

Original Research Article

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Impact on Physiological Parameters upon PRSV Infection in Different Genotypes of *Carica papaya* L. and *Vasconcellea* species

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ABSTRACT

Papaya is major tropical fruit gaining importance throughout the tropics and subtropics but there is a severe restriction in its production due to the serious disease known as Papaya Ring Spot Virus (*PRSV-P*). No papaya variety till date was identified as having durable resistance to *PRSV-P*; whereas several species of *Vasconcellea*, a close relative of papaya was reported to have heritable resistance to *Papaya Ring Spot Virus*. To understand these changes in physiological parameters upon *PRSV* infection this study was carried out by involving six *Carica papaya* varieties, two intergeneric hybrids of *Carica papaya* and *Vasconcellea cauliflora* and three *Vasconcellea* species. The physiological parameters viz., photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (E) were measured before inoculation, and subsequently 7 days post inoculation (7 dpi) with *PRSV*, 14 dpi and 30 dpi. The results revealed that the photosynthetic rate and stomatal conductance were found get reduced in the susceptible genotypes viz., Arka Surya, Arka Prabhath, Red Lady and Pusa Dwarf at all the stages of observation, while in tolerant genotypes such as Pusa Nanha, TNAU papaya CO8, they initially decreased at 7 dpi but found to be raised at 14 dpi but again declined during the observation on 30 dpi. In IGH1 and IGH2, P_N and g_s were found to be increased at 7 dpi but decreased afterwards. In susceptible genotypes, transpiration rate was found to be increased continuously upto 14 dpi and reduced at 30 dpi. The physiological parameters did not show any significant alterations in the photosynthetic rate, stomatal conductance and transpiration rate in the three *Vasconcellea* species evaluated

Keywords

Papaya (*Carica papaya* L., family Caricaceae), *Carica papaya* and *Vasconcellea cauliflora*

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Introduction

Papaya (*Carica papaya* L., family Caricaceae) is considered as fourth most important fruit crop worldwide due to its acceptance amongst the consumers and food processing industries. Although, it has originated from tropical

central America, the crop has spread throughout the tropics and sub tropics in other parts of the world due to its high nutritional value, consumer preference and ability to provide better revenue to the growers. Papaya fruit is a nutrition basket filled with vitamins, minerals, carbohydrates, proteins, iron,

calcium and phosphorous. It is a rich source of vitamins having an approximate composition of 2020 IU of vitamin A, 40 mg of vitamin B₁, and 46 mg of vitamin C per 100g of fruit (Dinesh, 2010).

The total area under cultivation of papaya has recorded a regular increase in the recent past but its production has not increased correspondingly owing to several biotic stresses such as various diseases caused by fungi, bacteria, phytoplasm and viruses. Among them, the global papaya production is seriously hampered by a devastating disease caused by virus known as *Papaya Ring Spot Virus (PRSV-P)* which is transmitted by aphids in a non-persistent manner. This deadly disease causes losses as high as 70% of expected yield (Manshardt and Drew, 1998). No papaya varieties till date were identified for resistance to *PRSV-P*; whereas several species of *Vasconcellea* was reported to have heritable resistance to *Papaya Ring Spot Virus* such as *V. cauliflora*, *V. cundinamarcensis* and *V. quercifolia* (Drew *et al.*, 1998). Evaluation of physiological parameters in relationship to *PRSV* incidence may help to identify resistant accessions and also genetic functions regulating tolerance to disease. Biomass production in plants greatly depends on the level of gas exchange between the plant and environment (Wenda-Piesik *et al.*, 2016). The process of photosynthesis is greatly affected by biotic or abiotic stress. This is possibly through the alteration in organelle structures, pigment production and stomatal regulation. The response of the stress developed in the plants can be initially detected by net photosynthetic rate and related parameters.

After infection caused by pathogens, effect of *PRSV* in the genotypes varies that in turn affects the plant growth characters via effect on physiological process. The present study was carried out to understand the differences

in changes in photosynthetic related parameters in genotypes having varied responses to *PRSV* by involving six *Carica papaya* varieties, two intergeneric hybrids of *Carica papaya* and *Vasconcellea cauliflora* and three *Vasconcellea* species

Materials and Methods

The experiment was conducted at ICAR-Indian Institute of Horticultural Research, Bengaluru during the period of June to August, 2017 under the insect proof glass house.

Source of virus inoculum

Susceptible cultivar ('Arka Prabath') was inoculated with Papaya Ring Spot Virus-P and maintained inside insect proof net house and was used as the source of inoculum.

