

Intergeneric and interspecific crossing in *Vitaceae*: an attempt for disease resistant types

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ABSTRACT

Wild grapes species are conserved, characterized and utilized at MACS-Agharkar Research Institute, Pune, in a crop improvement programme. In the present study, 20 species/ cultivars belonging to the family *Vitaceae* collected from different sources within India and abroad were used for making intergeneric as well as interspecific crosses. Out of 263 seeds obtained from 21 cross combinations, four hybrids were successfully grown from *V. rotundifolia* as one of the parents showed resistance against downy and powdery mildew. The hybrids were evaluated for eleven different fruit characters. These hybrids could be utilized as pre-breeding material in future hybridization programmes.

Keywords: Disease resistance, Interspecific hybridization, *Vitaceae*, *Vitis rotundifolia*

INTRODUCTION

India is one of the biodiversity rich countries of the world and fortunately harbors numerous wild relatives of grapes, which belong to the family *Vitaceae*. The grape is commercially important fruit crop of India. Grapes are considered as rich source of vitamins, minerals and unique natural products like resveratrol. Grapes are gaining lot of importance in daily diet due to its anti-oxidant, anti-carcinogenic, immunomodulatory, antidiabetic, antiatherogenic, neuroprotective, anti-obese and anti-aging properties (Yadav *et al.*, 2009).

Out of total grapes produced in India, about 78.83 per cent are produced only in Maharashtra, that to in a narrow belt parallel to eastern side of Western Ghats comprising Nasik, Pune, Solapur and Sangli districts (Anon., 2018) . Though the primary centres of origin of cultivated grape is the region between Caspian and Black seas and secondary centres of origin as North America, about 70 species consisting of 8 genera have been reported from India (Tetali *et al.*, 2013). Indian species belonging to the family *Vitaceae* have been reported from different biogeographical regions, maximum number of species are reported from North-Eastern region of India covering Assam, Nagaland and the adjacent regions (Chadha and Shikhamany, 1999). Other than this, the Western Ghats also reported to have a high number of species belongs to family *Vitaceae*.

All varieties which are in commercial cultivation are susceptible to fungal diseases. Powdery and downy mildew are two most common fungal diseases that are considered as severe diseases of grapes. They can cause total loss if infected during the flowering stages in warm and wet climate. Grape growers spend lot of money for chemical control of these diseases, which eventually increases the cost of cultivation. Management of these diseases on traditional grapevine varieties requires regular application of fungicides. The intensive use of chemicals has its own limitations because of their cost, risk on human health and negative environmental impact. Furthermore, some fungicide-resistant strains of *Plasmopara viticola* are now observed in the vineyard, decreasing the efficiency of these sprays. Plant breeding for disease resistance the most effective way to avoid grapevine diseases.

Wild relatives of grapes are gene banks for heritable resistance to diseases and pests (Wan *et al.*, 2007) and need to be conserved and evaluated as a valuable source for improvement of commercial grapes. Novel ways for genetic improvement using untapped genetic diversity available in crop wild relatives and closely related species must be explored (Tetali and Karkamkar, 2016). Keeping that in mind, grape improvement programme was initiated during 1970 at Maharashtra Association for cultivation of science later changed its name as MACS-Agharkar

Research Institute (an autonomous institute of DST, Govt. of India) in 1992 under All India Co-ordinated Research project- Fruits, of ICAR, Govt. of India, New Delhi. A large number of indigenous species of grapes were collected from different sources. These species were maintained and evaluated and being utilized in crop improvement programme. Many wild relatives of grapes belonging to the family *Vitaceae* are recorded to be resistant to most commonly observed fungal diseases of cultivated grapes. The use of wild relatives to improve crop performance is well established. There are many successful examples of using wild species as resistant rootstocks to control soil borne pathogens in various horticultural crops (Pereira *et al.*, 2018; Panth *et al.*, 2020). Hence, the collection was explored to harness their potential as rootstocks or as a parent in breeding program in grape improvement. Interspecific and intergeneric hybridization was carried out. The results are discussed in the present communication.

