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Screening of *Carica Papaya x Vasconcellea Cauliflora* Hybrids for Resistance to Papaya Ring Spot Virus (PRSV)

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Abstract

Carica papaya x *vasconcelleacauliflora* and intergeneric F_1 hybrids of these species were screened for resistance to severely infected papaya ringspot virus isolates of papaya ringspot virus. Artificial screening for papaya ringspot virus was carried out 27 days after sap inoculation. Out of twenty-nine F_1 hybrid plants of CO 7 x *Vasconcelleacauliflora,* only six plants were found free from PRSV symptoms. Similarly, out of fifty-five F_1 hybrid plants of PusaNanha x *Vasconcelleacauliflora*only twenty-three were found free from the symptoms and seventy plants out of 335 plants of CP50 x *Vasconcelleacauliflora* were found free from PRSV symptoms. The resistance of the hybrids and parents and their hybrids *viz*, CO 7 x *Vasconcelleacauliflora,* PusaNanha x *Vasconcelleacauliflora* and CP50 x *Vasconcelleacauliflora* were subjected to DAS ELISA test. Molecular marker *viz*, ISSR markers were used to check and verify the hybridity. ISSR markers showed confirmity on three hybrid progenies *viz*, CO7V3, CO7V5 and CO7V6 from CO 7 x*Vasconcelleacauliflora*.

Keywords: *Carica Papaya*, V.C. (*Vasconcelleacauliflora)*, CO7V3 (CO7 x *V.c*), PNV1(Pusa Nanha x *V.c)* CPV1(CP 50 x *V.c)* Intergeneric Hybrids, Papaya Ringspot Virus

Introduction

Papaya (*Carica papaya* L.), a delicious fruit tree, is affected by number of diseases caused by various pathogens and viruses. At present, it is cultivated throughout the world. Besides Central America, papaya is important as a commercial plant in Hawaii, South Africa, Australia, India, Ceylon, the Philippines and South-East Asia. The names papaw, pawpaw, paw-paw, melon pawpaw, papaya and papita are applied to Carica papaya L, the most commonly used being papaya and papaw. The papaya plant has short life, hence the area under cultivation varies greatly in different years. In India it is cultivated over an area of 97.7 thousand hectares with annual production of 3628.9 thousand MT. (NHB, 2020). In India, it is commercially cultivated in Andhra Pradesh, Gujarat, Maharashtra, Karnataka, West Bengal, Assam, Orissa, Madhya Pradesh, Manipur, Tamil Nadu and Bihar and certain extent in Kerala.

The papaya is popular as a backyard tree in many developing countries but increasingly becoming more important in commercial plantings for domestic markets and for export in countries like Mexico and Malaysia. The advantage in papaya cultivation is the rapid return of investment due to its early maturation, intensive cultivation and high yield. Most papayas in the tropics can be harvested 8 or 9 months after sowing and yields can range from 60 to 100 t/ha/year for improved varieties. The ripe fruit has a delicate aroma and sweetness and has high contents of vitamins A and C. One medium-sized papaya exceeds the Dietary Reference Intakes (DRI) of 3000 IU for vitamin A and 90 mg for vitamin C, established by the U.S. Food and Nutrition Board (OECD 2004). There is great diversity in the size, shape and quality of the fruit. In unselected germplasm or backyard trees, fruits are usually very large and not very palatable, but varieties such as 'Solo' and 'Eksotika', specifically selected for export or up-markets, are usually small for convenience in packaging and have much better taste and storage attributes. Papaya is usually eaten fully ripe when the flesh is soft and succulent. However, it can also be eaten raw, sliced into thin strips and eaten as vegetable or processed into various products such as candy, pickle or puree. The 'Eksotika' papayas imported by China are served as a delicacy in high-end restaurants: the half-cut fruit with seed scooped out is filled with 'sharks-fin' or 'birds-nest' and steamed before serving. The latex from unripe fruit and leaves contains a proteolytic enzyme papain, which can be used for tenderizing meat, chill-proofing beer, tanning leather and for making chewing gum. In pharmaceutics, papain is used for suppression of inflammation, treatment of gangrenous wounds and for various digestive ailments. As a proteolytic enzyme, it has exfoliating property that removes the dead surface cells of the skin, giving it a rejuvenated feeling. It is therefore popularly used in soaps, creams, shampoos and lotions in the cosmetic industry.

