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Screening of F_1 intergeneric hybrid progenies of papaya for papaya ringspot virus (PRSV) resistance

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ABSTRACT : The present investigation was undertaken to develop papaya ringspot virus (PRSV) resistant papaya hybrids through intergeneric hybridization. Intergeneric hybridization was done involving nine *Carica papaya* cultivars as female and *Vasconcellea cauliflora* as male. Intergeneric F_1 hybrids were artificially inoculated. 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 *V. cauliflora,* only six plants (CO 7V1 to CO 7V6) were found free from PRSV symptoms. Similarly, out of fifty five F_1 hybrid plants of Pusa Nanha x *V. cauliflora* only twenty three (PNV1 to PNV23) were found free from the symptoms and seventy plants (CPV1 to CPV70) out of 335 plants of CP50 x *V. cauliflora* were found free from PRSV symptoms. Molecular markers ISSR markers were used to check and verify the hybridity. The resistance of the hybrids and parents were subjected to DAS ELISA test. ELISA titre value varied from 0.216 to 0.927. Among the parents, the resistant male parent *V. 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 Pusa Nanha 0.952 and CP50 0.942. Among the hybrids ELISA titre value ranged from 0.218 to 0.29.

Keywords: *Carica papaya*, *Vasconcellea cauliflora*, intergeneric hybrids, papaya ringspot virus, resistance

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the most important fruits of tropical and subtropical regions of the world and belongs to family Caricaceae. India is the largest producer of papaya in the world. 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. Papaya is affected by a number of diseases caused by various pathogens and viruses. Now a days the most destructive disease of *C. papaya* worldwide is papaya ring spot caused by papaya ring spot virus (PRSV) -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).

Incidence of PRSV has been reported to be more than 90 per cent in India (Varma, 1996; Jagadish Chandra and Samuel, 1999) and rendering papaya orchards economically unviable (Hema and Theertha Prasad, 2003). The results of the roving survey for papaya ringspot incidence in Karnataka revealed the presence of the disease in all the districts ranging from 75 to 100 per cent except Udupi, Hassan and Kodaku (Kunkalilkar Suresh and Byadgi, 2004). In Tamil Nadu, the disease was first noticed in Coimbatore during 2003 (Jyoti Sharma *et al.,* 2004). The present study was conducted to evaluate intergeneric F_1 hybrids of papaya for their resistance to PRSV.

MATERIALS AND METHODS

Plant materials

Seedlings were artificially inoculated with papaya ring spot virus through artificial inoculation method. The seedlings showing initial resistance alone were taken to field for further evaluation. The details of the parents and F_1 seedlings are presented in Table 1.

Mechanical inoculation of PRSV toparents, 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

Table 1. Scale of disease incidence and intensity score

Reaction	Intensity score	Symptom
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	5 = Extensive mosaic and serious deformation of leaves, or plant death).

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 was given using the scale developed by Dhanam (2006). Details of the disease incidence and intensity score scale are presented in APPENDIX I.

Transplanting

Experiment was laid out in a Randomized Block Design with three replications. Forty five day old healthy seedlings along with parents (6 seedlings each) were planted at a spacing of 1.8 × 1.8 m and standard package of practices were followed during the period of study.

Hybridity confirmation using ISSR markers

To confirm the hybridity of these intergeneric progenies, ISSR marker analysis was carried out using six CO 7 x *V. cauliflora,* twenty three Pusa Nanha x *V. cauliflora* and seventy plants of CP50 x *V.cauliflora*. DNA from leaves of parents and F_1 was carried out following CTAB method (Doyle and Doyle, 1987). PCR reaction was performed using 6 (ISSR) primers. PCR reaction was carried out in total volume of 10 µl in 96 tubes PCR plates. Following were the master mix of solution for one reaction. For ISSR primers, reagents of 10 X Taq buffer + $MgCl_2$ (15 mM) on 1.0 µl, dNTP (2 mM)on 1.0 µl, Primers 10µM 1.0 µl (0.5µl each for combination), Taq polymerase (3 IU / µl) on 0.1 µl, Sterile double distilled water on 4.9 µl and Template DNA 10 ng / µl on 2 µl. Cycling profile- Touch down protocol was followed for all the primers. PCR cycles included initial denaturation at 94°C for 3 min followed by 19 cycles of 30 Sec (-0.5°C) denaturation at 94°C, annealing at 63°C for 30 sec and 1 min in extension at 72°C. Again 19 cycles of 15 Sec denaturation at 94°C, annealing at 55°C for 30 sec, 1 min in extension at 72°C, 10 min in final extension at 72°C and infinitive final hold at 4°C. Electrophoresis was performed in 1.5 per cent agarose with 120V for 2 hours.

Source of antiserum and positive sample: Antibody for PRSV and their positive samples were provided from DSMZ, Braunschweing, Germany. 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.