MATERIALS AND METHODS

Vitis species and wild species of family *Vitaceae* are collected from western Ghats of Maharashtra along with other regions of India as well as abroad and are being conserved at Institute's main premises and also the farm that is situated at Hol, Taluk Baramati, Dist. Pune (Maharashtra). Identification of species, locality and GPS data were recorded (Table 1). Some of the grape cultivars were collected from Ganesh Khind Fruit Research Station, Pune, in 1971, and some were acquired from Federal Research Institute for grapevine breeding, Germany, in 1978.

The present study was carried out during 2012 to 2019 at MACS-Agharkar Research Institute. The relationship between cultivated grapes and their wild relatives is given in Table 2. Pollen viability was worked out by the aceto-carmin test. Targeted hybridization programme was attempted at research

farm of this institute situated at Hol, Taluk Baramati using cultivated varieties *Vitis vinifera* (Anab-e-Shahi, Bhokri, Cheema Sahebi, Gulabi, James and Ribier), *V. labrusca* (Catawba) and *V. rotundifolia* (James) and species of *Ampelocissus*, *Cayratia*, *Cissus* and *Leea species* in hybridization programme.

Emasculations and pollinations were carried out by conventional procedure using selected cultivars/species in hybridization programme. 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 H₂O₂ (0.5M) and GA (1000 ppm) treatments for 24 h. each. The seeds were sown after treatment with fungicides in a mixture of soil, sand and FYM (1:1:1) in seedling trays. Germinated F₁ hybrids were transplanted into polythene bags, 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. 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 recorded.

Observations for powdery mildew incidence were recorded when disease symptoms were fully developed in natural conditions. 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.* (1995).

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

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

In the present study, out of 20 species/ cultivars, thirteen wild relatives of grapes were collected from Konkan and Western Ghats of Karnataka and Maharashtra. The list of species used in the present experiment, the place of collection including GPS data is given in the Table 1. They include 4 *Leea* species, 3 species of *Ampelocissus*, *Cayratia* and *Cissus* each and seven *Vitis* species.

The members of *Vitaceae* are characterized as small trees or climbing shrubs sometimes herbaceous and usually having tendrils on the opposite side of leaves. The family *Vitaceae* is closely associated with its sister family *Leeaceae* and can be distinguished by the presence of stipulate leaves, sunken ovary with 1-6 seeded berries. *Vitaceae* members are occurring in abundance in the Konkan region in the rainy season. Most of them have ephemeral habit. After sprouting in the rainy season, they complete their lifecycle and go into hibernation in the form of rhizome/tubers. *Ampelocissus* and *Cayratia* species are climbers. Members of family *Vitaceae* are mostly climbing shrubs, whereas *Leea* species are perennial shrubs or small trees in habit. Plant habit and distribution of species is given in Table 2a.

The variation in a leaf characters is shown in Table 2b. Leaves of *Vitis*, *Ampelocissus* and *Ampelopsis* species are simple lobed with cordate/palmate in shape whereas *Leea* species have simple/pinnate leaves with sheathing stipule as a differentiating character. Flower characters recorded are presented in Table 2c. *Vitis* species show tendril bearing panicle with compound raceme. Flowers of *Ampelocissus* spp. and *Ampelopsis* are tendril bearing pedunculated cymes. Almost all species show hermaphrodite penta or tetramerous flowers.

Cytological observations of different genera and species are presented in Table 2d. The diploid chromosome number of commonly cultivated species of *Vitis*, i.e. *Vitis vinifera* is $2n=38$ (Sax 1929) whereas it is 40 in American cultivated species of *Vitis rotundifolia* which is reported to be the source of resistance to downy and powdery mildew (Olmo, 1937). The chromosome number $2n=40$ is also observed in the genus *Ampelocissus*, *Ampelopsis* and *Parthenocissus*. The largest

chromosomal variation is observed in the genus *Cayratia* ranging from $2n=20$ to 120. (Shetty, 1958; Shetty and Raman, 1960; Vatsala, 1960, Patil *et al.*, 1980, Karkamkar *et al.*, 2010). In *Cissus* the diploid chromosome number ranged from $2n=22,24,28,32$ and 48 (Ghimpu, 1929) and *Tetrastigma* $2n=22,44$ and 52 (Eichhorn, 1938; Krishnaswamy *et al.*, 1954; Shetty 1958; Shetty and Raman 1960; Patil *et al.* 1980). The diploid chromosome number of *Leea* was recorded as $2n=24$ and 48.