Papaya is affected by number of diseases caused by various pathogens and viruses. Nowdays the most destructive disease of *C. papaya* worldwide is papaya ring spot caused by papaya ring spot virus-type P Litz, (1984), Manshardt, (1992), a definitive potyvirus species in the *Potyviridae* (Shukla *et al*, 1994). PRSV is grouped into two types, Type P (PRSV - P) infects cucurbits and papaya and type W (PRSV-W) infects cucurbits but not papaya (Gonsalves, 1998). Almost all cultivated varieties are highly susceptible. *Carica cauliflora* J, a wild species having non-edible fruits is known to be resistant for this viral disease (Jimenez and Horovitz, 1957). Now the species *cauliflora* has been grouped under the genera *Vasconcellea* (Vegas *et al*, 2003).

Control measures to check the viral incidence against PRSV-P include cultural practices, cross-protection and planting of tolerant cultivars (Gonsalves, 1994). None of these has been very successful and the development of virus resistant cultivars through conventional breeding is the only reliable tool for long term control. None of the *Carica papaya* cultivars has natural-resistance to PRSV-P. Even though interspecific hybridization of *Carica papaya* with other species attempted, a very little work has been done using *Vasconcellea cauliflora* which has the desirable gene for PRSV resistance (Jayavalli *et al*, 2015). Selection and sibmating of intergeneric progenies of papaya and evaluation of intergeneric progenies (F_6) for fruit characteristics and PRSV tolerance (Vasugi,2022), Disease resistances that have been identified in *Carica* species for PRSV-P resistance are *C. cauliflora, C. pubescens, C. quercifolia* and *C. stipulate* (Conovar, 1964, Horovitz and Jimenez, 1967). Papaya breeding in India can be broadly classified into three phases. Work carried out at Tamil Nadu Agricultural University, Pusa, Pantnagar, Pune and at Bangalore has resulted in the development of new varieties suitable for papain extraction and for table purpose. Information on the inheritance pattern has helped in identifying the parents as gene donors for several characters. In recent times the breeding is being carried out with the objective of developing lines resistant to PRSV (Dinesh, 2 010). Papaya ringspot virus type P(PRSV-P) is a major threat to the papaya industry worldwide. F1 hybrids have been produced when Carica papaya L. female flowers have been pollinated with pollen of the PRSV-P resistant species Vasconcellea quercifolia. A single dominant gene for PRSV-P resistance in *V. pubescens* has been mapped by use of

dominant, polymorphic randomly amplified DNA fingerprint (RAF) markers in F_2 interspecific population of *V. parviflora* (PRSV-P susceptible) and *V. pubescens* (PRSV-P resistant) Drew *et al*, 2007. Hydrogel capsules are a potential candidate for drug delivery and an interesting alternative to polyelectrolyte multilayer capsules which are under investigation in the last 20 years. Recently introduced polyelectrolyte complex capsules produced by spraying are non-biodegradable and not biocompatible, which limits their practical application, while biodegradable alginate capsules require complex coaxial electrospray ionization jetting. biodegradable alginate capsules cross-linked by calcium are successfully produced by hydrodynamic electrospray ionization jetting with the assistance of low frequency ultrasound. The size and shape of most capsules show significant differences with respect to different spraying distance, spraying mode, electrode shape and spraying concentration. Capsules in the shape of vase, mushrooms and spheres were successfully produced. Average capsule size can be adjusted from 10 μm to 2 mm. These capsules are used to encapsulate a model drug. Encapsulated paramagnetic particles enable defined directional motion under the propulsion of a rotating magnetic field, while model drugs can be released by ultrasound (Rutkowski *et al,* 2019

Disease resistance, increased yields and improved quality and storage traits are important objectives for breeding programmes of any crop. While significant improvements have been made with conventional hybridization techniques, programmes incorporating methods of genetic engineering offer opportunities for the transfer of genetic variability from other gene pools. Much of the review addresses transgenic virus resistance, which is the major application. Approaches related to improved quality traits and pharmaceutical productions are also examined (Melaine Randle, Paula Tennant, 2020 Hence, the development of virus resistant cultivars through conventional breeding is the only reliable tool for long term control and cost of production is very low compare to production of transgenic papaya. Under these circumstances, screening of *Carica papaya* x *Vasconcellea cauliflora* hybrids for resistance to papaya ringspot virus (PRSV) is attempted.

Materials and Methods

Present investigations on the breeding for papaya ring spot virus (PRSV) resistance in papaya (*Carica papaya* L.) were carried out in the College Orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

Artificial Screening for Papaya Ringspot Virus (Mechanical Inoculation of PRSV to Parents, F_1 Progenies)

One gram of infected leaves was ground in a pre-chilled mortar and pestle using 1 ml of 0.1M chilled sodium phosphate buffer (pH 7.2) containing β-mercaptoethanol and 0.01 M EDTA. The sap was rub inoculated using the pestle or glass rod on the young leaves of seedlings at 3 leaves stage previously dusted with carborundum powder 600 meshes. After 5 minutes, the excess sap was washed off by distilled water. The disease incidence and intensity score were given using the scale developed by Dhanam (2006 Details of the disease incidence and intensity score scale is presented in Table 1.

