MR
H-563 (James × Tas-A-Ganesh)	8.36	5.04	6.70	2.35	R
H-1299 (James × Kishmish Beli)	10.33	5.47	7.90	3.44	R
H-1301 (James × Kishmish Beli)	13.36	9.44	11.40	2.77	MR
H-1302 (James × Kishmish Beli)	7.4	2.67	5.04	3.34	R
H-1308 (James × Kishmish Beli)	12.65	10.21	11.43	1.73	MR

conditions, powdery mildew usually appears in December and peaks in January. Disease severity index was lowest in James among the parents, which suggest it to be the important source of resistance to powdery mildew. Natural incidence of powdery mildew was more in the year 2014 than in 2015. Out of eleven hybrids selected, six hybrids were rated as resistant and 5 hybrids showed moderately resistance based on the severity index. Hybrid with SI = 0 - 10 was considered as resistant.

Powdery mildew is an important disease of grape growing regions in the world and severely affects the production. Developing genetic resistance is important because of its sustainability. Hence, to induce powdery mildew disease resistance in grape hybrids, hybridization programme was undertaken. As shown in our studies James × Kishmish Beli hybrids (H-1299 and H-1302) were resistant and hybrids (H-1301 and H-1308) showed moderately resistant to powdery mildew along with desirable fruit characters. These hybrids are being tested in multi-location trials to evaluate the performance in different agro-climatic conditions for their commercial use. After success of multi-location trials for these hybrids, these will be available to farmers for cultivation.

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