Average pollen length has been observed to be maximum in *Leea macrophylla* (50.5 μm) and minimum in *Vitis* species var. *Cheema sahebi* (22.0 μm) (Table 2e). These observations are in conformity with earlier reports (Patil, 1998). Pollen viability plays a vital role in the success of crossing programme. Higher pollen viability (95%) is recorded in var. *Anab-e-Shahi* in commercially cultivated varieties of *Vitis vinifera* whereas it was lowest in *V. labrusca* and *V. rotundifolia*. *Cissus* species also showed higher pollen viability. These observations are in conformity with earlier reports (Patil, 2001 and 2006).

Spherical, Oblate, 1-4 seeded berries are observed in *Vitis* spp., *Ampelocissus*, *Ampelopsis*, *Parthenocissus*, and *Cissus* species show spherical, round, 1-4 seeded berries whereas berries of *Cayratia* and *Tetrastigma* are ellipsoidal oblate 2-4 seeded. Depressed, globular 3-6 berries are recorded in *Leea* species as described in Table 2f.

Disease reaction in general for downy mildew, powdery mildew and anthracnose observed in different genera and species are presented in Table 2g. Most of the genera from the family *Vitaceae* and *Leeaceae*, i.e. *Ampelopsis*, *Parthenocissus*, *Cissus*, *Cayratia*, *Tetrastigma* and *Leea*, are resistant to mildews and anthracnose diseases. In contrast, wide diversity in reactions to the diseases mentioned above is observed in wild species of *Vitis* and *Ampelocissus*.

On the basis of available data on chromosomal constitution combined with taxonomical affinities, preliminary attempt to cross cultivars and wild relatives were made. Data on the number of flower bunches combinations attempted and number of flowers emasculated and pollinated is given in the Table 3. *Vitis rotundifolia* cv James used in crossing programme is a known source of resistance of

Table 1: Collection and identification of grape germplasm

Sl. No.	Name of species	Source of collection	GPS data		
			Altitude (m)	Latitude (N)	Longitude (E)
1	<i>Ampelocissus indica</i>	Karwar	0	14° 49'22.8"	74° 07'41.8"
2	<i>Ampelocissus latifolia</i>	Ambolisawantwadi road	573	16° 29'45.1"	74° 07'07.2"
3	<i>Ampelocissus tomentosa</i>	Saked before Gargoti	300	16° 29'45.1"	74° 09'07.0"
4	<i>Cayratia auriculata</i>	Shimoga-Agumbe road	567	12° 51'42.8"	73° 33'20.1"
5	<i>Cayratia elongata</i>	Honavar-Gokarna road	14	14° 28'22.1"	74° 26'21.1"
6	<i>Cayratia trifoliata</i>	Honavar-Gokarna road	14	14° 28'22.1"	74° 26'21.1"
7	<i>Cissus quadrangularis</i> (Round)	Pune	569	18° 52'4.0"	73° 85'0.0"
8	<i>Cissus quadrangularis</i> (Winged)	Pune	569	18° 52'4.0"	73° 85'0.0"
9	<i>Cissus woodrowii</i>	Khambataki	120	16° 30'00.1"	74° 20'00.0"
10	<i>Leea crispa</i>	Ambolighat	215	15° 56'20.4"	73° 57'5.6"
11	<i>Leea indica</i>	Radhanagarighat	445	16° 29'54.1"	74° 03'09.9"
12	<i>Leea macrophylla</i>	Madoni Dadra Nagar Haveli	30	20° 02'58.3"	73° 13'47.2"
13	<i>Leea sambucina</i>	Ambolighat	215	15° 56'20.4"	73° 57'5.6"
14	<i>Vitis vinifera</i> var. Bhokri (IC-0616667)	GFRS, Pune	570	18° 53'00"	73° 87'00"
15	<i>V. vinifera</i> var. Anab-e-Shahi (IC-0616636)	GFRS, Pune	570	18° 53'00"	73° 87'00"
16	<i>V. vinifera</i> var. Cheemasahabi (IC-0620725)	GFRS, Pune	570	18° 53'00"	73° 87'00"
17	<i>V. labrusca</i> var. Catawba (IC-0612104)	FRIGVB, Germany	130	51° 78'00"	11° 15'00"
18	<i>V. labrusca</i> var. Concord (IC-0612112)	FRIGVB, Germany	130	51° 78'00"	11° 15'00"
19	<i>V. vinifera</i> var. Gulabi (IC-0612119)	GFRS, Pune	570	18° 53'00"	73° 87'00"
20	<i>V. rotundifolia</i> var. James (IC-0623206)	GFRS, Pune	570	18° 53'00"	73° 87'00"