Reactions	Intensity scores	Symptoms
Apparently healthy (AH)	0-1	0 = No disease symptoms
Moderately resistant (MR)	1-2	1 = Slight mosaic on leaves 2 = Mosaic patches and / or necrotic spots on leaves
Moderately susceptible (MS)	2-3	3 = Leaves near apical meristem deformed slightly, yellow, and reduced in size
Susceptible (S)	3-4	4 = Apical meristem with mosaic and deformation
Highly susceptible (HS)	4 and above	5 = Extensive mosaic and serious deformation of leaves, or plant death).

Table 1: Scale of disease incidence and intensity score

Source of antiserum and positive sample

Antibody for PRSV and their positive samples were provided from DSMZ, Braunschweing, Germany.

Enzyme Linked Immunosorbent Assay (ELISA)

DAS-ELISA was performed for the detection of PRSV by following the manufacturer's instructions (DSMZ Gmbh, Braunschweig, Germany). Purified IgG was diluted in coating buffer (1:1000) and 200 µl was added to each well of a micro titer plate (Grainer). The plates were then incubated at 37°C for 2 to 4 hours and thereafter plates were washed with PBS-T using wash bottle, soaked for a few minutes and repeat washing for twice. Plates were blotted by tapping upside down on tissue paper. 200 µl aliquots of the test sample (extracted in sample extraction buffer) were added to duplicate wells. The plates were incubated overnight at 4°C. The plates were washed as in earlier and added with 200 µl of the anti-virus conjugate (1:500) to each well and incubated at 37°C for 2 hours. Then the plates were washed three times as done earlier. Finally, 200 µl of freshly prepared substrate (10 mg ρ-nitro phenyl phosphate (Sigma 104-105) dissolved in 10 ml of freshly prepared substrate buffer) was added to each well and incubated in dark at room temperature for 20 to 45 minutes or as long as necessary to obtain clear reactions. Spectrometric measurement of absorbance was then read at 405 nm (EL 800, BIO-TEK Instrument Inc, and USA The reaction was stopped by adding 50 µl of 3 M NaOH. Buffer served as negative control.

Hybrid confirmation by molecular markers

DNA extraction from leaves of parents and F_1's was carried out following CTAB method (Doyle and Doyle, 1987).

PCR amplification

PCR reaction was performed using 10 (SSR) and 6 (ISSR) primers. The reagents that required for performing PCR reaction are as follows. The details of the primers are presented in APPENDIX I.

Protocol

PCR reaction was carried out in total volume of 10 µl in 96 tubes PCR plates. Following was the master mix of solution for one reaction.

For ISSR primers

Reagents	For 10 µl Reaction	Final concentration
10 X Taq buffer + $MgCl_2$ (15mM)	1.0 µl	1X
dNTP (2 mM)	1.0 µl	0.2 mM
Primers 10 µ M	1.0 µl (0.5µl each for combination)	1.0 µM
Taq polymerase (3 IU / µl)	0.1 µl	0.31 IU
Sterile double distilled water	4.9 µl	-
Template DNA10 ng / µl	2 µl	20 ng

Cycling profile

Touch down protocol was followed for all the primers.

S.No	Name of the primers	Sequence of the primers
1.	UBC - 807	5' AGA GAG AGA GAG AGA GT 3'
2.	UBC - 810	5'CAC ACA CAC ACA CAC AA 3'
3.	UBC - 815	5'CTC TCT CTC TCT CTC TG 3'
4.	UBC - 817	5' GAG AGA GAG AGA GAG AT 3'
5.	UBC - 856	5' ACA CAC ACA CAC ACA CYA 3'
6.	UBC - 861	5'ACC ACC ACC ACC ACC ACC 3'

APPENDIX I. List of ISSR Primers with sequences used in the analysis

Electrophoresis was performed in 1.5 per cent agarose with 120V for 2 hours. PAGE electrophoresis was carried out for SSR's silver staining protocol as performed following Benbouza *et al*. (2006).

