Determination of PRSV Tolerant Papaya Plants from 
$SBF_6$ Generation of Intergeneric Population Using 
Ranking Model System

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Authors’ contributions

This work was done in collaboration among all the six authors. Authors KS and AJJ designed the study. Author RMA performed the experiment, statistical analysis and wrote the first draft of the manuscript. Authors KS and AJJ supervised the study and analysed the data. Authors PP, PM and CNC corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The Full-sib mated sixth ($SBF_6$) generation of intergeneric population of papaya from the cross CP 50 x Vasconcellea cauliflora was evaluated to select the progenies with PRSV tolerance coupled with better yield. In order to identify the well performing progenies, the mean performance and genetic parameters were estimated. Interestingly, the results of the variability study revealed that the genotypic coefficient of variation on number of fruits, leaf area and number of leaves were higher and making it as the selectable traits. The correlation analysis was made to understand the degree of relationship between the PRSV tolerance and various morphological traits. The result
showed that plant height and number of fruits were significantly and negatively correlated with percent disease index (PDI) with the r value of -0.273 and -0.261 respectively. By considering the number of fruits as the selectable trait, a ranking model system was developed and the results showed 5 ranks of 53 progenies. Among them, 13 well performing progenies from Rank IV and Rank V were selected for further evaluation. The ranking model system helps the researchers by making the selection process statistically much easier and efficient.

Keywords: Intergeneric; PRSV; ranking model.

1. INTRODUCTION

Papaya belongs to the family Caricaceae with the somatic chromosome number 2n = 18 [1] and the genome size of 372 Mbp [2]. It is originated from Central America and distributed to the Tropical Asia in the early fifteenth century. It is widely cultivated in the tropical regions because of its economic, nutritional and medicinal value. But nowadays, the production of papaya in the tropical region was drastically declining due to the diseases caused by fungi and virus. Among them, the major devastating disease is the Papaya Ring Spot disease caused by Papaya Ring spot Virus (PRSV); transmitted through the vector aphids mainly Myzus persicae in a non-persistent manner [3]. As a control measure, various strategies were developed. Among them, the most promising and a durable strategy was the development of intergeneric hybrids. The intergeneric hybridization was done between the cultivated papaya and the crop wild relative Vasconcellea cauliflora which is a resistant source for PRSV [4]. Most of the countries had attained the advanced generation of intergeneric population, but, the difficult part at this stage was the wise selection of plants with PRSV tolerance coupled with a better yield. Hence, the present study was made to understand the interactive relation between the PRSV tolerance and various morphological and yield traits. Also, a ranking model was developed to make the selection process much easier and efficient.

2. MATERIALS AND METHODS

2.1 Plant Material

The intergeneric hybridization was made between a PRSV tolerant papaya line ‘CP 50’ [5] and Vasconcellea cauliflora by Jayavalli [6] at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The plants were evaluated and the well performed plants were selected, full-sib mated and forwarded for next generation designated as SBF1. Likewise, the intergeneric hybrids were evaluated subsequently for five generations. In SBF5 generation; CPV-1-14, CPV-2-15, CPV-2-19, CPV-3-8 and CPV-3-12 were the well performed plants which was forwarded to SBF6 generation as family rows.

2.2 Cultivation Details

The SBF6 generation of intergeneric population was raised at University Orchard, Tamil Nadu Agricultural University with the spacing of 1.8 m x 1.8 m. Intercultural operations, manuring and irrigation was followed based on the standardized package of practices by Tamil Nadu Agricultural University.