FRIGVB, Germany: Federal Research Institute for Grape Vine Breeding, Germany; GFRS, Pune : Ganesh Khind Fruit Research Station, Pune

Table 2: Relationship between cultivated grapes and their wild relatives

a) Plant habit and distribution				
Sr. No.	Genera	Habita	Distribution	
			World	India
1	<i>Vitis</i> sp.	Climbing shrub	America, Europe, Asia	Maharashtra, Karnataka, Telangana, Tamil Nadu, Punjab, Haryana
2	<i>Ampelocissus</i>	Weak climber/creeper	Europe, Asia	Western Ghats, Eastern Ghats
3	<i>Ampelopsis</i>	Ceriferous liana	Europe, Asia	Himalayan region
4	<i>Parthenocissus</i>	Ceriferous liana	Asia, Africa	South and North India
5	<i>Cissus</i>	Weak climber/ erect shrubs	Asia, Africa	Deciduous forest
6	<i>Cayratia</i>	Large/ weak climbers	Asia, Africa	Deciduous forest
7	<i>Tetrastigma</i>	Large woody evergreen climbers	Asia, Africa	Evergreen forest
8	<i>Leea</i>	Herb/shrub/small tree	Tropical Asia and Africa and rare in Australia	North east Western Ghats, Andaman

Contd.

b) Variation in leaf characters

Sr. No.	Genera	Nature	Shape
1	<i>Vitis</i> sp.	Simple lobed	Cordate/ palmate
2	<i>Ampelocissus</i>	Simple lobed digitate	Cordate/ lanceolate
3	<i>Ampelopsis</i>	Simple lobed pinnate	Cordate/Ovate
4	<i>Parthenocissus</i>	Usually trifoliolate	Ovate lanceolate
5	<i>Cissus</i>	Simple rarely trifoliolate	Cordate, ovate, lanceolate
6	<i>Cayratia</i>	3,5,7or 9 foliolate, leaflet, digitate/pedate	Lanceolate, obovate, ovate
7	<i>Tetrastigma</i>	3,5,7 foliolate, pedate, rarely simple	Lanceolate, acuminate
8	<i>Leea</i>	Simple/pinnate with sheathing stipule	Lanceolate, ovate, oblong

