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Original Research Paper

Correlation of Leaf Parameters with Incidence of *Papaya Ring Spot Virus* in Cultivated Papaya and its Wild Relatives

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ABSRACT

Papaya ring spot virus (PRSV) disease has been the major impediment in papaya cultivation. The disease is transmitted through three aphid vectors and field tolerance towards this disease varies among *Carica papaya* cultivars as well as within the *Vasconcellea* genus. Leaf morphological traits are known to have influence on the probing preferences of aphids. Hence, this study was conducted to know whether the leaf parameters could contribute to the incidence of PRSV possibly by influencing the probing or feeding behaviour of aphid vectors. Leaf parameters *viz*., leaf thickness, leaf epicuticular wax content, presence and type of trichomes, trichome density were correlated with disease incidence at field conditions. The result revealed that leaf thickness along with epicuticular wax content had significant negative correlation with disease incidence. Similarly, trichome density had negative impact on disease incidence at 99.92% significance level. High epicuticular wax content and high trichome density in *V. cauliflora* and *V. cundinamarcensis* were found to be negatively associated with low to very low infection indicating that these parameters may have limited the vector transmission significantly.

Keywords: Epicuticular wax content, Papaya, Trichome density and *Vasconcellea.*

INTRODUCTION

Papaya is ranked as the third most traded tropical fruit (excluding bananas). The area and production of papaya is on the increase in recent years due to its wide ecological adaptability, easiness in cultivation, high palatability, early fruiting, year round bearing, higher productivity and economic returns. Papaya is a nutrition basket filled with vitamins (2020 IU of vitamin A, 40 mg of vitamin B_1 and46 mg ofvitamin C per 100g of fruit), minerals, carbohydrates, proteins, iron, calcium and phosphorous (Dinesh, 2010). However, the production of papaya is hampered by a serious outbreak of viral disease caused by Papaya ring spot virus (PRSV-P). This virus affects production and productivity by decreasing photosynthetic capacity of plant, and subsequently leading to stunted growth, deformed and inedible fruitsand early mortality. PRSV is transmitted by several species of aphids in a non-persistent manner. Generally, aphids do not colonize papaya and transmission of PRSV is through transient aphid vectors.*Aphis gossypii* is the predominant vector followed by *A. craccivora* and *Myzus persicae*. Recent study suggested that *M. persicae* is more efficient than the other two species with 52.5 % transmission after the first inoculation access period (IAP) (Kalleswaraswamy and Krishnakumar, 2008).

Field tolerance towards PRSV varies between the *Carica papaya* varieties as well as the *Vasconcellea* species. *Vasconcellea* species *viz*., *V. cundinamarcensis*,*V. candicans*, *V. stipulata*, *V. cauliflora* and*V. quercifolia* are reported to be resistant to PRSV. Even though the genetic variability within the *Carica* genus for PRSV resistance is very low, a few varieties have shown tolerance to PRSV. This might be due to the non-preference of aphids to these varieties or species in addition to the innate resistance mechanism in *Vasconcellea* genepool. Morphological traits such as higher density of simple and glandular trichomes, epicuticular waxes and leaf thickness are reported to hinder the aphid attack in plants (Bin, 1979; Guerrieri and Digilo, 2008;

Wojcicka, 2015). A preliminary study was carried out to explore the possibility of aphid tolerance in papaya varieties as well as *Vasconcellea* species.

MATERIALS AND METHODS

Plant material

The experiment was carried outat ICAR- Indian Institute of Horticultural Research, Bengaluru during the period of June 2016 to August 2017 under open field conditions consecutively in two different locations during *kharif* and *rabi* seasons.The experiment plot surrounded by papaya crop with already established with PRSV incidence was selected. Six cultivars of *Carica papaya* namely Arka Surya, Arka Prabhath, Red Lady, Pusa Dwarf, Pusa Nanha and CO8, two intergeneric hybrids of Arka Surya and *V. cauliflora* (IGHI and IGHII) and four wild relatives *V. cauliflora*, *V. goudotiana*, *V. cundinamarcensis* and *V. parviflora* were used in this study. Twenty plants of each accession in three replicates were maintained as per the randomized block design.

