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Study on resistance to papaya ringspot virus (PRSV) in intergeneric hybrid population of papaya CV washington (*Carica papaya* L.) and *Vasconcellea cauliflora*

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Abstract
An intergeneric hybridization programmee was conducted between papaya cultivar Washington (*Carica papaya* L) and *Vasconcellea cauliflora*. The objective was to incorporate the PRSV resistantce from *V. cauliflora* in to papaya CV Washington. From the cross six plant types were studied. Among these selections, present population study of promising papaya selection GKPS-2-7 (F_7 generation) was conducted at ZARS, NARP, Pune during 2015-16. The performance of this promising selection population was studied in comparison with popular cultivars *viz.* Arka Prabhat and Red Lady. From this study it was observed that, at 7 months after planting genotype GKPS-2-7 recoded minimum ring spot infection (1.24 as *Per cent* Disease Index) However, cultivars Arka Prabhat and Red Lady recorded maximum papaya ring spot infection (54.00 and 100 as *Per cent* Disease Index respectively). This selection is to be explored for further development of a papaya ring spot resistant cultivar.

Keywords: Papaya, PRSV, Population study

1. Introduction
Papaya (*Carica papaya* L) is one of the most important fruit crops of tropics. Papaya is cheapest fruit like banana and preferred by all in different levels of age group and economy. It is believed to be native of Tropical America; probably Southern Mexico from where it was distributed to most of the Caribbean and Asian countries during the 16th century [5]. It is a rich source of nutrients such as vitamins, a carotenoids (2020 IU/100 g), vitamin C (46 g / 100 g), B vitamins, dietary fiber and the minerals *viz.* potassium and magnesium. In India it is cultivated on 1.33 lakh ha area with production of 56.39 lakh MT and productivity of 42.30 MT ha^{-1}. In respect of Maharashtra it is cultivated on 11,000 ha area with production of 5.00 lakh MT and productivity of 45.50 MT ha^{-1}) [1].
The total area under cultivation in the country has recorded a regular increase. However, its productivity has been decreasing. This might be due to the losses caused by different diseases caused by fungi, bacteria, phytoplasma and viruses. There are many economically important diseases of papaya the most important among them is a papaya ring spot caused by virus [18]. Management of PRSV through rouging of infected plants, use of insecticides against insect vectors and cross protection generally have not been effective in controlling the disease. Up till now, natrual resistance to PRSV has not been identified in any papaya cultivar. Thus, with aim of developing a resistant papaya cultivar, an intergeneric hybridization programme was conducted between papaya CV Washington (*Carica papaya* L) with *Vasconcellea cauliflora* during the year 1998.
Various efforts have been made to incorporate the resistance genes from other genera in the family Caricaceae namely, *Vasconcellea cauliflora*,*V. quercifolia*, *V. stipulata* and *V. pubescens*. Intergeneric hybrids between papaya and PRSV resistant species have been produced by number of investigators with aid of embryo rescue techniques [11, 13]. However not much progress has been made and overcame the virus disease.

Materials and Methods
An intergeneric hybridization programmee was conducted between papaya CV Washington (*Carica papaya* L) and *Vasconcellea cauliflora* at NARP, Ganeshkhind, Pune during the year1998-99. With aid of embryo rescue techniques, different six selections were made in F_2 generation *viz.* GKPS-1, GKPS-2, GKPS-3, GKPS-4, GKPS-5 and GKPS-6. During the year 2014 by applying selection pressure on F_6 generation, PRSV resistant selection (GKPS-2-7)

was made from GKPS-2. After obtaining hopeful findings from F_7 generation, to prove quantative resistance against PRSV it was felt necessary to test papaya genotype GKPS-2-7 on large scale. In this context, total 113 healthy seedlings of GKPS-2-7 were planted along with check cvs. *viz;* Arka Prabhat and Red Lady in well drained, fertile, clay loam soil with spacing of 2.25 X 2.25 m on 16th March 2015. All recommended crop management practices except use of insecticides were followed. Each plant was manured with 20 kg FYM before transplanting and 200 g N : 200 g P : 200 g K in four equal split doses at 1, 3, 5 and 7 month after transplanting. During the crop growth no any insecticide spray was applied, the object behind this was to test the natural resistance in uncontrolled vector population and thereby under good disease pressure.