Plant material

Six cultivars of *Carica papaya* namely Arka Surya, Arka Prabath (check), Red Lady, Pusa Dwarf, Pusa Nanha and CO8, two intergeneric hybrids of the cross Arka Surya and *Vasconcellea cauliflora* (IGH1 and IGH2) and three wild relatives *V. cauliflora*, *V. goudotiana* and *V. cundinamarcensis* were involved in this study. Three replications of twenty plants in each accession were maintained as per Randomized Block design.

Method of inoculation

Infected leaves from the susceptible cultivar maintained in the insect proof net house were homogenized in 0.1 M potassium phosphate buffer (1:5 w/v) (pH 7.0), two drops of β -mercaptoethanol and pinch of sodium sulphite. The homogenate was squeezed through cotton wool and used as standard inoculum. The papaya plants at five leaf stage, the basal three leaves were mechanically inoculated on the

upper surface of the leaves with the standard inoculum, using carborundum powder through 600 mesh as an abrasive. After 5 minutes of inoculation, the excess sap was washed off by distilled water. Control and inoculated plants were maintained separately in insect proof glass house and retained a temperature of 25-28°C and humidity of 75-80 percent.

Symptom expression

Initial symptom of chlorotic spot on leaves at the site of inoculation at nursery stage was observed within 11-13 days post inoculation (dpi) in *Carica papaya* genotypes such as Arka Prabhath, Arka Surya, Pusa Dwarf and Red Lady, whereas in Pusa Nanha and TNAU Papaya CO8, the symptom expression was delayed and appeared at 23rd day and 17 dpi respectively. Intergeneric hybrids viz., IGH1 and IGH2 showed the initial symptom of chlorotic spots on 18th day. No symptom expression was observed in the three wild relatives.

Measurement of physiological parameters

Leaves just above the inoculated ones (considered as 'systemic leaves') were observed both prior to inoculation as well as post inoculation after 7 days, 14 days and 30 days, for physiological parameters. The gas exchange parameters were measured between 9.30 AM to 11.00 AM prior to inoculation as well as post inoculation after 7 days, 14 days and 30 days, using Portable Photosynthesis System, LI-6400 Xt (LiCor. Lincoln, Nebraska, USA). The instrument was properly calibrated to record net photosynthetic rate P_N ($\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance g_s ($\text{mol m}^{-2} \text{s}^{-1}$) and transpiration rate E ($\text{m mol m}^{-2} \text{s}^{-1}$).

Results and Discussion

Based on symptom expression on the plants and growth, the genotypes evaluated were

classified as susceptible (Arka Surya, Arka Prabhath, Red Lady and Pusa Dwarf), tolerant (Pusa Nanha, TNAU papaya CO8, IGH1 &2) and resistant (*Vasconcellea* sp). The differential rates or changes in the physiological parameters viz., Net Photosynthetic Rate (P_N), Stomatal conductance (g_s) and transpiration rate (E) post inoculation as compared to pre inoculation stages in these genotypes are presented here. The observations are furnished in Table 1.

Net photosynthetic rate (P_N)

The net photosynthetic rate in plants before inoculation ranged from 3.17 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in *V. goudotiana* to 9.72 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in Arka Prabhath among the genotypes evaluated. Before inoculation, higher photosynthetic rate (9.72 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was observed in Arka Prabhath followed by Arka Surya (9.26 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and Pusa Dwarf (8.17 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The photosynthetic rates were observed to be lower in *V. goudotiana* (3.17 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and intermediate in *V. cauliflora* (4.46 $\mu\text{mol m}^{-2} \text{s}^{-1}$), TNAU Papaya CO8 (5.23 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and *V. cundinamarcensis* (6.66 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

At 7dpi, it ranged from 4.46 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in *V. cauliflora* to 8.69 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in IGH2. At 14 dpi, P_N reduced to a range of 4.16 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in *V. cauliflora* to 8.53 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in IGH2. The P_N range was from 1.73 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in Arka Surya to 6.51 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in *Vasconcellea cundinamarcensis* at 30 dpi (Table 1).