Results and discussion

Screening of F_1 progenies through artificial inoculation against PRSV under glass house conditions

Carica papaya and *Vasconcellea cauliflora* were produced via intergeneric hybridization. Intergeneric hybrid seedlings along with parents were raised and artificially inoculated with PRSV under glass house conditions for screening. Observation for PRSV was done 27 days after inoculation. Out of 29 intergeneric hybrid seedlings involving CO 7 x *V.c* six were found to be apparently free from the disease. Similarly in the cross-combination Pusa Nanha x *V.c* out of 55 seedlings, 23 seedlings were found to be apparently free from PRSV. In the cross-combination CP 50 x *V.c* out of 335 seedlings, 70 seedlings were apparently free from PRSV disease. However, all the parents except *Vasconcellea cauliflora* showed typical PRSV symptoms after artificial inoculation (Table 2). In a perennial crop like papaya, field screening for diseases is very difficult since, it requires a larger area for planting. Hence, screening in glass houses in the nursery stage proved quick and rapid method.

Parents / Hybrids	Total number of plants inoculated	Disease scoring (number of plants in each category)						Number of plants without symptom 27 days after inoculation
		0	1	2	3	4	5	
CO 7	5	0	0	0	0	0	5	0
PusaNanha	5	0	0	0	0	0	5	0
CP 50	5	0	0	0	0	0	5	0
Vasconcellea cauliflora	5	5	0	0	0	0	0	5
CO 7 x *Vasconcellea cauliflora*	29	6	0	0	0	10	13	6
PusaNanha x*Vasconcelleacauliflora*	55	23	0	0	0	15	17	23
CP 50 x *Vasconcellea cauliflora*	335	70	0	0	0	100	165	70

Table 2: Screening of F_1 progenies through artificial inoculation against PRSV under glass house conditions

Cycling profile

Touch down protocol was followed for all the primers.

Typical PRSV symptom of mottling of leaves and water-soaked lesions on stems were observed in the susceptible parents and the hybrids. However, six out of 29 seedlings in CO 7 x *V.c*, 23 out of 55 in Pusa Nanha *V.c* and 70 out of 335 in CP 50 *V.c* were found to be completely free from PRSV symptoms (Plate 1). Regarding the female parents, all were found to exhibit the virus symptoms uniformly after sap inoculation. Symptom free F_1 hybrids were transplanted in the main field for further evaluation. The failures of PRSV symptoms to develop on the manually inoculated hybrid plants indicate the incorporation of genes resistant to PRSV. Further, the wild genus *V. cauliflora* was found to be completely resistant to the strain PRSV prevalent in Coimbatore area of Tamil Nadu, India (Manoranjitham*et al*, 2008).

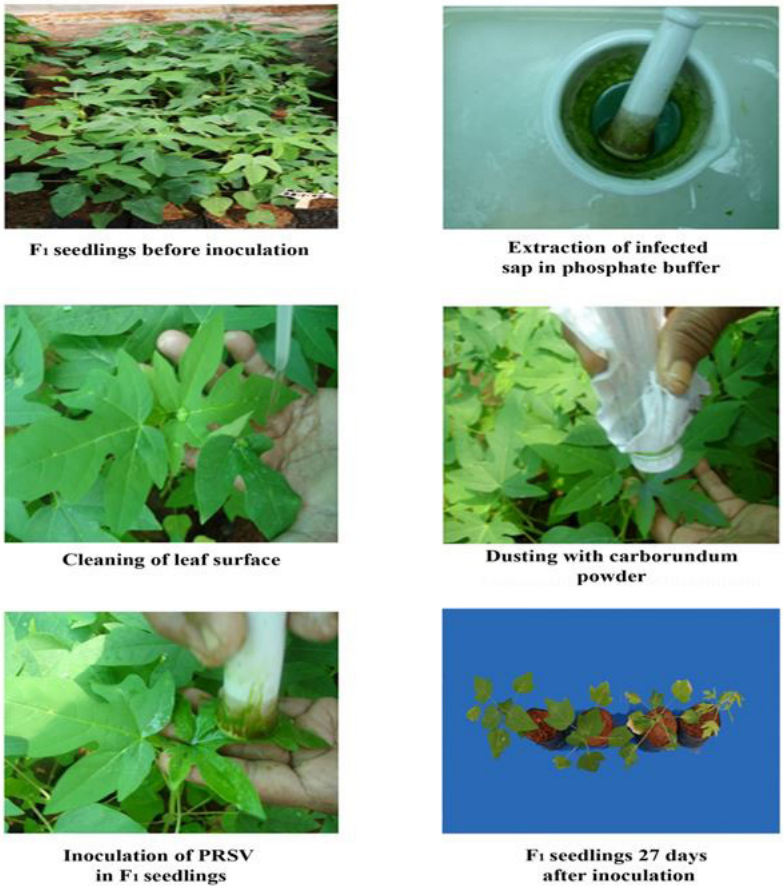
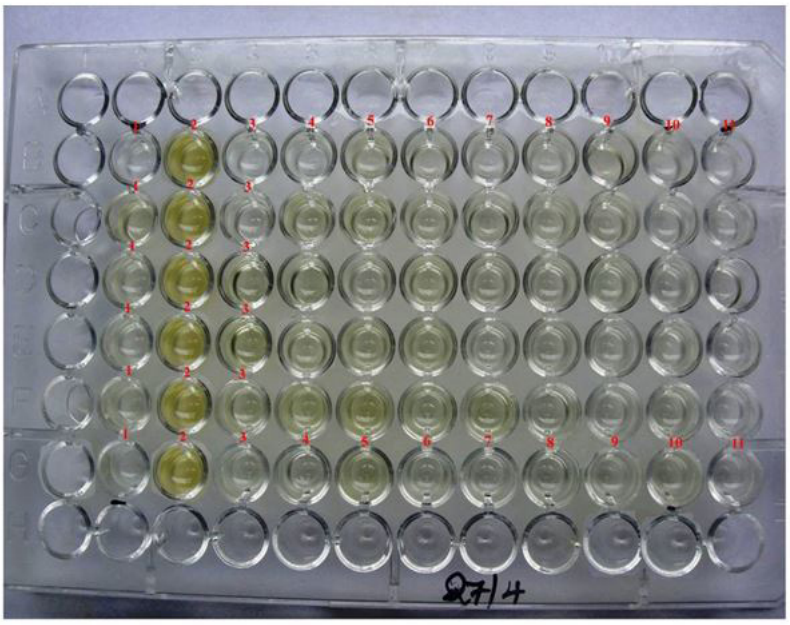