c) Variation in flower characters

Sr. No.	Genera	Inflorescence	Flowers
1	<i>Vitis</i> sp.	Tendrill bearing panicle, or compound raceme	Hermaphrodite, Pentamerous
2	<i>Ampelocissus</i>	Pedunculate cyme tendril bearing	Polygamo-monoecious, female pseudo hermaphrodite
3	<i>Ampelopsis</i>	Pedunculatecorymbose cyme	Hermaphrodite, 4-5 merous
4	<i>Parthenocissus</i>	Terminal or leaf opposed dichotomous cyme ending in umbellus	Hermaphrodite pentamerous
5	<i>Cissus</i>	Umbellately divided cyme	Hermaphrodite, Tetramerous
6	<i>Cayratia</i>	Axillary, corymbose, pseudoterminal or umbel	Hermaphrodite, Tetramerous
7	<i>Tetrastigma</i>	Axillary, corymbose, cyme,2-3 chotamous	Polygamous, dioecious, stigma 4 lobes, tetramerous
8	<i>Leea</i>	Pedunculatecorymbose or cyme	Hermaphrodite, staminoidal tube pentamerous

d) Cytological studies

Sr. No.	Genera	Chromosome number (2n)	Reference
1	<i>Vitis</i> sp.	38, 40	Sax 1929; Olmo 1937; Krishnaswamy <i>et al.</i> 1954, Shetty & Raman, 1960; Patil <i>et al.</i> 1980
2	<i>Ampelocissus</i>	40	Vatsala 1960; Shetty & Raman 1960
3	<i>Ampelopsis</i>	40	Sax 1929
4	<i>Parthenocissus</i>	40	Sax 1929; Shetty 1958
5	<i>Cissus</i>	22,24,26,28,32,48	Ghimpu 1929; Krishnaswamy <i>et al.</i> , 1954; Shetty 1958; Shetty & Raman 1960; Vatsala 1960; Patil <i>et al.</i> 1980
6	<i>Cayratia</i>	20,22,24,30,40,60,80,120	Shetty, 1958; Shetty & Raman, 1960; Vatsala 1960; Patil <i>et al.</i> , 1980
7	<i>Tetrastigma</i>	22,44,52	Krishnaswamy <i>et al.</i> 1954; Shetty 1958; Shetty & Raman 1960; Patil <i>et al.</i> 1980
8	<i>Leea</i>	24,48	Vatsala, 1960; Shetty & Raman, 1960; Patil <i>et al.</i> , 1980

powdery and downy mildew (Tetali *et al.*, 2018). Inter-specific crosses involving *V. rotundifolia* were successful in the field. The cultivars of *Vitis vinifera* (Anab-e-Shahi, Bhokri, Cheema-sahebi, Gulabi) were used as male parents and *V. rotundifolia* cv. James were used in hybridization as female parent

and vice-versa. Total 263 seeds were obtained but only four seeds were viable. Pre-zygotic barriers which might have prevented the growth of the pollen tube through the style, and possible post-zygotic barriers, which might have prevented fertilization resulting into failure to produce a

e) Variation in pollen characters

Sl. No.	Name of species	Pollen size (μm)		Wall thickness (μm)	Protoplasmic area (μm) ²	Pollen viability (%)
		Length	Diameter			
1	<i>Ampelocissus</i> spp.	30.7	30.5	2.16	559	88
2	<i>Cayratia</i> spp	41.6	39.9	2.70	963	77
3	<i>Cissusquadrangularis</i> (Round)	39.5	38.3	2.53	899	92
4	<i>Cissusquadrangularis</i> (Winged)	38.8	37.5	2.56	839	80
5	<i>Cissuswoodtrowii</i>	44.4	43.2	2.04	1238	96
6	<i>Leeacrispa</i>	44.2	40.1	3.77	942	96
7	<i>Leeaindica</i>	43.9	42	3.80	984	76
8	<i>Leeamacrophylla</i>	50.5	46.3	3.50	1347	93
9	<i>Leeasambucina</i>	46.1	42.9	3.60	1127	42
10	<i>Vitis vinifera</i> var. Bhokri	25.6	24.8	2.20	338	82.6
11	<i>Vitis vinifera</i> var. Anab-e-Shahi	25.0	23.7	2.40	298	95
12	<i>Vitis vinifera</i> var. Cheemashebi	22.0	21.0	1.80	254	80
13	<i>Vitis labrusca</i> var. Catawba	24.4	23.4	2.00	310	41
14	<i>Vitis labrusca</i> var. Concord	24.3	23.6	1.90	319	45
15	<i>Vitis vinifera</i> var. Gulabi	22.2	21.2	1.70	265	93
16	<i>Vitis rotundifolia</i> var. James	24.3	23.7	1.91	319	40
	Mean	34.09	32.63	2.54	687.56	76.04
	SD+/-	10.21	9.35	0.73	391.43	21.37