2.3 Traits Observed

In this study, the parameters viz., plant height, stem girth, number of leaves, leaf area, petiole length, petiole girth, first fruiting height, number of fruits and percent disease index (PDI) were recorded. The observations were recorded in 100 plants during the first fruit harvest stage. The plant height was measured at 15 cm above the ground level to the apex region of the crown and expressed in centimetres. Stem girth was measured using the measuring tape at 15 cm above the ground level and expressed in centimetres. The number of leaves was counted manually. The leaf parameters like leaf area, petiole length and petiole girth was recorded in the sixth leaf from top of the plant. The leaf area was calculated based on the prediction model described by Karikari [7]. Petiole length and girth was measured using the measuring tape and expressed in centimetres. The first fruiting height was measured from the ground level to the node of first fruit to be matured and was expressed in centimetres. The number of fruits per tree was calculated by counting the number of fruits manually. The scoring technique for PRSV disease was followed based on the chart suggested by Dhanam [8]. PDI was calculated...
were calculated for various traits to understand the nature of its heritability. Both the phenotypic (PCV) and genotypic coefficient of variations (GCV) were higher in the traits viz., number of leaves, leaf area and the number of fruits in the SBF<sub>6</sub> population (Table 1). Hence, the selection of progenies based on the traits with narrow difference between both PCV and GCV coupled with high heritability will be more effective. The PCV values were higher in plant height, first fruiting height, petiole length and girth; the enhancement of this phenotypical expression may be due to the environmental effect. Johnson, et al. [11] has mentioned that selection based on both the heritability and genetic advance together will make the selection more effective rather than calculating it alone. In this study, both the heritability and genetic advance as per cent of mean (GAM) was higher in all the observed eight parameters and selection will be effective for the studied parameters. It was also observed that the heritability is due to the additive gene effect and hence the selection will be more effective.

3.3 Associative Analysis

The correlation coefficient was analysed to find the strength or degree of association between different traits. In this study, the correlation analysis was made to understand the degree of relationship between the PRSV tolerance and various morphological traits. Hence, the Pearson’s correlation coefficient was computed with PDI over the various morphological traits. The result showed that the seven variables viz., plant height, stem girth, number of leaves, petiole length, petiole girth, leaf area and first fruiting height were negatively correlated with PDI (Table 2). The plant height was significant at 1% level with the r value of -0.273 whereas the petiole length (-0.216), leaf area (-0.221) and first fruiting height (-0.251) were significant at 5% level. The negative correlation coefficient denotes that the variables were negatively correlated i.e., when the disease incidence is heavy the plant height will be significantly lower than the healthy plant. The traits like plant height, stem girth, petiole length, petiole girth, number of leaves and leaf area were positively and significantly correlated with the number of fruits. It is a well-known fact that a plant that is tall, sturdy with a good foliage yields better. Since, the papaya flowers borne on the leaf axils; the number of leaves was directly related to the number of fruits (Fig. 1). If the petiole girth is more, then the time taken to senesce of that leaf will also be long. Hence, it

3. RESULTS AND DISCUSSION

3.1 Performance of SBF<sub>6</sub> Generation for the Observed Traits

In hybridization programme, the progenies or family with high mean value was selected as the superior segregants. In this study, all the five families recorded more leaf area, petiole length, petiole girth and number of fruits, and low first fruiting height and less per cent disease index than the mean value of its parent ‘CP 50’ (Table 1). Since, the plants are in the advanced generation, occurrence of heterosis and selection response leads to the better performance of the progenies than its parent. The ultimate goal of the intergeneric hybridization in papaya was to identify the best performing segregants with PRSV tolerance and good yield. Among the five families, the lower PDI of 58.33% was recorded in CPV-2-15 with higher the leaf area of 2027.52 cm<sup>2</sup> and petiole length of 65.16 cm whereas CPV-1-14 recorded 35.70 number of fruits with the maximum petiole girth of 8.60 cm. The desired growth traits such as the minimum plant height of 159.51 cm and first fruiting height of 69.27 cm, and higher stem girth of 31.03 cm and 34.17 number of leaves were recorded in CPV-3-12 family.

3.2 Variability

Apart from the mean performance, the selection process may not be much effective without the variability study. Hence, the genetic parameters were calculated for various traits to understand the following formula

\[
PDI = \frac{\sum Xi}{NX \text{ max rating}} \times 100
\]