Plate 1: Confirmation of PRSV resistance in F_1 seedlings

ELISA titre value for parents and F_1 hybrids

The Enzyme Linked Immunosorbent Assay (ELISA), a powerful immunological test (Clark and Adams, 1977), is extensively used for detecting, identifying and quantifying viruses in many plant species (Clark, 1994). Parents and their hybrids *viz*, CO 7 x *V.c*, Pusa Nanha x *V.c* and CP50 x *V.c* were subjected to DAS- ELISA test.

Parents and F_1 progenies involving CO 7 and *Vasconcellea cauliflora* were subjected to DAS- ELISA test ELISA titre value varied from 0.216 to 0.972. Among the parents, the resistant male parent *Vasconcellea cauliflora* had recorded the lowest titre value of 0.216. However, the susceptible female parent CO 7 recorded the highest titre value of 0.972, followed by PusaNanha (0.952) and CP 50 (0.942) **(Plate 2).**



1- Buffer
2- *Carica Papaya*
3- *Vasconcellea cauliflora*
4-11- Intergeneric F_1 hybrids

Plate 2: Confirmation of PRSV resistance in in intergeneric F1 hybrids by ELISA

Among the hybrids involving CO7 and *V.c*, ELISA titre value varied from 0.243 to 0.266 (Table 3). Among the hybrids involving Pusa Nanha x *V.c*, ELISA titre value varied from 0.218 to 0.286 (Table 4). Among the hybrids involving CP50 x *V.c*, ELISA titre value varied from 0.218 to 0.299 (Table 5).

Sl.No	Parentsand their hybrids	OD value at 405nm
1.	*Vasconcelleacauliflora*	0.216
2.	CO 7	0.972
3.	Buffer	0.102
4.	CO7V1	0.266
5.	CO7V2	0.259
6.	**CO7V3**	**0.243**
7.	CO7V4	0.261
8.	**CO7V5**	**0.245**
9.	**CO7V6**	**0.247**

CO 7V (CO 7 x *Vasconcellea cauliflora)*

Table 3: ELISA titre value for parentsand F_1 population involving CO7 (apparently free from PRSV after inoculation)

Sl.No	Parentsand their hybrids	OD value at 405nm	Sl.No	Parentsand their hybrids	OD value at 405nm
1.	*Vasconcelleacauliflora*	0.216	14.	**PNV11**	**0.220**
2.	PusaNanha	0.952	15.	PNV12	0.266
3.	Buffer	0.102	16.	**PNV13**	**0.223**
4.	**PNV1**	**0.219**	17.	PNV14	0.268
5.	PNV2	0.278	18.	PNV15	0.284
6.	**PNV3**	**0.218**	19.	PNV16	0.286
7.	PNV4	0.275	20.	PNV17	0.285
8.	PNV5	0.251	21.	PNV18	0.286
9.	**PNV6**	**0.220**	22.	PNV19	0.275
10.	PNV7	0.278	23.	PNV20	0.280
11.	**PNV8**	**0.222**	24.	**PNV21**	**0.224**
12.	**PNV9**	**0.218**	25.	PNV22	0.270
13.	PNV10	0.287	26.	PNV23	0.274

