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Short communication

Characterization of *Vasconcellea cauliflora* for morpho-horticultural traits under climatic conditions of Pune, India

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ABSTRACT

***Vasconcellea cauliflora* is one of the few species in the family Caricaceae that have shown resistance against *Papaya ringspot virus* strain papaya (PRSV-P) which is a global limiting factor in papaya (*Carica papaya* L.) cultivation. *V. cauliflora* has a potential role in introgression of gene(s) of PRSV-P resistance in *C. papaya*. It was introduced in India for academic and research purposes. Therefore, very little information is available about its morpho-horticultural traits under Indian climate and its reaction against local isolates of PRSV-P. *V. cauliflora* plantation maintained at ICAR-Indian Agricultural Research Institute, Regional Station, Pune since 2011 provided valuable information on morpho-horticultural traits under climatic conditions of Pune and its reaction against local isolate of PRSV-P. Plants gained a height of 0.89 m and collar diameter of 6.94 cm in one season which increased in subsequent years. Yellowish white and waxy female flowers were 3.9 cm long. Pollen viability was 86%. Average fruit yield was 4.3 kg/plant in the first year, which increased to 8.4 kg/plant in the third year. Berry type fruits were long, round or intermediate in shape with smooth skin texture. The average fruit length and width was 6.8 cm and 3.7 cm, respectively. Ridges and grooves were prominent in long fruit shape as compared to intermediate and round ones. Seedlings showed resistance against PRSV-P under field conditions and when infected by challenge inoculation in glasshouse. The plants remained disease-free even when exposed to severe disease pressure under field conditions for more than three years. There is a renewed interest in *V. cauliflora* as a source of gene(s) for resistance against PRSV-P infection, especially after overcoming the crossing barrier with *C. papaya* in India.**

Key words: *Carica papaya*, traits, PRSV resistance, wild species, crossing barrier.

Papaya (*Carica papaya* L.) is a popular fruit crop cultivated mainly in Asia, Latin America and Africa. India produced two-thirds of world's papaya from one-fourth of the global area. *Papaya ringspot virus* strain Papaya (PRSV-P) infection is a global limitation for papaya cultivation. PRSV-P infection causes major yield losses and severely affects fruit quality. There are no prophylactic or therapeutic control measures to combat the disease. Since there is no source of PRSV-P resistance in the genus *Carica,* resistant varieties could not be developed by conventional breeding approach (Sharma and Tripathi, 11). However, some related plants, e.g., highland/mountain papaya (*Vasconcellea species*), exhibit varying degrees of PRSV-P resistance. The genus *Vasconcellea* is a group of 'wild type' papaya comprising 20 species and one hybrid. Earlier, all species of the genus *Vasconcellea* were classified in the genus *Carica.* Later on, based on molecular taxonomical revelation, all these species were rehabilitated in a separate genus, *Vasconcellea* (Aradhya *et al*., 1; Badillo 2; Badillo, 3). *V. cauliflora* is an important species as it is one of the few *Vasconcellea* species that shows resistance against PRSV-P. Although *V. cauliflora* is found in wild in Central America, it is maintained by various research organizations in different parts of the world for their academic and research interests.

One such plantation is maintained at ICAR-Indian Agricultural Research Institute (IARI), Regional Station (RS), Pune, India for academic and research purposes. Some morphological characters of the species were expected to be affected by the climate of Pune which is situated 560 metres above sea level on the Deccan plateau, while mountain/highland papaya thrives well at higher altitudes. Although *V. cauliflora* plants were earlier raised in India, few reports described their morphological characters and reaction to local isolate of PRSV-P (Jayavalli *et al*., 9). Such information is of crucial importance because of cross- incompatibility issue between *V. cauliflora* and *C. papaya*, and conflicting reports of *V. cauliflora* resistance against different strains of PRSV-P (Conover, 6; Sharma *et al*., 10). Considering their importance in the breeding programme, a study was undertaken to record morpho-horticultural traits contributing towards growth and yield of *V. cauliflora* under local climate and its reaction to the Pune isolate of PRSV-P.

V. cauliflora seeds were procured from ICAR-Indian Institute of Horticultural Research, Bengaluru,

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India in 2010. Since then, its subsequent generations were raised by sib mating among selected plants. A subset of morphological traits based on list of papaya descriptor of International Board for Plant Genetic Resources was recorded on one year old plants. Morphological traits associated with the plant growth, flowering and fruit yield were recorded. Height of the plant was measured from ground level to the top apical bud. Stem girth was measured from 15 cm above the ground level. First fruiting height and length of the fruiting column were measured from the ground level. Morphological characters of flowers were recorded. The fruit yield (kg per tree) was calculated by weighing all ripen fruits produced from a plant. Morphological characters of fruits and seed were recorded from ripen fruits. The traits were recorded up to three years, the average life of *V. cauliflora* under Pune climate. Each set of data is an average value of 25 plants.