Observations

Characters such as leaf thickness, leaf pubescence, epicuticular wax content and trichome density were recorded after three months of transplanting in the field, since symptoms appeared in susceptible genotypes after three months of transplanting. Leaf thickness was measured using Digital Vernier Caliper (Mitutoyo, Digimatic Caliper). Presence of leaf pubescence was visually recorded while the type of the pubescence was observed under stereo-microscope (Leica M205A) and Scanning Electron Microscope (Hitachi, TM3030 plus, Tabletop microscope). Epicuticular wax content was estimated as described by Ebercon *et al.* (1977). Trichome density was calculated as number of trichomes per centimetre area. These observations were correlated with per cent infection (PI) and per cent disease index (PDI). PDI was calculated after first symptom development in field condition at fortnightly intervals and calculated as per the formula.

$$\text{PDI} = \frac{\in \mathbf{n}}{5N} \times 100$$

Where, n = individual ratings, N= total number of leaves/ plant; 5= maximum rating

The individual ratings (n) were given using the scale adopted by Dhanam (2006) and ranged from 0 to 5 (0 = no disease symptoms; 1 = slight mosaic on leaves; 2 = mosaic patches and/or necrotic spots on leaves; 3 = leaves near apical meristem deformed slightly, yellow, and reduced in size; 4 = apical meristem with mosaic and deformation; 5 = extensive mosaic and serious deformation of leaves, or plant dead).

RESULTS AND DISCUSSION

Leaf thicknessat three months of transplanting ranged from 0.22 mm to 0.40 mm. The thickest leaf was observed in Pusa Nanha (0.40 mm) which was on par with *V. cundinamarcensis* (0.38 mm) and *V. cauliflora* (0.37 mm), followed by *V. parviflora* (0.35 mm). Among the accessions, thinnest leaves were noticed in TNAU papaya CO8, Arka Parbhath and IGH2 (0.22 mm) which was on par with IGH1 and Arka Surya (0.23 mm).

The accessions such as Arka Surya, Arka Prabhath, Red Lady, Pusa Dwarf, Pusa Nanha, TNAU Papaya CO8, IGH1, IGH2 and *V. goudotiana* lack leaf pubescence on both dorsal and ventral surfaces (Fig.1). However, the wild species viz., *V. cauliflora*, *V. cundinamarcensis* and *V. goudotiana* had leaf pubescence with higher trichome density on ventral surface than dorsal surface. Trichome density was highest in *V. cundinamarcensis* (192.75/cm^2) followed by *V. cauliflora* (25.25/cm^2) and *V. goudotiana* (14.88/cm^2).

The type of trichome on the leaves were also observed under scanning electron microscope. *V. cundinamarcensis* consisted of single celled non-glandular trichomes, whereas *V. cauliflora* and *V. goudotiana* comprised of multicellular glandular trichomes (Fig.2). Trichomes were present as extension of veins in *V. cauliflora* and *V. goudotiana*, while these were distributed throughout the leaf surface in *V. cundinamarcensis*.

Studies suggested that trichome density has more impact on entry of aphids rather than the type of trichomes, as higher trichome density blocked aphids (Musetti and Neal, 1997). It is the first feature affecting the selection behaviour of an aphid. Most of the resistant varieties or wild relatives are characterized by presence of trichomes (Bin, 1979). However, the glandular trichomes might have produced toxic exudates or acyl sugars that repel aphids (Goffreda *et al*., 1989).

Table.1. Leaf thickness, leaf epicuticular wax content, trichome density, PRSV percentage infection, disease intensity score and PDI at field condition