Observations on growth, yield and yield contributing characters and incidence of papaya ring spot (PRS) disease were recorded. Those plants which don't showed viral PRS symptoms were indexed with Enzyme Linked Immunosorbent Assay (ELISA). The individual plants at regular interval were visually observed for PRS disease with following scale.

Table 1: Different levels of scale based on the symptoms exhibited by the plants.

Scale	Host response	Reaction
0	Non symptomatic	Resistant
1	Only a few tiny chloratic spots	Tolerant
2	Weak mosaic symptoms with oily rings on stem and petioles	Tolerant
3	Severe mosaic symptoms	Moderately susptible
4	Very severe mosaic symptoms without distortion of leaf	Susptible
5	Very severe mosaic symptoms with distortion of leaf	Highly Susptible

PDI:- *Per cent* Disease Index was calculated by following formula

$$\textbf{PDI} = \frac{\sum xi}{N \times \text{max rating}} \times 100$$

Where,

PDI = *Per cent* Disease Index
Xi = Sum of all ratings
N = Numbers of plants observed

Sib-mating

For sib-mating, male and female plants of the same population were selected based on the disease intensity, petiole colour, petiole arrangement and yield. Female flowers which were about to open the next day were bagged on the previous day evening. Pollen grains were collected from fully matured, unopened flowers of the desired male parent from the same population. Pollination was done between 6.30 and 8.30 AM. At the time of pollination, the top of bags of the female flowers was opened and the pollens collected from desired male parent were dusted on the stigma and bagged again. If either of the parents of sib mated fruit found infected by virus at early stage, such seeds were discarded. The fruits, whose both parents showing visually no PRS infection or at late stage were selected for extracting seed for raising the next generation.

Enzyme Linked Immunosorbent Assay (ELISA)

The Enzyme Linked Immunosorbent Assay (ELISA), a powerful immunological test [3] is extensively used for indexing, identifying and quantifying viruses in many plant species [4]. ELISA test is a more sensitive and convenient than back inoculation tests when large numbers of plants have to be screened. This test could be an effective component of a reliable method for screening *C. papaya* / *C. cauliflora* hybrid plants for PRSV indexing. Antibodies for PRSV and their positive samples were obtained. Double antibody sandwich ELISA (DAS-ELISA) was performed for the detection of PRSV by following the proper laboratory procedure. 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 ºC for 2 to 4 hrs and thereafter plates were washed with PBS-T using wash bottle, soaked for a few minutes and washing repeated twice. Plates were blotted by tapping upside down on tissue paper. 200 µl of aliquots of the test sample (extracted in sample extraction buffer) were added to duplicate wells. The plates were incubated overnight at 4 ºC. The plates were washed as mentioned earlier and added with 200 µl of the anti-virus conjugate (1:500) to each well and incubated at 37 ºC for 2 hrs. Then the plates were washed 3 times as indicated earlier. Finally 200 µl of freshly prepared substrate in 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 20-45 minutes or as long as necessary to obtain clear reactions. The reaction was stopped by adding 50 µl of 3M NaOH. Buffer served as negative control. Positive control was also included. Spectrometric measurement of absorbance was then read at 405 nm.

Traits under study

First fruiting height was measured from ground level to the height at which first flower appeared and expressed in centimeters. Total soluble solids of the fruit was determined by 'ERMA' hand refractometer and expressed as ºBrix. Disease intensity score was recorded at 30 days interval from first month after planting till maturity of crop (15 months).

Statistical analysis

The variation of individual in the population of genotype GKPS-2-7 for papaya ring spot symptoms were analyzed using MS excel and expressed in terms of *per cent* Disease Index (PDI).

Results and Discussions

Incidence of papaya ring spot virus (PRSV) disease

The present population study of papaya genotype GKPS-2-7 revealed that, this genotype found quantitatively resistant to PRSV as against check cultivars Arka Prabhat and Red Lady. The population mean of (113 plants) genotype GKPS-2-7 recorded minimum PRS incidence (1.24, 2.12, 4.42, 9.46, 10.44, 14.87, 30.44, 50.44 and 67.08 as PDI) at 7,8,9,10,11,12,13,14 and 15 months after transplanting, respectively (Table 1) .

However, check cultivar Red Lady recorded heavy incidence at very early i.e. at 2 months after planting (28.00 as PDI). This cultivar recorded highest disease (100 as PDI) at age of 7 months after planting. The cv. Arka Prabhat recorded highest disease (100 as PDI) at age of 9 months after planting (Table 2 and Table 3). However, population mean of PRS disease index in genotype GKPS-2-7 was 1.24 and 4.42 in seventh and ninth month age, respectively. The check cvs. v*iz.* Arka Prabhat and Red Lady showed 100 % population under 05 rating on 7 and 9 months of age respectively. As against in GKPS-2-7, more than 50% population was under 0 rating till

12 months of crop age.