RESULTS AND DISCUSSION

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

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. Observation for PRSV was done 27 days after inoculation. A total number of 29 seedlings in CO 7 x *V.cauliflora,* 55 plants in Pusa Nanha x *V.cauliflora*and 335 plants in CP50 x *V.cauliflora* were artificially inoculated with papaya ringspot virus through sap inoculation method. Typical PRSV symptom of mottling of leaves and water soaked lesions on stems were observed in the susceptible parents and the hybrids. 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 (Table 1). 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).

Hybridity confirmed intergeneric hybrids

Three intergeneric hybrids of CO 7 x *Vasconcellea cauliflora* crosses out of six, eight intergeneric hybrids of Pusa Nanha x *Vasconcellea cauliflora* crosses out of 23 and seven intergeneric hybrids of CP 50 x *Vasconcellea cauliflora* crosses out 70 were tested for hybridity. 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 (Fig. 1) but present in CO 7 x*Vasconcellea cauliflora* (CO7V3). The same primer produced distingusible band between Pusa Nanha x*Vasconcellea cauliflora* (PNV9) which was used for the identification of true hybrid (Fig. 2). 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 (Fig. 3). These primers were helpful to identify F_1's in cross CO 7 x*Vasconcellea cauliflora,* Pusa Nanha x *Vasconcellea cauliflora*and CP 50 x *Vasconcellea cauliflora.* The hybridity confirmed F_1 plants were forwarded to F_2.

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.

ELISA titre value for parents and F_1 hybrids

The Enzyme Linked Immunosorbent Assay (ELISA), a powerful immunological test (Clark and Adams,

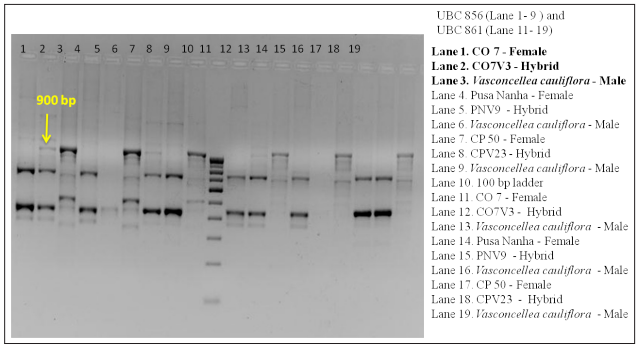

Fig. 1. ISSR Marker profile of parents and F_1s

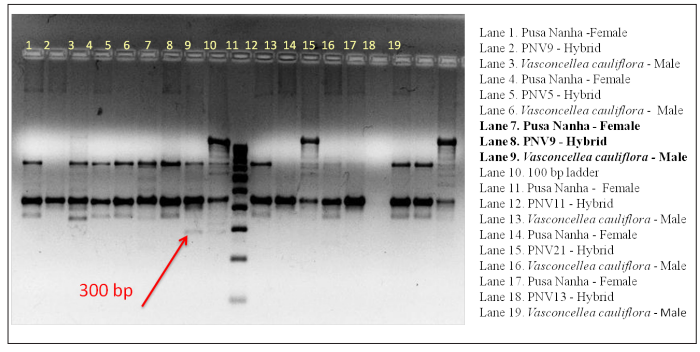

Fig. 2. ISSR marker UBC 856 profile of parents and F_1s

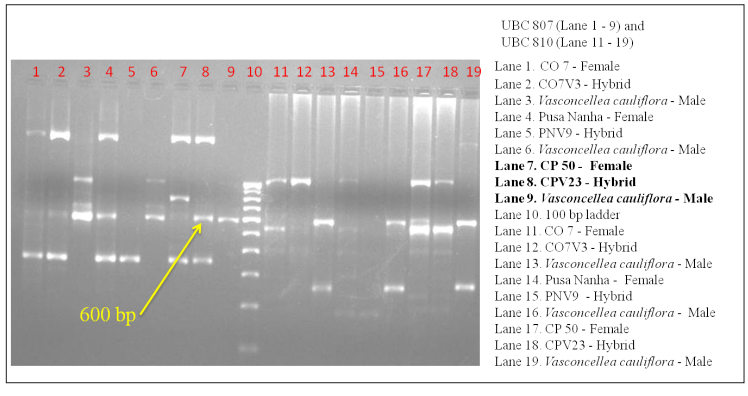

Fig. 3. ISSR marker profile of parents and F_1s

1977), is extensively used for detecting, identifying and quantifying viruses in many plant species (Clark, 1994). This test could be a component of a reliable method for screening *C. papaya* x *C. cauliflora* hybrid plants for PRSV resistance. In this study, the resistance of the hybrids and parents was assessed by serological test. Parents and their hybrids *viz*., CO 7 x *V.cauliflora*, Pusa Nanha x *V.cauliflora* and CP50 x *V.cauliflora* 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). Among the hybrids involving CO7 and *Vasconcellea cauliflora*, ELISA titre value varied from 0.243 to 0.266 (Table 3). Among them, the cross combination CO7V3, confirmed hybrid through molecular markers, was found to record the lowest titre value of 0.243 followed hybrid CO7V5 (0.245) and CO7V6 (0.247).