Reaction of *V. cauliflora* to Pune isolate of PRSV-P was recorded at seedling stage. A set of ten plants with three replications, with susceptible *C. papaya* as the control, were challenge inoculated by sap inoculation (mechanical) method in a glasshouse. One gram of infected leaves was ground in a pre-chilled mortar and pestle using 10 ml of 0.1 M chilled sodium phosphate buffer (pH 7.2) containing β-mercaptoethanol and 0.01 M EDTA. The sap was rub-inoculated using the pestle on the young leaves of seedlings at three-leaf stage. Excess sap was washed off by distilled water after five minutes. The disease incidence was recorded from six to eight weeks after inoculation. Another set of *V. cauliflora* plants were exposed to severe disease pressure when planted in field surrounded with severely infected *C. papaya*. The presence/absence of PRSV-P in inoculated seedlings and plants exposed to field conditions was confirmed by Enzyme Linked Immunosorbent Assay (ELISA, Clark and Adams, 4) using PRSV specific antibody (Agdia Inc., USA).

Plants showed a shrub or tree like growing habit. Plant height of one year old *V. cauliflora* was 0.89 m which went up to 2.31 m in the third year. Collar diameter rose from 6.9 cm in the first year to 22.2 cm in the third year. 'Fruiting height' and 'fruiting column length' increased with the age of the plant. Fruiting height was 39 cm from the ground, while average length of the fruiting column was 36 cm in the first year. Fruit yield was 4.3, 6.2 and 8.4 kg/plant in the first, second and third year, respectively (Table 1). Many plants failed to survive after three years due to fungal root rot. Jayavalli *et al*. (9) reported similar growth data (plant height, collar diameter and first fruiting height) and negative PRSV-P reaction for one year old *V. cauliflora* plants from Tamil Nadu Agricultural University (TNAU), Coimbatore, India

Both female and male flowers were yellowish white having waxy texture. Lanceolate female flowers were pentamerous. Average length of female flowers was 3.9 cm (range: 3.5-4.4). Berry type fruits were round/long/intermediate in shape with smooth skin texture. Only one type of fruit shape was observed on a plant. Stalk-end of fruits was generally flattened or inflated, but sometimes it was pointed. The blossom end scar was small. Average fruit length and width were 6.8 cm (range: 5.25-11.50 cm) and 3.7 cm (range: 2.25-4.00 cm), respectively. The ratio between length and width was 1.8. Various fruit shapes are depicted in Fig. 2. Average fruit weight was 34.2 g (range: 43-73 g). Ridges on fruits were deep in long fruits and intermediate in round ones. Average number of seed per fruit was 30 (range: 22-35), weighing 1.02 g (range: 0.83-1.17 g). Test weight of seed was 3.3 g. Brown colour seeds were spherical with opaque surface. Seed surface was glossy due to high mucilage (Fig. 1, Table 2).

Average number of pollens per view was seven with average size of 3.1 µ. Out of which 86% pollens were viable. Pollen germination was 27% after four hours. The pollen tube growth was 20, 60 and 91 µ after four, eight and twelve hours (Table 3). Although data is not readily available for Pune climate to compare the result, they conform to the findings of Cohen and Spiegel-Roy (5) for papaya pollens.

No PRSV-P symptoms were observed on *V. cauliflora* six weeks after sap inoculation of the virus whereas severe symptoms were recorded on all inoculated susceptible control (*C. papaya*). Further confirmation of PRSV-P infection was done by ELISA using specific antibody for PRSV. The

Table 1. Growth, fruiting characters and fruit yield up to first three years of plant age.

Year	Plant height (m)	Collar diameter (cm)	Fruiting height (cm)	Fruiting column Length (cm)	Fruit yield (kg/plant)
2011-12	0.89 (±0.11)	6.94 (±1.20)	39.00 (±9.62)	36.00 (±2.24)	4.3 (± 0.60)
2012-13	1.28 (±0.23)	12.92 (±6.09)	40.00 (±16.96)	64.00 (±18.17)	6.2 (± 0.99)
2013-14	2.31 (±0.02)	22.15 (±1.97)	64.00 (±11.94)	144.00 (±9.62)	8.4 (± 1.01)

Figure in parentheses are standard deviation

Table 2. Morpho-horticultural traits of one year old *V. Cauliflora*.