PNV (PusaNanha x *Vasconcelleacauliflora*)

Table 4: ELISA titre value for parentsand F_1 population involving PusaNanha (apparently free from PRSV after inoculation)

Sl.No	Parents and their hybrids	OD value at 405nm	Sl.No	Parents and their hybrids	OD value at 405nm	Sl.No	Parents and their hybrids	OD value at 405nm
1.	*Vasconcelleacauliflora*	0.216	26.	**CPV23**	**0.218**	51.	CPV48	0.286
2.	CP 50	0.942	27.	CPV24	0.285	52.	CPV49	0.289
3.	Buffer	0.102	28.	CPV25	0.279	53.	CPV50	0.279
4.	**CPV1**	**0.222**	29.	**CPV26**	**0.226**	54.	CPV51	0.277
5.	CPV2	0.285	30.	CPV27	0.282	55.	CPV52	0.279
6.	CPV3	0.286	31.	CPV28	0.284	56.	CPV53	0.288
7.	CPV4	0.292	32.	CPV29	0.296	57.	CPV54	0.299
8.	CPV5	0.294	33.	CPV30	0.292	58.	CPV55	0.269
9.	CPV6	0.277	34.	**CPV31**	**0.221**	59.	**CPV56**	**0.219**
10.	CPV7	0.278	35.	CPV32	0.281	60.	CP V57	0.297
11.	CPV8	0.287	36.	CPV33	0.286	61.	CPV58	0.295
12.	CPV9	0.282	37.	CPV34	0.284	62.	CPV59	0.294
13.	CPV10	0.285	38.	CPV35	0.285	63.	CP V60	0.279
14.	CPV11	0.284	39.	CPV36	0.280	64.	CP V61	0.286
15.	**CPV12**	**0.232**	40.	CPV37	0.283	65.	CPV62	0.287
16.	CPV13	0.285	41.	CPV38	0.284	66.	CP V63	0.299
17.	CPV14	0.295	42.	**CPV39**	**0.220**	67.	CP V64	0.298
18.	CPV15	0.292	43.	CPV40	0.287	68.	CP V65	0.295
19.	CPV16	0.290	44.	CPV41	0.284	69.	CP V66	0.294
20.	CPV17	0.284	45.	CPV42	0.296	70.	CP V67	0.289
21.	CPV18	0.282	46.	CPV43	0.298	71.	CP V68	0.287
22.	CPV19	0.275	47.	CPV44	0.296	72.	CP V69	0.285
23.	CPV20	0.289	48.	CPV45	0.298	73.	CP V70	0.296
24.	CPV21	0.292	49.	CPV46	0.289			
25.	CPV22	0.294	50.	CPV47	0.295			

CPV (CP50 x *Vasconcellea cauliflora*)

Table 5: ELISA titre value for parents and F_1 population involving CP 50 (apparently free from PRSV after inoculation)

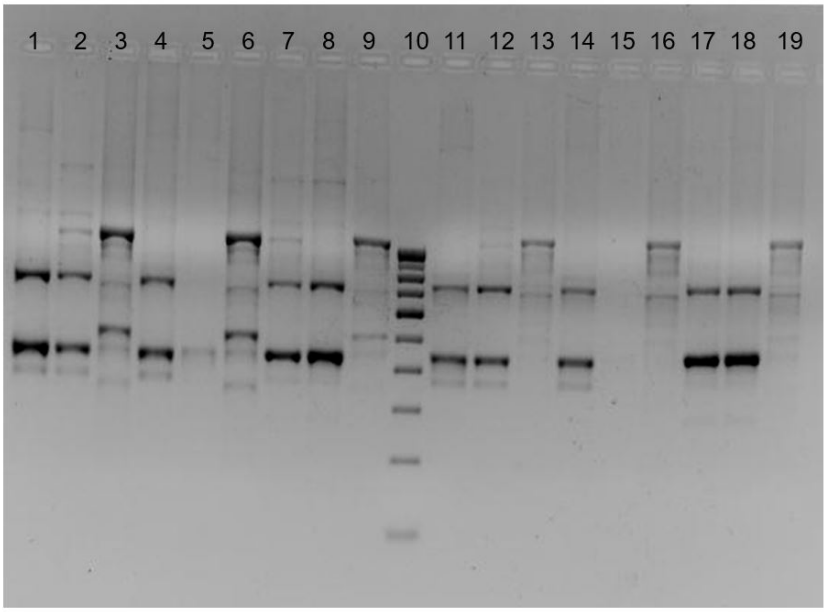
The cross combinations namely CO7V3, CO7V5 and CO7V6 were found to record lower titre values proving their tolerance to PRSV. Similarly, cross combination involving crosses *viz*, PNV1, PNV3, PNV9, PNV6, PNV8, PNV13 and PNV21 were found to record lower titre values proving their tolerance to PRSV. F_1 progenies namely CPV1, CPV12, CPV23, CPV31, CPV39, CPV26 and CPV56 were found to record lower titre values proving their tolerance to this virus (Plate 3, 4). This observation confirms the earlier report of Manshardt (1992) who studied the intergeneric hybrids involving *C. cauliflora x C.papaya* hybrids. Similar studies using ELISA test had been conducted previously to identify PRSV-P infected *C. papaya* (Gonsalves and Ishii, 1980, Thomas and Dodman, 1993).