Accessions	Leaf thickness (mm)	Leaf epicuticular wax content (µg/cm²)	Trichome density (number/cm²)	Per cent infection (%)	Disease intensity score	Per cent Disease index (%)
Arka Surya	0.23[de]	95.00[ef]	Nil	100.00[a] (89.71)	4/5	65.71[a] (54.16)
Arka Prabhath	0.22[e]	109.38[de]	Nil	100.00[a] (89.71)	4/5	61.25[b] (51.51)
Red Lady	0.27[cd]	143.75[c]	Nil	100.00[a] (89.71)	4/5	51.04[c] (45.60)
Pusa Dwarf	0.29[c]	134.38[c]	Nil	100.00[a] (89.71)	4/5	62.50[ab] (52.25)
Pusa Nanha	0.40[a]	114.38[d]	Nil	25.33[e] (29.77)	1	1.56[f] (9.34)
TNAU Papaya CO 8	0.22[e]	96.25[ef]	Nil	100.00[a] (89.71)	3/4	35.64[d] (36.65)
IGH1	0.23[de]	94.38[f]	Nil	81.33[d] (64.66)	3/4	21.46[e] (27.59)
IGH2	0.22[e]	106.25[def]	Nil	86.67[c] (68.60)	3/4	25.24[e] (28.24)
V. goudotiana	0.26[cde]	114.38[d]	Nil	87.66[b] (70.35)	4	25.00[e] (29.97)
V. cauliflora	0.37[ab]	200.00[a]	25.25[b]	0.00[f] (0.286)	0	0.00[g] (0.286)
V. cundinamarcensis	0.38[ab]	170.00[b]	192.75[a]	0.00[f] (0.286)	0	0.00[g] (0.286)
V. parviflora	0.35[b]	105.00[def]	14.88[b]	23.62[e] (29.32)	1	2.46[f] (9.02)
Mean	0.29	123.59	77.63	67.05 (59.32)		29.32 (28.74)
CV (%)	9.96	3.32	3.17	1.61		5.02
SE(d)	0.012	3.353	1.231	0.78		1.18
Tukey HSD at 1%	0.045	14.667	4.26	1.73		1.91

Values inparentheses are arc sine transformed values

A correlation was drawn between the leaf parameters and disease scoring (Table.2). Epicuticular wax content in leaves was negatively and significantly correlated with percentage of infection. Epicuticular waxes are complex mixture of long chain aliphatic and cyclic components such as fatty acids, hydrocarbons, alcohols, aldehydes, ketones, esters, terpenoids, sterols, flavanoids and phenolic substances. Higher epicuticular wax content might be a reason for reduction in aphid landing or movement, which in turn could have contributed, to inhibition of the sap transmission of PRSV. Similar negative effects of leaf epicuticular waxes were reported on neonate larval movement of *Spodoptera frugiperda* on *Zea mays* (Ostrand et al., 2008), resistance in cabbage to aphids *Bravicoryne brassicae*, sorghum to green bug (*Schizaphis graminum*) and winter wheat to English grain aphid (*Sitobion avenae*) (Shepherd *et al.*, 1999). The presence of thick wax content might have affected the probing and feeding by aphids, thereby rejecting the particular variety or species.

Leaf thickness was negatively correlated with PDI and infection percentage. Leaf thickness was positively contributed by the epicuticular wax content, which indirectly influences the disease tolerance or resistance. Thick cell wall has been attributed to resist the feeding activity of aphids (Guerrieri and Digilo, 2008).

The association analysis reveals that there is a significant negative correlation between trichome density and infection percentage. Surface resistance is the first barrier against aphid attack (Wang *et al.*, 2004). Either or both of epicuticular wax content or presence of trichomes is known to hinder the aphid movement and stylet insertion (Bin, 1979).

Table.2. Correlation between disease incidence and leaf parameters

	Epicuticular Wax Content	Leaf Thickness	Trichome Density	Per centage Disease Index	Per centage Infection
Epicuticular Wax Content	**1.00**	0.62*0.031	0.76*0.004	-0.360.247	-0.61*0.033
Leaf Thickness		**1.00**	0.390.201	-0.71*0.009	-0.90*<.0001
Trichome Density			**1.00**	-0.390.201	-0.53*0.076
Percentage Disease Index				**1.00**	0.87*0.000
Percentage Infection					**1.00**

First value represents ‘r’ value and second value represents p value. *<0.05 p value shows significant correlation.