f) Variation in fruit characters

Sr. No.	Genera	Berries	Seeds
1	<i>Vitis</i> sp.	Spherical, oblate, 1-4 seeded	Pyriiform/obovoid furrowed
2	<i>Ampelocissus</i>	Spherical, round, 2-3 seeded	Oblong, Obovoid, convex on back
3	<i>Ampelopsis</i>	Spherical, round, 2-4 seeded	Obovoid, 2 grooved
4	<i>Parthenocissus</i>	Round spherical, 2-4 seeded	Obovoid, globose, convex on back
5	<i>Cissus</i>	Round oblate, usually 1 seeded	Ellipsoidal, pyriform, smooth
6	<i>Cayratia</i>	Ellipsoidal, oblate, 2-4 seeded	Obcordate, oblong, angular
7	<i>Tetrastigma</i>	Ellipsoidal, oblate, 1-4 seeded	Globose oblong, pyriform, smooth
8	<i>Leea</i>	Depressed, globular, 3-6 seeded	Wedge shape, with hard testa

g) Variation disease resistance (R Resistant, S Susceptible, Moderately resistant)

Sr. No.	Genera	Downy mildew	Powdery mildew	Anthraco nose
1	<i>Vitis</i> sp.	S / MR	S / MR	R / S
2	<i>Ampelocissus</i>	R/S	R/S	R / S
3	<i>Ampelopsis</i>	RR	RR	RR
4	<i>Parthenocissus</i>	RR	RR	RR
5	<i>Cissus</i>	RR	RR	RR
6	<i>Cayratia</i>	RR	RR	RR
7	<i>Tetrastigma</i>	RR	RR	RR
8	<i>Leea</i>	RR	RR	RR

viable seed in hybrid. Berries might have been developed through parthenocarpy in most of the fruits (Royo et al., 2016). 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) relative compatibility among the species (ii) genetic behaviour of male parent and (iii) artificial pollination may not be as effective as natural pollination (Patil et al., 1992).

According to Olmo, reciprocal approach to introduce the better fruit quality of *vinifera* has been

