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Intergeneric hybridization in papaya for 'PRSV' tolerance

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ABSTRACT

'Papaya ringspot virus' is one of the major impediments in papaya cultivation. Intergeneric crossing carried out using the 'PRSV' resistant wild species *Vasconcellea cauliflora* as male parent, with the papaya variety 'Arka Surya' as female parent resulted in the intergeneric hybrid progenies. The progenies found resistant after artificial inoculation were field planted. The progenies segregated for castor and papaya type leaves with fruits having yellow and pink pulp. The tolerant hermaphrodite progenies selected based on the fruit characteristics were selfed. Five generations of single plant selection was carried out by selfing the hermaphrodite tolerant progenies having castor type leaf. The hybrids in the sixth generation were validated using ISSR markers. The banding pattern was obtained with the primers UBC-815, UBC-834, UBC-836, UBC-841 and UBC-844 showed that they are intergeneric hybrids.

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1. Introduction

Papaya (Carica papaya L.) is one of the important fruit crops that are being grown commercially in India. One of the main impediments in its cultivation is the infestation of Papaya ringspot virus (PRSV), which is a major limiting factor in papaya production. The virus causes diverse symptoms that include vein clearing, mottling, malformed leaves, filiformy, ring spots and streaks on fruits, stem and petioles and stunting. The virus was first recorded in from western regions of India in 1958 (Capoor and Varma, 1958) and since then it has spread throughout the country. A breeding programme using this source in Florida (USA) resulted in the release of 'Cariflora', a dioecious cultivar with moderate resistance and fruit quality (Conover et al., 1986). The virus isolates have been bio-typed to papaya infecting (Type P) and non-papaya infecting (Type W) types. PRSV belongs to the poty virus group of plant viruses and is transmitted by several species of aphids in a nonpersistent manner (Purcifull et al., 1984). All the known varieties of papaya are susceptible to this disease. However, some of the wild species like Vasconcellea cauliflora have been found to be resistant to this disease (Jimenez and Horovitz, 1958; Horovitz and Jimenez, 1967). Work carried out earlier has shown that use of Sucrose resulted in viable seed set from the intergeneric hybridization (Dinesh et al., 2007). The intergeneric hybridization was carried

out with *V. cauliflora* as male parent with Arka Surya as female parent. The objective of the study was to work out a methodology for developing 'PRSV' tolerant intergeneric progeny by single plant selection and selfing of hermaphrodites and validating these progenies in successive generations by ISSR markers and by screening for tolerance/resistant in every generation.

2. Materials and methods

The *C. papaya* var 'Arka Surya' was used as the female parent for intergeneric crossing. It is a gynodioecious variety bearing medium sized fruits of $600-800\,\mathrm{g}$ with pink pulp colour and high TSS of $13-14^\circ$ Brix. It was derived from the parentage Sunrise Solo × Pink Flesh Sweet. The male parent *V. cauliflora* is a dioecious wild species having castor type leaf and producing non-edible fruits with yellow pulp. Crossing was carried out in the morning hours between 9 and $11\,\mathrm{a.m.}$ The pollen fertility and viability were confirmed using Alexander's stain (Alexander, 1987). The stigma of the female parent was smeared with Sucrose (5%) and then the pollen was smeared on top of that. On an average 50 crosses were made per plant and the fruits were harvested at the right stage of maturity and seeds were extracted.

The virus inoculum was prepared by homogenizing infected papaya leaves in 0.1 mol/L phosphate buffer, pH 7, and applied by gentle rubbing onto carborundum-dusted leaves. Plants were inoculated three times at two week intervals. Assessment of symptoms was done one month after the final inoculation and plants were assayed for virus concentration by ELISA as per the protocol developed (Magdalita et al., 1998).

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Fig. 1. Schematic diagram.

Hybridity confirmation through molecular characterization was done by extracting the genomic DNA using CTAB method (Lodhi et al., 1994) yielded good amount of DNA. DNA recovery varied from 750 ng/ μ l to 3640.25 ng/ μ l. The ratio of DNA to protein ranged from 1.85 to 3.08. 25 μ l of total reaction mixture was prepared each by adding 2.5 μ l of reaction buffer, 5 μ l of primer, 2.5 μ l of DNTPs, Taq DNA polymerase of 0.25 μ l along with 9.75 μ l of water and 5 μ l of template DNA. PCR conditions were optimized for informative and reproducible finger print profile. Ten ISSR primers (viz., UBC 807, 810, 814, 815, 817, 834, 836, 841, 844, 856) were used for validating.

3. Results and discussion

The schematic diagram for the procedure adopted for developing progenies from the intergeneric crosses is given in Fig. 1.

As early as in 1957, the interspecific crossing relationship in papaya was studied (Sawant, 1958). Interspecific hybridization generated a hybrid progeny tolerant to 'PRSV', however, on back-crossing tolerance was lost (lyer et al., 1987).

The methodology (Fig. 1), mentioned has been adopted to develop intergeneric lines tolerant to 'PRSV. In this procedure, back-crossing has not been carried out and only sibmating and selection has been carried out depending on the quality of the fruits by sibmating. The intergeneric hybridization carried out by the use of 5% Sucrose, resulted in a seed set of 13.1% per fruit from which 10 seedlings were raised (Dinesh et al., 2007). The procedure shows that in the F₁ generation there is segregation in the ratio of 3:1 for papaya to castor type plants. The two sets of population generated thus, one with castor leaf type and another with papaya leaf type were maintained by selfing the hermaphrodites. The successive generations thus raised were subjected to screening and the tolerant one taken to the field (Figs. 2 and 3). The progeny

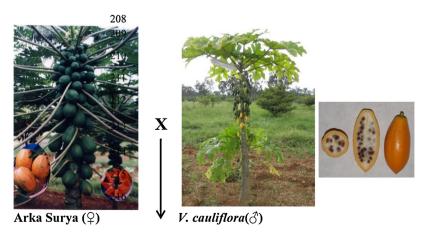


Fig. 2. Hybridization using the female and male parents.

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