Plate 3: Field view of parents and intergeneric F1hybids

Plate 4: Field view of Intergeneric F1 hybrids

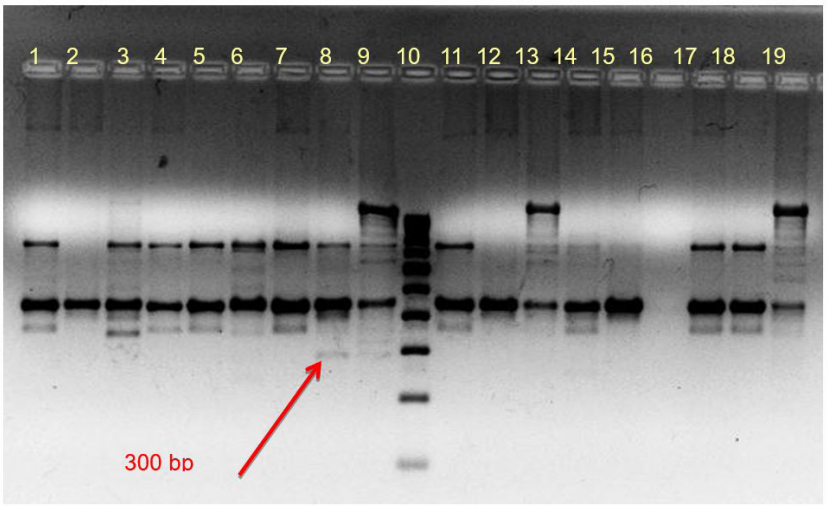
Hybridity confirmation using ISSR markers

The main objective of resistance breeding is the introgression of one or more resistant genes from the donor parent into the elite variety. Precise identification of plants using morphological markers to distinguish the true hybrid and out cross seeds is difficult as the phenotypic marker to differentiate male and female plants should be available (Zamir and Tadmore, 1986). In the absence of that proceeding for further generations to tag the useful genes conferring resistance, molecular marker will be a reliable tool to discriminate the hybrids and the parental lines. In the present investigation, to verify the hybridity and the level of resistance derived from *V. cauliflora*, a study was carried out using Inter- simple sequence repeats (ISSR). To detect hybridity, there must be polymorphism between the parents. Polymorphic bands which are present in male parent should be present in all the hybrids and should not be present in female parent (Magdalita *et al,* 1998).



Lane 1. CO 7 - Female
Lane 2. CO7V3 - Hybrid
Lane 3. Vasconcelleacauliflora - Male
Lane 4. PusaNanha - Female
Lane 5. PNV9 - Hybrid
Lane 6. Vasconcelleacauliflora - Male
Lane 7. CP 50 - Female
Lane 8. CPV23 - Hybrid
Lane 9. Vasconcelleacauliflora - Male
Lane 10. 100 bp ladder
Lane 11. CO 7 - Female
Lane 12. CO7V3 - Hybrid
Lane 13. Vasconcelleacauliflora - Male
Lane 14. PusaNanha - Female
Lane 15. PNV9 - Hybrid
Lane 16. Vasconcelleacauliflora - Male
Lane 17. CP 50 - Female
Lane 18. CPV23 - Hybrid
Lane 19. Vasconcelleacauliflora - Male