At 7dpi, significant decrease in P_N was observed in the six *Carica papaya* cultivars evaluated while there was significant increase in P_N in IGH1, IGH2, *V. goudotiana* and *V. cundinamarcensis* while there was no significant change in P_N in *V. cauliflora*. At 14 dpi, except in *V. goudotiana*, *V. cauliflora*, Pusa Nanha and TNAU papaya CO8, in the

other genotypes there was significant reduction in P_N as compared to P_N at 7dpi. At 30 dpi, except in *V.cauliflora*, *V.goudotiana* and *V. cundinamarensis*, in all the other genotypes the P_N was significantly reduced as compared to P_N levels at 14 dpi. At 30 dpi, the photosynthetic rates were comparatively higher in TNAU Papaya CO8, IGH1, *V.cauliflora* and *V.goudotiana* indicating that these genotypes have some adaptable mechanism to maintain reasonable rates of P_N as compared to other genotypes.

Based on symptom expression on the plants and growth, the genotypes evaluated were classified as susceptible (Arka Surya, Arka Prabhat, Red Lady and Pusa Dwarf) tolerant (Pusa Nanha, TNAU papaya CO8, IGH 1 &2)) and resistant (*Vasconcellea* sp)

In the susceptible genotypes, assimilate rate decreased continuously as the days progressed after *PRSV* inoculation. Similar results were obtained after *SCYLV* infection in sugarcane, *Sunflower chlorotic mosaic virus* in sunflower, phytoplasma in grapevine (Lehrer and Komor, 2009; Arias *et al.*, 2003; Bertamini *et al.*, 2002). Generally RuBisco activity is the major limiting factor which eventually leads to chlorophyll breakdown (Izguirre-Mayoral *et al.*, 2002) and it may be the cause of chlorotic symptoms in observed susceptible genotypes. The diversion of carbohydrates to virus replication in susceptible genotypes also may reduce the RuBisco synthesis and thereby the reduction in photosynthetic rate.

Stomatal conductance (gs)

The stomatal conductance ranged from 0.35 mol m⁻²s⁻¹ (Arka Prabhat) to 0.77 mol m⁻²s⁻¹ (Arka Surya) in control plants of *Carica papaya* genotypes and intergeneric hybrids, while it ranged from 0.17 mol m⁻²s⁻¹ (*V. cauliflora*) to 0.39 mol m⁻²s⁻¹ (*V. cundinamarcensis*) in the *Vasconcellea*

species evaluated in the present study. Before inoculation, highest stomatal conductance of 0.77 mol m⁻²s⁻¹ was registered in Arka Surya and was on par by IGH2 (0.67 mol m⁻²s⁻¹) and lowest was recorded in *V. cauliflora* (0.17 mol m⁻²s⁻¹) succeeded by *V. goudotiana* (0.32 mol m⁻²s⁻¹) and *V. cundinamarcensis* (0.39 mol m⁻²s⁻¹).

Consequent to *PRSV* inoculation at 7 dpi, gs ranged from 0.14 mol m⁻²s⁻¹ in *V. cauliflora* to 0.55 mol m⁻²s⁻¹ in Arka Surya. At 14 dpi, gs ranged between 0.13 mol m⁻²s⁻¹ in Arka Prabhat and 0.54 mol m⁻²s⁻¹ in *V. cundinamarcensis*. The gs ranged from 0.05 mol m⁻²s⁻¹ in Pusa Dwarf to 0.54 mol m⁻²s⁻¹ in *V. cundinamarcensis* at 30 dpi.

Stomatal conductance (gs) was decreased in Arka Surya, Arka Prabhat, Red Lady, Pusa Dwarf, TNAU Papaya CO8, IGH1 and IGH2 at 7 dpi. At 14 dpi, further decrease as compared to 7 dpi was observed in Arka Surya, Arka Prabhat and TNAU papaya CO8, while, other genotypes remain on par with gs levels at 7 dpi. At 30 dpi, except Pusa Nanha and IGH1, in all other genotypes the gs was significantly reduced as compared to gs levels at 14 dpi.

In resistant genotypes viz., *V. cauliflora* and *V. goudotiana* the stomatal conductance showed non-significant differences after inoculation, while in *V. cundinamarcensis*, increase in stomatal conductance was recorded after inoculation at 7dpi, 14 dpi and 30 dpi. The genetic background of high stomata conductance in *V. cundinamarcensis* is worth further study.

In the susceptible genotypes, similar to photosynthetic rate, stomatal conductance decreased continuously as the days progressed after *PRSV* inoculation. The reduction in photosynthetic rate might be due to the limited stomatal conductance.