Among the hybrids involving PusaNanha x *V.cauliflora*, ELISA titre value varied from 0.218 to 0.286 **(Table 2).**Among the hybrid combinations, the combinations PNV3 and PNV9 recorded the lowest titre value of 0.218 followed by PNV1 (0.219), PNV6 (0.220), PNV11 (0.220), PNV8 (0.222) and PNV13 (0.223). All the above said hybrid combinations were confirmed as true hybrids through molecular marker studies.

Among the hybrids involving CP50 x *V.cauliflora*, ELISA titre value varied from 0.218 to 0.299 (Table 3). Among them, the cross combination CPV23 a confirmed hybrid through molecular markers, was found to record the lowest titre value of 0.218 followed by other confirmed hybrids *viz*., CPV56 (0.219), CPV39 (0.220), CPV31 (0.221), CPV1 (0.222), CPV26 (0.226) and CPV12 (0.232).

The results revealed that the lowest value of 0.216 was recorded by the resistant male parent.*V. cauliflora* however; all the female parents used for this study recorded very high titre values proving their susceptibility. Manoranjitham *et al.* (2008) reported that *V.cauliflora* registered the lowest titre value which clearly indicated its natural resistance to PRSV. They also reported that *V.cauliflora* is resistant to all the strains of PRSV which are prevalent in Coimbatore conditions.

Among the parents, the gynodioecious female parent CO7 was found to be highly susceptible than the other two dioecious female parents *i.e*. Pusa Nanha and CP 50.Thirugnanavel (2010) also reported that tolerant genotypes recorded the lower ELISA absorbance value than

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

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
Pusa Nanha	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
Pusa Nanha x*Vasconcelleacauliflora*	55	23	0	0	0	15	17	23
CP 50 x *Vasconcellea cauliflora*	335	70	0	0	0	100	165	70

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

Parents and their hybrids	OD value at 405nm	Parents and their hybrids	OD value at 405nm	Parents and their hybrids	OD value at 405nm
Vasconcellea cauliflora	0.216	*Vasconcellea cauliflora*	0.216	*Vasconcellea cauliflora*	0.216
CO 7	0.972	Pusa Nanha	0.952	CP 50	0.942
Buffer	0.102	Buffer	0.102	Buffer	0.102
CO7V3	0.243	PNV1	0.219	CPV1	0.222
CO7V5	0.245	PNV3	0.218	CPV12	0.232
CO7V6	0.247	PNV6	0.220	CPV23	0.218
		PNV8	0.222	CPV26	0.226
		PNV9	0.218	CPV31	0.221
		PNV11	0.220	CPV39	0.220
		PNV13	0.223	CPV56	0.219

Fig. 4. Field view of intergeneric F_1 hybrids

the susceptible ones. Among the genotypes tested, tolerant genotype CP 50 recorded the lowest value of 0.187 at flowering and 0.198 at harvest.

In the present study, the cross combinations *viz*., C7V3, CO7V5 and CO7V6 were recorded the lowest titre values. Similarly the crosses namely PNV1, PNV3, PNV6, PNV8, PNV9, PNV13 and PNV21 were observed the lowest titre values. CP50 x *V.Cauliflora* progenies *viz*., CPV1, CPV12, CPV23, CPV26, CPV31, CPV39 and CPV56 were recorded the lowest titre values proved their tolerance to this virus . 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).

Reaction of parents and F_1 hybrids after transplanting under field conditions

The study revealed varied levels of tolerance for PRSV by the parents and their hybrids (Figure 4). All the hybrids which were artificially inoculated with PRSV, but not showing virus symptoms, and their parents the male parent *V.Cauliflora* was not showed the PRSV symptoms but the female parents CO7 , Pusa Nanha and CP50 showing virus symptoms in the main field. This may be due to the fact that tolerance is affected by many factors including inherent genetics, time of infection and climatic conditions (Vimla Singh *et al*., 2005).

CONCLUSION

Based on the disease intensity score, reaction to the papaya ringspot virus and yield performance, selected F_1 combinations *viz*., CO 7 x *Vasconcellea cauliflora* (CO7V3), Pusa Nanha x *Vasconcellea cauliflora* (PNV9) and CP 50 x *Vasconcellea cauliflora* (CPV23) were advanced to F_2 generations.