Parameter	Description / average value (range)
Qualitative	
Fruit shape	Berry
Fruit shape	Round to elongated
Leaf shape	Palmate
Flower shape	Mostly lanceolate
Flower colour	Yellowish white
Flower texture	Waxy
Stalk end fruit shape	Flattened or inflated, sometime pointed
Size of blossom end scar	Small
Fruit skin texture when ripe	Smooth
Ridging on fruit surface	Deep to shallow
Seed surface lustre	Glossy
Seed shape	Spherical
Seed surface type	Opaque
Seed mucilage	Intermediate
Colour of seed coat	Brown
Quantitative	
Flower length (cm)	3.9 (3.5-4.4)
Fruit length (cm)	6.8 (5.25-11.50)
Fruit width (cm)	3.7 (2.25-4.00)
Fruit weight (g)	34.2 (43-73)
No. of seeds/fruit	30 (22-35)
Dry weight of seed per fruit (g)	1.0 (0.83-1.17)
Test seed weight (g)	3.3

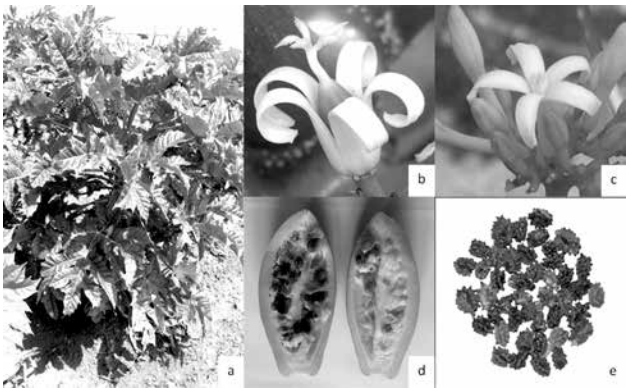

Fig. 1. A typical *Vasconcellea cauliflora* plant (a), female (b) and male (c) flowers, cut fruit (d) and seed (e).

Fig. 2. Variation in fruit shape of *V. Cauliflora* under Pune climate.

OD value at 405nm absorbance of ELISA reaction for inoculated *V. cauliflora* was 0.123 whereas the OD value for inoculated *C. papaya* plant was 1.7. ELISA value for healthy papaya plants was in the range of 0.102 to 0.111 (Table 4). This confirmed resistant status of *V. cauliflora* against Pune isolate of PRSV-P. Jayavalli *et al*. (Jayavalli *et al.*, 9) reported negative reaction of *V. cauliflora* to Coimbatore isolate of PRSV-P while analysing papaya intergeneric hybrids for morphological traits. In another report from the same place, *V. cauliflora* did not show PRSV-P symptoms 27 days after infecting them, using artificial inoculation method (Sudha *et al.*, 12) under glasshouse conditions.

V. cauliflora showed negative reaction against PRSV-P (Pune isolate) in challenge inoculation under glasshouse condition, and it remained virus-free for the entire life of three years when cultivated along with severely infected papaya plants under open field condition. This indicates that *V. cauliflora* will remain an important source of resistance genes for breeding programme. There is a renewed interest in

Table 3. Pollen characteristics of *V. cauliflora*.

Values	Pollen size (µ)	Pollen observed (No.)	Viable pollen (No.)	Pollen germinated after 4 h (No.)	Growth of pollen tube in length (µ) after			
					2 h	4 h	8 h	12 h
Mean	3.10	6.87	5.93 (86.32%)	1.60 (26.98%)	20.00	20.40	60.73	91.07
S.D.	0.54	2.53	2.60	1.06	10.11	16.23	31.95	31.21

Average of 15 field views, S.D.; standard deviation

Table 4. Confirmation of PRSV in artificially inoculated *V. cauliflora* plants by ELISA.

	Tested plants					
	V. cauliflora			*C. papaya*		
	PRSV inoculated	Buffer inoculated	Reaction	PRSV inoculated	Buffer inoculated	Reaction
ELISA value (OD 405 nm)	0.123	0.102	Negative	1.7	0.111	Positive

V. cauliflora especially after overcoming the crossing barrier with *C. papaya* by the use of 5% sucrose solution at IIHR, Bangalore (Dinesh *et al*., 7) and TNAU, Coimbatore (Jayavalli *et al.*, 8).

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