Table.1 Changes in parameters related to photosynthesis in response to *PRSV* infection

Varieties/ Wild relatives	Photosynthetic Rate (P _N) (□ mol m ⁻² s ⁻¹)					Stomatal conductance (g _s) (mol m ⁻² s ⁻¹)					Transpiration rate (E) (mmol m ⁻² s ⁻¹)				
	BI	7dpi	14dpi	30dpi	Tukey's HSD at 1%	BI	7dpi	14dpi	30dpi	Tukey's HSD at 1%	BI	7dpi	14dpi	30dpi	Tukey's HSD at 1%
Arka Surya	^a 9.26 ^b	^b 8.38 ^b	^c 6.57 ^b	^d 1.73 ^k	0.25	^a 0.77 ^a	^b 0.55 ^a	^c 0.48 ^a	^c 0.11 ^b	0.14	^c 3.43 ^d	^b 3.86 ^{cd}	^a 4.44 ^f	^d 1.19 ^g	0.18
Arka Prabhath	^a 9.72 ^a	^b 7.36 ^c	^c 5.44 ^d	^d 2.22 ^j	0.16	^a 0.35 ^{de}	^b 0.23 ^{de}	^c 0.13 ^c	^c 0.12 ^b	0.04	^b 2.93 ^f	^c 2.85 ^f	^a 3.97 ^g	^d 2.55 ^e	0.04
Red Lady	^a 7.06 ^{de}	^b 6.27 ^f	^c 5.28 ^{de}	^d 2.74 ^g	0.22	^a 0.47 ^{bc}	^b 0.21 ^{de}	^{bc} 0.18 ^c	^c 0.10 ^b	0.09	^d 2.19 ⁱ	^c 3.21 ^e	^a 5.65 ^b	^b 3.34 ^c	0.11
Pusa Dwarf	^a 8.17 ^c	^b 6.71 ^e	^c 6.10 ^c	^d 2.12 ^j	0.31	^a 0.45 ^{cd}	^b 0.32 ^{cd}	^b 0.33 ^b	^c 0.05 ^b	0.08	^b 3.90 ^c	^b 4.17 ^c	^a 4.83 ^e	^c 1.00 ^h	0.29
Pusa Nanha	^a 6.70 ^{ef}	^b 6.14 ^g	^a 6.71 ^h	^c 3.22 ^f	0.41	^a 0.49 ^{bc}	^a 0.51 ^{ab}	^b 0.14 ^c	^b 0.13 ^b	0.05	^c 1.16 ^j	^a 4.14 ^c	^b 1.65 ^j	^b 1.75 ^f	0.14
TNAU papaya CO8	^a 5.23 ^g	^b 4.97 ^h	^a 5.16 ^e	^c 4.72 ^b	0.19	^a 0.43 ^{cd}	^b 0.28 ^{cd}	^c 0.15 ^c	^c 0.15 ^b	0.12	^b 6.67 ^a	^c 3.62 ^d	^a 6.94 ^a	^d 3.44 ^c	0.11
IGH1	^b 6.43 ^f	^a 8.59 ^a	^c 5.37 ^d	^d 4.54 ^c	0.24	^a 0.56 ^b	^b 0.24 ^{de}	^b 0.31 ^b	^b 0.25 ^a	0.14	^c 4.13 ^b	^b 4.57 ^b	^a 5.30 ^c	^b 4.57 ^a	0.18
IGH2	^b 7.33 ^d	^a 8.69 ^a	^a 8.53 ^a	^c 2.49 ^h	0.22	^a 0.67 ^a	^b 0.41 ^{bc}	^b 0.32 ^b	^c 0.15 ^b	0.17	^c 4.18 ^b	^b 4.85 ^b	^a 4.95 ^d	^d 2.96 ^d	0.10
<i>V. cauliflora</i>	4.46 ^h	4.46 ^j	4.16 ^g	4.35 ^d	NS	0.17 ^f	0.14 ^e	0.15 ^c	0.14 ^b	NS	2.62 ^g	2.95 ^{ef}	2.48 ⁱ	2.91 ^d	NS
<i>V. goudotiana</i>	4.17 ⁱ	4.79 ⁱ	4.92 ^f	4.16 ^e	NS	0.32 ^e	0.34 ^{cd}	0.32 ^b	0.34 ^a	NS	^c 2.38 ^h	^a 5.44 ^a	^b 3.64 ^h	^c 2.42 ^e	0.58
<i>V. cundinamarcensis</i>	6.66 ^f	7.09 ^d	6.56 ^b	6.51 ^a	NS	0.49 ^{bc}	0.54 ^{ab}	0.54 ^a	^a 0.55 ^a	0.12	3.15 ^e	3.75 ^d	3.92 ^g	3.64 ^b	NS
Mean	6.74	6.68	5.62	3.53		0.46	0.34	0.28	0.17		3.34	3.95	4.34	2.71	
CV (%)	1.51	0.49	0.83	0.56		6.30	11.27	7.28	16.26		0.73	2.50	0.64	1.56	
SE (d)	0.08	0.03	0.04	0.02		0.02	0.03	0.02	0.02		0.02	0.08	0.02	0.03	
Tukey's HSD at 1%	0.36	0.12	0.17	0.07		0.104	0.14	0.072	0.100		0.088	0.353	0.099	0.151	