Table 3: Hybridization in *V. vinifera* and wild relatives of grapes

Sr. no.	Parental combinations	Combinations attempted	Flowers pollinated	Berry set	Berry harvested	Seed extracted	Seed germination
1	<i>V. vinifera</i> var. <i>Bhokri</i> x <i>Ampelocissus latifolia</i>	1	32	10	6	4	0
2	<i>V. vinifera</i> var. <i>Anab-e-Shahi</i> x <i>Leea indica</i>	9	1002	202	109	70	0
3	<i>V. vinifera</i> var. <i>Gulabi</i> x <i>Cayratia elongata</i> .	5	247	75	58	17	0
4	<i>V. vinifera</i> var. <i>Bhokri</i> x <i>Cissus woodrowii</i>	14	2294	1633	232	107	0
5	<i>V. vinifera</i> var. <i>Gulabi</i> V. x <i>rotundifolia</i> var. <i>James</i>	2	95	10	10	24	1
6	<i>V. labrusca</i> var. <i>Catawba</i> x <i>Cissus woodrowii</i> .	5	140	1	1	1	0
7	<i>V. labrusca</i> var. <i>Concord</i> x <i>Cayratia trifoliata</i> .	3	243	50	37	46	0
8	<i>V. labrusca</i> var. <i>Concord</i> x <i>Leea crispa</i> .	1	249	64	42	18	0
9	<i>V. rotundifolia</i> var. <i>James</i> x <i>V. vinifera</i> var. <i>Cheemasahebi</i>	2	70	11	11	26	1
10	<i>V. rotundifolia</i> var. <i>James</i> x <i>V. vinifera</i> var. <i>Gulabi</i>	2	50	26	21	35	3
11	<i>Cayratia auriculata</i> x <i>V. vinifera</i> var. <i>Gulabi</i> .	4	578	0	0	0	0
12	<i>Cissus woodrowii</i> x <i>V. vinifera</i> var. <i>Cheemasahebi</i> .	6	170	0	0	0	0
13	<i>V. vinifera</i> <i>Bhokri</i> x <i>Ampelocissus latifolia</i>	1	28	4	4	0	0
15	<i>V. vinifera</i> <i>Bhokri</i> x <i>Cissus woodrowii</i>	2	43	0	0	0	0
16	<i>V. vinifera</i> var. <i>Ribier</i> x <i>Cissus elongata</i>	2	47	4	4	3	0
17	<i>V. vinifera</i> var. <i>Ribier</i> x <i>C. quadrangularis</i> (<i>winged</i>)	1	17	0	0	0	0
18	<i>V. vinifera</i> var. <i>Cheemasahebi</i> x <i>Cissus quadrangularis</i> (<i>ornamental type</i>)	2	150	0	0	0	0
19	<i>V. vinifera</i> var. <i>Cheemasahebi</i> x <i>C. quadrangularis</i> (<i>round</i>)	3	319	0	0	0	0
20	<i>V. vinifera</i> var. <i>Cheemasahebi</i> x <i>Leea sambucina</i>	1	17	0	0	0	0
21	<i>V. vinifera</i> <i>Gulabi</i> x <i>C. quadrangularis</i> (<i>round</i>)	1	62	0	0	0	0
	Total	49	5055	2035	485	263	5

Table 4: Evaluation of successful hybrids

Hybrid Characters	H 265 (James x Gulabi)	H 267 (James x Gulabi)	H 584 (James x Cheema sahebi)	H 297 (Gulabi x James)
Bunch Maturity	Uneven	Uneven	Even	Uneven
Berry colour	Brick Red	Green	Bluish black	Black
Berry shape	Ellipsoidal	spherical	Spherical	Obovoid
Bunch weight (g)	80.7	90.9	68.15	35
100 berry weight (g)	125	154	171	190
Berry length (cm)	14.3	14.7	10.94	13.8
Berry width (cm)	12.3	13.84	10.81	13.26
Seeds/berry	2.19	0.75	1.63	1.88
TSS (^o B)	22.81	17.25	21.5	21.5
Skin thickness	Medium	Thin	Thin	Medium
Juice colour	White	White	White	White
Disease severity index (PM)+/- SD	7.07+/- 4.54	8.00 +/-2.30	7.33+/- 1.46	17.22 +/- 2.66

hindered by the lack of cross compatibility when *rotundifolia* is used as the female parent (Olmo, 1937). In the present experiment cross compatibility was observed when we used *V. rotundifolia* as female parent. This may be due to the use of different cultivar of *V. rotundifolia* on present study.

After planting these seedlings in the field, they started fruiting after 3-4 years. Hybrids were evaluated for eleven different fruit characters. Performance of hybrids based on resistance and quality of fruits are shown in Table 4. Three hybrids H 265, H 267 and H 584 recorded severity index less than 10 hence considered as tolerant for powdery mildew under natural field conditions. H 297 showed moderate resistance (SI =17.22) based on severity index.

In Maharashtra, powdery mildew usually appears in December and peaks in January. Disease severity index was lowest in James, which reveals its importance as source of resistance to powdery mildew. It can also be noted from the table that all hybrids produced small bunches having seeded berries which may not be useful to be used as table purpose in present conditions. But it can be used as pre-breeding material for transferring the disease resistance. One or two more backcrosses may be

required to attain the berry size and quality to be used for table purpose. Thus, modern tools like somatic hybridization, embryo rescue, gene expression experiments may be taken into consideration for successful utilization of wild germplasm to incorporate traits of interest in cultivated varieties of grapes.

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