Higher field tolerance to PRSV was observed in ‘Pusa Nanha’, which had thicker leaves. The PRSV incidence was significantly low in both *V. cauliflora* and *V. cundinamarcensis, which* registered thicker leaves, higher epicuticular wax content and denser trichomes. The preliminary study broadly indicates that leaf thickness, presence of trichomes in higher density and epicuticular wax content in papaya is likely to play a definitive role towards reduction in PRSV incidence probably by restricting the aphid vector incidence.

Higher leaf thickness, epicuticular wax content and trichome density in papaya is found to have negative impact on the incidence of Papaya ring spot virus in papaya and these factors may have a role to play in restricting the virus transmission by aphid vector. Future research needs to be focussed on the biochemical constituents of glandular trichomes and its effect on aphids.

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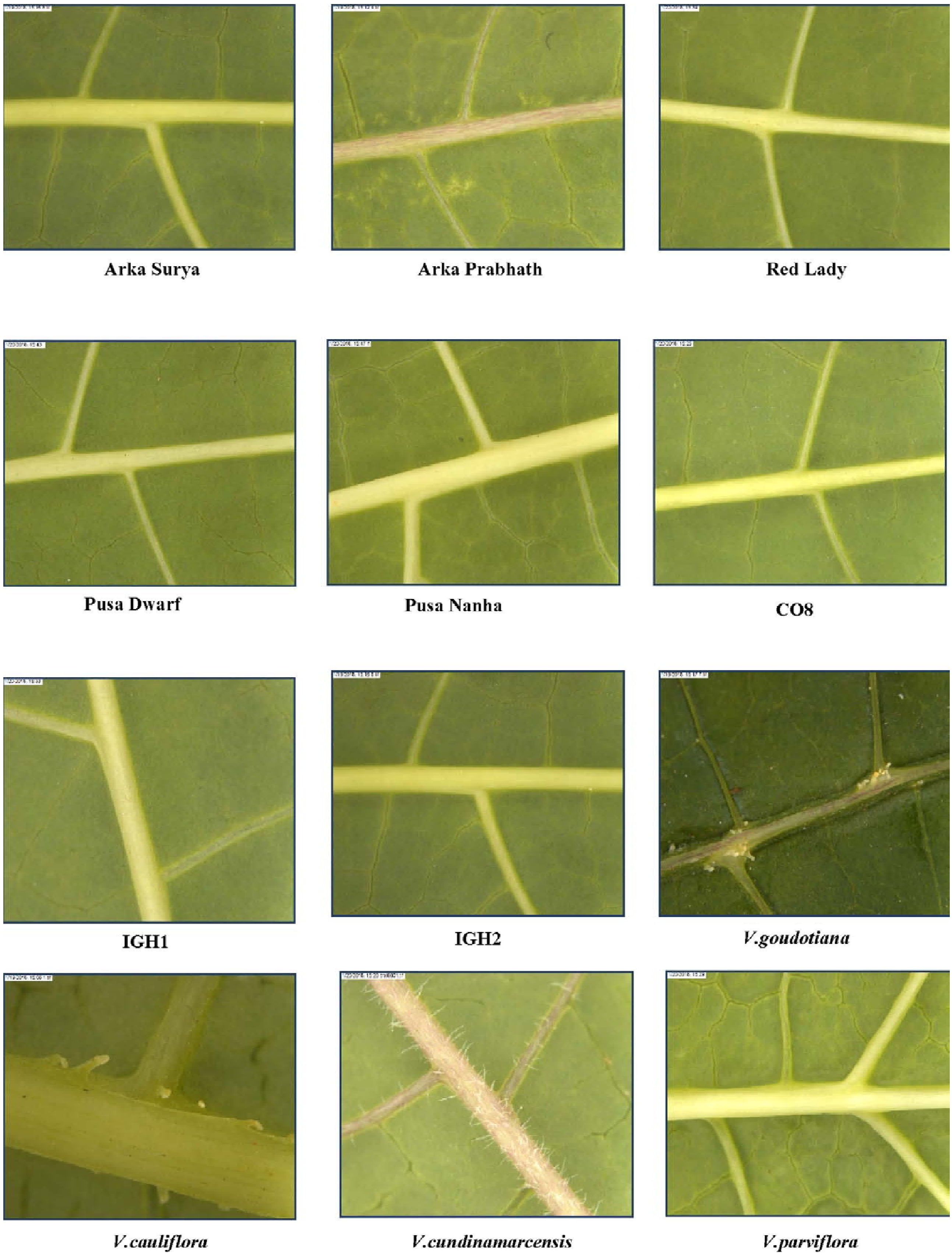