Where, \(Xi\) = sum of all scores
\(N\) = number of plants observed

2.4 Statistical Analysis

The descriptive statistics and Pearson Correlation analysis were analysed using the software Genstat version 15.0. The genetic parameters like phenotypic and genotypic coefficient of variation was calculated using the formulas from Sivasubramanian and Madhava Menon [10], heritability from Robinson, et al. [11] and genetic advance by Johnson, et al. [12].
Table 1. Genetic parameters for morphological and yield traits in SBF6 intergeneric population

| Genetic parameters | Family/parent | Plant height (cm) | Stem girth (cm) | No. of leaves | Leaf area (cm²) | Petiole length (cm) | Petiole girth (cm) | First fruiting height (cm) | Number of fruits | PDI (%) |
|--------------------|---------------|------------------|-----------------|---------------|-----------------|---------------------|-------------------|-----------------------------|-----------------|---------|
| Mean ± SE          | CPV-1-14      | 238.00 ± 8.20    | 29.50 ± 1.09    | 32.83 ± 1.78  | 1951.57 ± 113.68| 61.40 ± 2.12        | 8.60 ± 0.29        | 107.70 ± 3.56               | 35.70 ± 3.42    | 62.32   |
|                    | CPV-2-15      | 219.34 ± 7.24    | 28.44 ± 1.13    | 32.50 ± 1.25  | 2027.52 ± 100.49| 65.16 ± 1.82        | 6.16 ± 0.23        | 106.71 ± 3.53               | 31.83 ± 2.67    | 58.33   |
|                    | CPV-2-19      | 216.48 ± 7.09    | 25.95 ± 1.00    | 20.84 ± 0.57  | 770.93 ± 47.11  | 42.90 ± 2.23        | 5.46 ± 0.14        | 108.75 ± 4.21               | 30.19 ± 2.83    | 61.11   |
|                    | CPV-3-8       | 181.43 ± 5.15    | 23.95 ± 1.09    | 20.30 ± 0.67  | 1093.38 ± 55.63 | 50.18 ± 2.08        | 6.89 ± 0.21        | 94.76 ± 3.55                | 27.24 ± 2.91    | 66.14   |
|                    | CPV-3-12      | 159.51 ± 5.15    | 31.03 ± 1.00    | 34.17 ± 1.14  | 1656.68 ± 85.06 | 46.41 ± 1.43        | 5.61 ± 0.12        | 69.27 ± 2.75                | 27.38 ± 3.59    | 68.06   |
|                    | CP 50         | 205.00 ± 4.08    | 25.67 ± 0.27    | 20.33 ± 0.72  | 551.33 ± 125.75 | 42.00 ± 1.89        | 4.33 ± 0.27        | 114.33 ± 2.84               | 25.00 ± 1.70    | 100.00  |
|                    | CPV           | 20.27            | 19.73           | 29.15         | 40.66           | 22.73               | 23.13              | 46.41                       | -               |         |
|                    | GCV (%)       | CPV              | 18.29           | 18.75         | 26.61           | 32.56               | 19.28              | 19.05                       | 18.44           |         |
|                    | Heritability (%) | CPV           | 81.37           | 90.31         | 83.37           | 64.14               | 71.92              | 67.84                       | 74.89           | 77.62   |
|                    | GAM (%)       | CPV              | 33.98           | 36.71         | 50.06           | 53.72               | 33.68              | 32.32                       | 32.87           | 74.21   |

Table 2. Pearson correlation between the morphological traits and percent disease index to the yield

| Parameters          | Plant height | Stem girth | Number of leaves | Petiole length | Petiole girth | Leaf area | First fruiting height | PDI | Number of fruits |
|---------------------|--------------|------------|------------------|----------------|---------------|-----------|-----------------------|-----|------------------|
| Plant height        | 1.000        | 0.534      | 0.264*           | 0.578*         | 0.524*        | 0.385*    | 0.715*                | -0.273 | 0.504* |
| Stem girth          | 1.000        | 0.550*     | 0.543*           | 0.387*         | 0.586*        | 0.142     | -0.135                | 0.674* | -     |
| Number of leaves    | 1.000        | 0.396*     | 0.160            | 0.614          | 0.033         | -0.097    | 0.214*                | -     | -     |
| Petiole length      | 1.000        | 0.538*     | 0.730*           | 0.269*         | -0.216*       | 0.567*    | -0.161                | 0.448* | -     |
| Petiole girth       | 1.000        | 0.472*     | 0.283*           | -0.021*        | 0.465*        | -0.251    | 0.019                 | 1.000  | -     |
| Leaf area           | 1.000        | 0.098      | -0.221*          | 0.019          | 1.000         | -0.261*   | 1.000                 | -     | -     |
| First fruiting height | 1.000   | 0.098      | -0.221*          | 0.019          | 1.000         | -0.261*   | 1.000                 | -     | -     |
| PDI                 | 1.000        | 0.098      | -0.221*          | 0.019          | 1.000         | -0.261*   | 1.000                 | -     | -     |