This has been previously reported in tobacco cultivars infected by poty viruses *PVY* and *Potato virus A* (Ryslava *et al.*, 2003). Reduction in stomatal conductance also indicated a high accumulation of viral aggregates inside the guard cells (Schnkova *et al.*, 2005). This might negatively affect the stomatal conductance in susceptible genotypes. In contrast, the virus replication in resistant genotypes was possibly restricted by different defense mechanisms and hence may cause limited accumulation of viral aggregates and thus non-significant reduction in stomatal conductance.

Transpiration rate (E)

Among the genotypes, highest transpiration rate of $6.67 \text{ mmol m}^{-2}\text{s}^{-1}$ prior to inoculation was observed in TNAU Papaya CO8, followed by IGH2 ($4.18 \text{ mmol m}^{-2}\text{s}^{-1}$) and IGH1 ($4.13 \text{ mmol m}^{-2}\text{s}^{-1}$) (Table 1) and lower values were recorded in Pusa Nanha ($1.16 \text{ mmol m}^{-2}\text{s}^{-1}$) and Red Lady ($2.19 \text{ mmol m}^{-2}\text{s}^{-1}$). At 7 dpi, the transpiration rate ranged from $2.85 \text{ mmol m}^{-2}\text{s}^{-1}$ in Arka Prabhath to $5.44 \text{ mmol m}^{-2}\text{s}^{-1}$ in *V. goudotiana*. The transpiration rate at 14 dpi was ranged from $1.65 \text{ mmol m}^{-2}\text{s}^{-1}$ in Pusa Nanha to $6.94 \text{ mmol m}^{-2}\text{s}^{-1}$ in TNAU papaya CO8. At 30 dpi, the levels of E ranged from $1.00 \text{ mmol m}^{-2}\text{s}^{-1}$ in Pusa Dwarf to $4.57 \text{ mmol m}^{-2}\text{s}^{-1}$ in IGH1.

In Arka Surya, Arka Prabhath, Red Lady, Pusa Dwarf, TNAU papaya CO8, IGH1 and IGH2 transpiration rate increased significantly at 14 dpi but declined significantly at 30 dpi. In Pusa Nanha, significant increase in transpiration rate was observed at 7 dpi and further declined at 14 dpi and 30 dpi as compared to 7 dpi. Transpiration rates remained unchanged in *V. cauliflora* and *V. cundinamarcensis*, while in *V. goudotiana* the increase in transpiration rate was recorded at 7 dpi and declined further at 14 and 30 dpi as compared to 7 dpi.

In susceptible genotypes, the transpiration rate was found increased significantly upto 14 days post inoculation and further declined probably with lower metabolic rates. This results was contradictory to Bertamini *et al.*, (2005), who reported significant reduction in transpiration after grapevine leaf roll-associated virus in grapevine. Disrupted water supply was associated in water stress leading to symptoms viz., chlorosis, wilting. Generally reduced stomatal conductance was associated with transpiration rate. In this study increase in transpiration rate in susceptible genotypes after *PRSV* inoculation was observed and this might be because of the stress developed inside the plant system as the plant tissues may be in demand for more assimilates to regain its homeostasis. The physiological parameters in *Vasconcellea* species were comparatively less affected or not significantly impacted by *PRSV* infection indicating that the resistance mechanisms prevailing in these genotypes have the capacity to maintain physiological functioning of the plant inspite of viral incidence. The physiological parameters were found to be influenced greatly within a short span of infection (7 dpi) and the tolerant or resistant genotypes were able to maintain the important physiological parameters related to photosynthesis at a reasonable level subsequently (14 or 30 dpi) and recover as compared to severely susceptible genotypes. The genetic basis of such regulation and adaptation in the *Vasconcellea* species and as well as in the tolerant *Carica papaya* genotypes viz., Pusa Nanha, TNAU Papaya CO.8 and IGH-1 which helps to achieve homeostasis for effective functioning of photosynthetic system further needs to be studied.

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