As the genotype GKPS-2-7 recorded late PRS apperance this helps to escape heavy economic losses from PRS infection up tp 11 months after planting (10.44 as PDI). By this time fruits on the plants were set and they were ready for harvest. Thus delayed disease outbreak appearance and slow progress of of the disease is promising to minimize yield losses.

Horticultural performance (Table 4)

Mean value is the important factors for selection; mean serves as a basis for eliminating undesirable crosses. Selection for the improvement of quantitative characters can be effective only when the segregating generations possess the potential variability. The population means of GKPS-2-7 revealed that, this genotype recorded 81.36 days for flowering, 75.71 cm fruit bearing height, 33.93 fruits plant^{-1} with 1.01 kg average fruit weight. The mean fruit yield plant^{-1} was 34.11 kg. The CV Red Lady recorded 28.56 fruits yield plant^{-1} with average fruit weight of 1.52 kg. The CV Arka Prabhat recorded 26.97 kg fruit yield $^{-1}$ with 0.96 kg as average fruit weight.

Serological test

In the present study, plants showing visually '0' rating from 6th to 13th month of age were indexed as negative through ELISA serological test. Manoranjitham *et al* reported that the wild papaya *V. cauliflora* had the lowest titre value thus indicating its natural resistance to PRSV [16]. They also reported that *V. cauliflora* is resistant to all the strains of PRSV which are prevalent in Coimbatore conditions. Similar results were observed by Jayavalli [12]. Thirugnanavel who also reported that tolerant genotypes recorded lower ELISA absorbance value than the susceptible ones in papaya [19]. Eighteen progenies from Pusa Nanha / *Vasconcellea cauliflora* (C1-7, C1-10, C1-13, C121, C1-24, C1-28, C1-38, C1- 43, C1-46, C1-52, C1-73, C1-82, C1-84, C1-91, C1-92, C1-99, C1-101 and C1-108), 5 progenies from CP 50 / *Vasconcellea cauliflora* (C2-15, C2-16, C2-26, C2-27 and C2-28) and only one progeny from the cross CO 7 / *Vasconcellea cauliflora* (C3-5) were found to record lower titre values ranging from 0.243 to 0.285, proving their tolerance to PRSV and were selected for further evaluation in the field. Similar studies using ELISA test had been conducted previously by Thomas and Dodman to identify PRSV-P infected *C. papaya* [20].

Table 1: Performance of GKPS-2-7 on papaya ring spot virus (PRSV).

Sr. No	Age	Total plants	Plant population under PRS rating						PDI
			0	1	2	3	4	5	
1	6 month	113	113	0	0	0	0	0	0.00
2	7 months	113	107	5	1	0	0	0	1.24
3	8 months	113	105	5	2	1	0	0	2.12
4	9 months	113	103	2	3	3	2	0	4.42
5	10 months	113	94	2	4	5	7	0	9.46
6	11 months	113	90	5	7	5	5	1	10.44
7	12 months	113	81	7	9	7	7	2	14.87
8	13 months	113	24	42	24	14	5	4	30.44
9	14 months	113	0	21	43	28	11	10	50.44
10	15 months	113	0	3	23	40	25	22	67.08

Table 2: Performance of Red Lady on papaya ring spot virus (PRSV).

Sr. No	Age	Total plants	Plant population under PRS rating						PDI
			0	1	2	3	4	5	
1	1months	10	10	0	0	0	0	0	0.00
2	2 months	10	3	1	5	1	0	0	28.00
3	3 months	10	0	2	5	3	0	0	42.00
4	4 months	10	0	0	2	6	1	1	62.00
5	5 months	10	0	0	0	1	6	3	84.00
6	6 months	10	0	0	0	0	4	6	92.00
7	7 months	10	0	0	0	0	0	10	100.00

Table 3: Performance of Arka Prabhat on papaya ring spot virus (PRSV).