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)
can bear the weight of the fruit throughout the fruit development phase and makes the fruit retentive. The strong petiole may also help to delay the vascular degeneration caused by PRSV. The PDI was negatively and significantly correlated with the number of fruits with the r value of -0.261. That is, when the disease incidence is heavy the fruit setting capacity of the tree gets decreased or vice versa. It can also be explained as the higher the severity of disease infection lesser the yield or lesser the severity of disease infection higher the yield. Contrarily, in SBF₆ population, the PRSV tolerance was seen along with the gradation of yield difference i.e., inspite of susceptibility to PRSV, the progeny manages to give a better number of fruits. This may be due to the presence of more number of leaves with higher leaf area of that progeny (Fig. 1). Considering number of leaves and leaf area are contributed probably by many genes. The study of such gene determining the resistance or tolerance through modulation of resistance mechanism needs to be further studied. Thus, the correlation study indeed helped to understand the PRSV tolerance in SBF₆ population.

### 3.4 Ranking Model

Considering the result of the variability study which revealed that the selection of progenies based on the number of fruits will be more effective. Also, by considering the identification of PRSV tolerant genotype from the SBF₆ population, a ranking model was made to negotiate the number of fruits with the PRSV score. The number of fruits per tree and PRSV score for the observed 100 plants of SBF₆ population was projected as the scatter plot in the Fig. 2a) and 2b) respectively. The model includes four rows and three columns of ranks (Fig. 3a). The rows includes the PRSV score from 3 to 9 (the scores observed in the SBF₆ intergeneric population during first fruit harvest stage) and the columns includes the number of fruits with mean + 3SD, mean + 2SD and mean + SD. The ranking was given in the sense that the first priority was given to the PRSV score and secondly the number of fruits. By applying the ranking model system into the data of Fig. 1a and 1b, 47 progenies were found to have the number of fruits less than the mean value of 30.18. The rest of the 53 progenies having the number of fruits more than the mean value were grouped into low (Mean + SD = 31 to 45 number of fruits), medium (Mean + 2SD = 46 to 60 number of fruits) and high (Mean + 3SD = 61 to 75 number of fruits). These 53 progenies were then interrelated to its corresponding PDI to calculate its rank. The result showed that three progenies in Rank IV, ten progenies in Rank V, twenty eight progenies in Rank VI, two progenies in Rank VIII and ten progenies in Rank IX (Fig. 3b). From this, the three progenies from Rank IV viz., CPV-1-14-26, CPV-1-14-28 and CPV-2-15-7 and ten progenies from Rank V viz., CPV-1-14-19, CPV-1-14-29, CPV-1-14-39, CPV-2-15-21, CPV-2-15-36, CPV-3-12-14, CPV-3-8-2, CPV-3-8-5, CPV-3-8-22 and CPV-2-19-27 were considered to be the superior genotypes having moderately tolerant to PRSV with higher the number of fruits. Hence, these genotypes of the segregating population alone can be selected for evaluating the fruit and quality attributes, subsequently forwarding to the next generation.
Fig. 1(a-d). Comparison of PRSV susceptible and tolerant plant

c) PRSV tolerant plant
d) Fruit column of PRSV tolerant plant

Fig. 2. Scatter plot showing the a) number of fruits and b) PRSV score for the observed 100 plants of SBF6 generation of intergeneric population during first fruit harvest stage

Fig. 3. a) Ranking model system showing the likelihood between the number of fruits and papaya ring spot disease incidence b) Applying the ranking model system in SBF6 generation of intergeneric population
4. CONCLUSION

The complex nature of the inter-relation between PRS disease severity, plant yield and other morphological traits can be apparently unravelled using the correlation study to understand the PRSV tolerance in the progeny. The variability studies revealed an understanding on heritable traits with genetic advance which can be considered for selection while forwarding to next generation. The ranking model system may help the researchers to differentiate the well performing progenies from the population of an advanced generation and thus making the selection of plants more efficient and wiser. Based on the interpretation from the mean performance and the ranking model system, the selected thirteen progenies can be forwarded to the next generation to stabilize the PRS disease resistance and yield.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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