Fig. 1: Stereo microscope view of leaf ventral surface pubescence

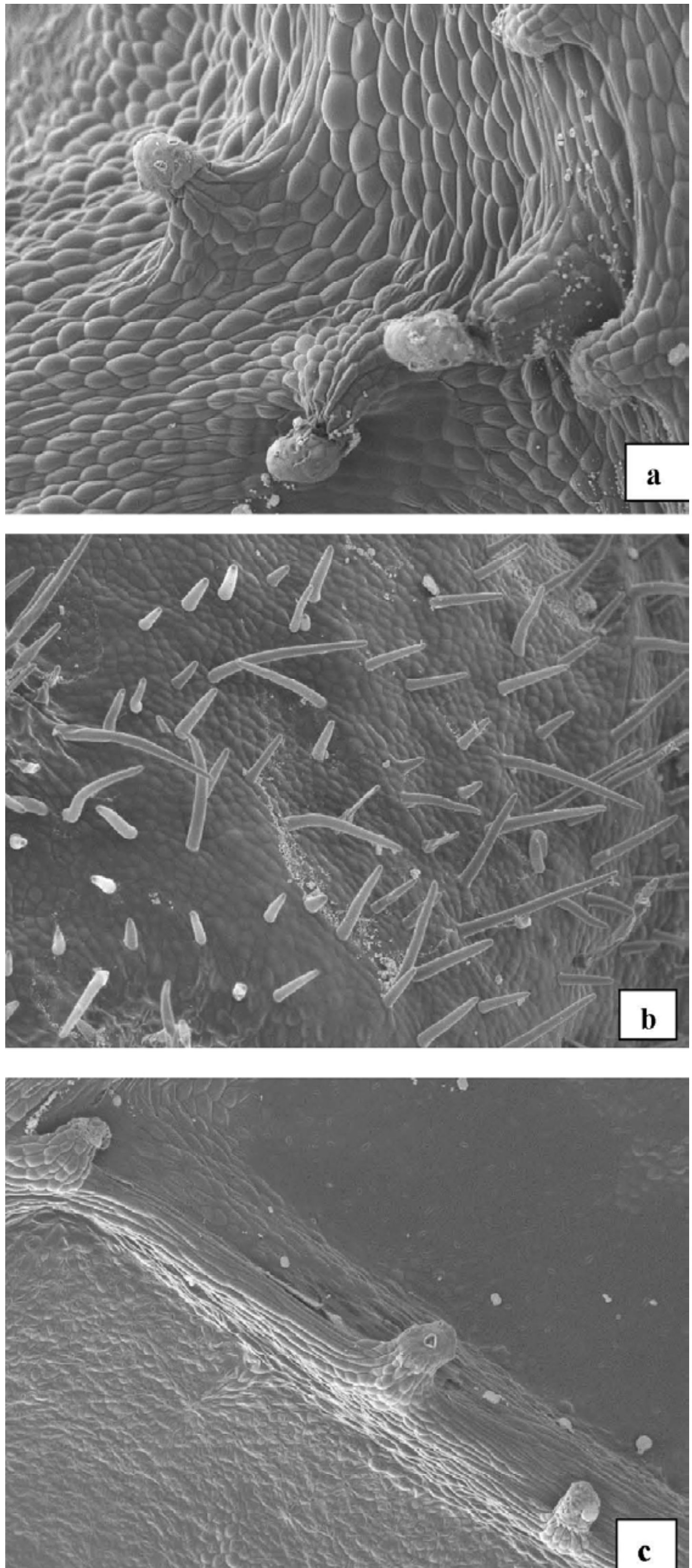

Fig. 2: Scanning electron microscope view of leaf ventral surface pubescence at 500µm
a) *V. cauliflora* (b) *V. cundinamarcensis* (c) *V. goudotiana*

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