Sr. No	Age	Total plants	Plant population under PRS rating						PDI
			0	1	2	3	4	5	
1	1 months	10	10	0	0	0	0	0	0.00
2	2 months	10	10	0	0	0	0	0	0.00
3	3 months	10	8	2	0	0	0	0	4.00
4	4 months	10	7	2	1	0	0	0	8.00
5	5 months	10	6	3	1	0	0	0	10.00
6	6 months	10	1	2	6	1	0	0	34.00
7	7 months	10	0	1	4	3	1	1	54.00
8	8 months	10	0	0	0	0	8	2	84.00
9	9 months	10	0	0	0	0	0	10	100.00

Table 4. Horticultural performance of GKPS-2-7.

Sr. no	Days for 1st flow.	Height at 1st flow. Female cm.	No of fruits plant^{-1}.	Average fruit weight (kg.)	Yield kg Plant^{-1}.	Sr. no	Days for 1st flow.	Height at 1st flow. Female cm.	No of fruits plant^{-1}.	Average fruit weight (kg.)	Yield kg Plant^{-1}.
1	79.00	81.00	43.00	0.98	42.14	16	76.00	63.00	36.00	1.07	38.52
2	89.00	79.00	14.00	1.10	15.40	17	81.00	78.00	31.00	1.10	34.10
3	81.00	83.00	35.00	0.97	33.95	18	84.00	68.00	21.00	0.89	10.69
4	81.00	79.00	49.00	1.27	62.23	19	82.00	74.00	28.00	1.20	33.60
5	84.00	69.00	32.00	0.89	28.40	20	76.00	68.00	46.00	0.89	40.94
6	91.00	66.00	29.00	0.78	22.62	21	82.00	79.00	31.00	1.09	33.79
7	79.00	67.00	41.00	0.88	36.08	22	78.00	83.00	26.00	1.10	28.60
8	79.00	72.00	34.00	1.02	34.68	23	81.00	67.00	34.00	0.89	30.26
9	82.00	91.00	29.00	1.21	35.09	24	84.00	76.00	29.00	0.68	19.72
10	84.00	86.00	38.00	0.89	33.82	25	86.00	86.00	38.00	0.87	33.06
11	83.00	81.00	41.00	1.12	45.92	26	91.00	68.00	26.00	1.02	26.52
12	84.00	69.00	36.00	1.06	38.16	27	86.00	68.00	33.00	0.98	32.34
13	79.00	72.00	31.00	0.98	30.38	28	81.00	79.00	24.00	0.79	18.96
14	87.00	66.00	36.00	1.10	39.60	29	76.00	82.00	57.00	1.16	66.12
15	79.00	69.00	41.00	0.98	40.18	30	86.00	72.00	26.00	1.23	31.98

Table 4: Horticultural performance of GKPS-2-7.

Sr. no	Days for 1st flow.	Height at 1st flow. Female cm.	No of fruits plant^{-1}.	Average fruit weight (kg.)	Yield kg Plant^{-1}.	Sr. no	Days for 1st flow.	Height at 1st flow. Female cm.	No of fruits plant^{-1}.	Average fruit weight (kg.)	Yield kg Plant^{-1}.
31	82.00	79.00	26.00	0.98	25.48	39	76.00	84.00	12.00	1.10	13.20
32	81.00	71.00	38.00	1.02	38.76	40	78.00	73.00	29.00	1.16	33.64
33	78.00	61.00	46.00	1.10	50.60	41	74.00	71.00	36.00	0.86	30.96
34	79.00	68.00	31.00	0.87	26.97	42	81.00	69.00	32.00	1.06	33.92
35	78.00	74.00	56.00	1.26	70.56	43	82.00	74.00	49.00	1.13	55.37
36	81.00	76.00	31.00	0.73	22.63	44	79.00	78.00	36.00	0.98	35.28
37	84.00	84.00	29.00	1.19	34.51	45	76.00	62.00	51.00	0.71	36.21
38	79.00	76.00	23.00	0.81	18.63	46	82.00	72.00	21.00	1.16	24.36

	Population range , mean and Standard error				
Character	Days for 1st flow.	Height at 1st flow. Female cm.	No of fruits plant^{-1}.	Average fruit weight (kg.)	Yield kg Plant^{-1}.
Range					
Mean	81.31	75.71	33.93	1.01	34.11
SE	0.57	1.26	1.43	0.02	1.83

Conclusion

The present study suggested that the genotype GKPS-2-7 found to be promising based on the disease intensity score, reaction to the papaya ring spot virus and mean performance for morphological, yield and quality attributes. This genotype may be explored for further improvement in papaya against PRS disease.

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