REFERENCES

Clark, M.F., Adams, A. N.1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology*, **34**: 475-83.

Clark. 1994. Immunodiagnosis methods using polyclonal and monoclonal antibodies. In: The identification and characterization of pest organisms. (Hawksworth, D.L., ed). Wallingford, UK; CAB International, 377-393.

Dhanam, S. 2006. Studies on papaya ringspot disease (*Carica papaya* L.). A part of M.Sc. Thesis**.** Tamil Nadu Agricultural University, Coimbatore.

Doyle, J.J. and Doyle, J. L.1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin***, 19**: 11-15.

Gonsalves, D. 1998. Control of papaya ringspot virus in papaya: a case study. *Annual Review of Phytopathology***, 36**: 415-437.

Gonsalves, D. 1994. Papaya ringspot. **In:** Compendium of Tropical Fruit Diseases. (Ploetz, R.C, ed). MN, USA: APS Press.67.

Gonsalves, D. and Ishii, M. 1980. Purification and serology of papaya ringspot virus. *Phytopathology***, 70:** 1028-32.

Hema, M.V. and Theertha Prasad, D. 2003. Management of papaya ringspot virus-need for transgenic approach. *Indian Journal of Agricultural Biochemistry***, 16**: 1-11.

Jagadish Chandra, K. and Samuel, L. D. K. 1999. Viral and phytoplasmal diseases of papaya in India. **In:** Diseases of Horticultural Crops –Fruits. (Ed.) Verma, L.R. and Sharma, R.C. Indus Publishing Co., New Delhi.

Jimenez, H. and Horovitz, S. 1957. Cruzabilidad entree species de Carica. *Agronomy Tropical***,7:**207-215.

Jyoti Sharma, Jain, R. K. and Varma, A. 2004. Detection of papaya ringspot virus in naturally infected papaya plants by reverse transcription polymerase chain reaction. *Indian Phytopathology,* **57:** 237-239.

Kunkalilkar, S. and Byadgi, A. S. 2004. Molecular characterization, cloning of coat protein gene, epidemiology and management of papaya ring spot virus. *Indian Phytopathology,* **57:** 330-331.

Litz, R.E, 1984. Papaya. **In:** Evans DA Sharp WR, Ammirato PV & Yamada Y,eds. Handbook of Plant Cell Culture, Vol 2. New York, NY, USA: Macmillan, 349-368.

Manshardt, R.M. 1992. Papaya. **In:** Hammerschlag FA, Litz FA & Litz RE, eds. biotechnology of perennial fruit crops. Wallingford, UK; CAB International, 489-511.

Manoranjitham, S.K., Auxcilia, J., Balamohan, T. N., A. Thirugnanavel and Rabindran, R. 2008. Confirmation of PRSV resistance in wild type *Vasconcelleacauliflora* through sap inoculation studies. S-II: Genetic resources and crop Improvement. Second International Symposium on Papaya. 9-12 December, Madurai India

Praveen, K.S. 2005.Interspecific hybrid progeny evaluation in papaya (*Carica papaya* L.). M.Sc. Thesis. University of Agricultural Sciences, Bangalore.

Ruas, P.M., Raus, C. F., Rampim, V.P. Carvalho, E.A. Raus and Sera, T. 2003. Genetic relationship in *Coffea* species and parentage determination of interspecific hybrids using ISSR (Inter-simple sequence repeat) markers. *Genetics and Molecular Biology*, **26** (3):345-349.

Shukla, D.D, C.W. Ward and A.A. Brunt.1994. The polyviridae. Wallingford, UK: CAB International.

Thirugnanavel, A. 2010. Breeding for PRSV resistance in papaya (*Carica papaya* L.) through germplasm screening and intergeneric hybridization. Ph. D Thesis. Tamil Nadu Agricultural University Coimbatore.

Thomas, J.E. and Dodman, R. L. 1993. The first record of papaya ring spot virus –type P from Australia. *Australasian Plant Pathology*, **22**, 1-7.

Varma, A.K. 1996. Viral and Mycoplasmal diseases of papaya (*Carica papaya* L.) Disease Scenario in Crop Plants, Vol. I-Fruits and Vegetables (Eds). Agnihort, V.P., Om Prakash, Ram Krishnan and Mishra, A.K. International Books and Periodical Supply Service, New Delhi.

Vegas, A.,Trujillo, G., Sandrea, Y. and Mata, J. 2003. Obtention, regeneration and evaluation of intergeneric hybrids between *Carica papaya* and *Vasconcellea cauliflora*. *Intersciencia*, **28**(12):710-714.

Vimla Singh, G.P., Rao and Shukla, K. 2005. Response of commercially important papaya cultivars to papaya ring spot virus in eastern U.P. conditions. *Indian Phytopathology*, 58: 212-216.

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