Figure 1: ISSR marker profile for parents and F_1s



Lane 1. PusaNanha -Female
Lane 2. PNV9 - Hybrid
Lane 3. Vasconcelleacauliflora - Male
Lane 4. PusaNanha - Female
Lane 5. PNV5 - Hybrid
Lane 6. Vasconcelleacauliflora- Male
Lane 7. PusaNanha - Female
Lane 8. PNV9 - Hybrid
Lane 9. Vasconcelleacauliflora - Male
Lane 10. 100 bp ladder
Lane 11. PusaNanha- Female
Lane 12. PNV11 - Hybrid
Lane 13. Vasconcelleacauliflora - Male
Lane 14. PusaNanha - Female
Lane 15. PNV21 - Hybrid
Lane 16. Vasconcelleacauliflora - Male
Lane 17. PusaNanha - Female
Lane 18. PNV13 - Hybrid
Lane 19. Vasconcelleacauliflora -Male

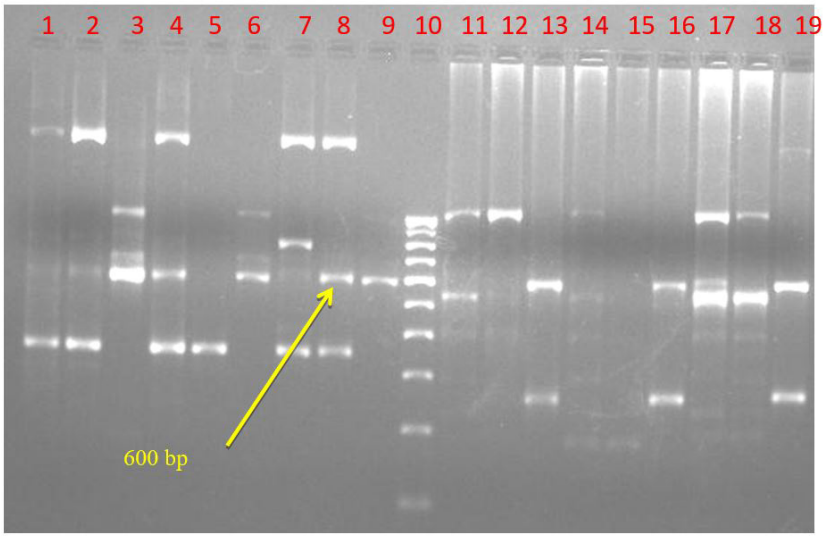
Figure 2: ISSR marker UBC 856 profile for parents and F_1

The primer UBC - 856 produced unique banding patterns in *Vasconcellea cauliflora* (male parent) in which five bands were prominent, out of which third and fifth were absent in female parent (Figure.1) but present in CO 7 x *Vasconcellea cauliflora* (CO7V3). The same primer produced distingusible band between PusaNanha x*Vasconcellea cauliflora* (PNV9) which was used for the identification of true hybrid (Figure.2).

[Internet]. 147(3):355-8.

Ruas et al. (2003) used Inter-simple sequence repeat (ISSR) markers and successfully evaluated the genetic divergence among the eight Coffea species. To confirm the hybridity of intergeneric hybrids involving Carica papaya x V. cauliflora, Praveen (2005) also used ISSR markers and confirmed successfully.

In case of UBC- 807 primer, one prominent band was observed in male parent which was absent in female parent but present in CP 50 x*Vasconcellea cauliflora* (CPV23) hybrid (Figure.3). These primers were helpful to identify F_1's in cross (CO7V3, CO7V5 and CO7V6), (PNV1, PNV3, PNV6, PNV8, PNV9, PNV11, PNV13 and PNV21) and (CPV1, CPV23, CPV12, CPV26, CPV31, CPV39 and CPV56). The hybridity confirmed F_1 plants were forwarded to F_2.



UBC 807 (Lane 1 - 9) and
UBC 810 (Lane 11 - 19)

Lane 1. CO 7 - Female
Lane 2. CO7V3 - Hybrid
Lane 3. Vasconcellea cauliflora - Male
Lane 4. PusaNanha - Female
Lane 5. PNV9 - Hybrid
Lane 6. Vasconcellea cauliflora - Male
Lane 7. CP 50 - Female
Lane 8. CPV23 - Hybrid
Lane 9. Vasconcellea cauliflora - Male
Lane 10. 100 bp ladder
Lane 11. CO 7 - Female
Lane 12. CO7V3 - Hybrid
Lane 13. Vasconcellea cauliflora - Male
Lane 14. PusaNanha- Female
Lane 15. PNV9 - Hybrid
Lane 16. Vasconcellos cauliflory- Male
Lane 17. CP 50 - Female
Lane 18. CPV23 - Hybrid
Lane 19. Vasconcellea cauliflora - Male

Figure 3: ISSR marker profile for parents and F_1s

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