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A comparative study of changes in quality attributes of fruit pulp due to *Papaya Ring Spot Virus-P* infection in *Carica papaya* and its wild relatives

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Abstract
Production of papaya is hampered by a deadly disease known as *Papaya Ring Spot Virus*. When infected in the early stages of growth, fruit production is severely affected and upon the later stages of infection, fruits of lower quality were produced. Maintaining the balance between fruit quality and disease resistance will be toilsome for breeders during screening of the germplasm or hybrid progenies. This study was conducted in order to identify papaya genotypes which have lower fluctuations in fruit quality in response to the viral infection. Six cultivars of *Carica papaya* namely Arka Surya, Arka Prabhath, Red Lady, Pusa Dwarf, Pusa Nanha and TNAU papaya CO8, two intergeneric hybrids of Arka Surya and *Vasconcellea cauliflora* (IGH1 and IGH2) and three wild relatives *V. cauliflora, V. goudotiana* and *V. parviflora* were evaluated in this study. Fruit quality parameters viz., total soluble solids, total sugars, reducing sugars, titrable acidity, ascorbic acid and carotene concentration were estimated in these eleven genotypes which was inoculated at nursery stage, vegetative and flowering stage. When the plants were infected at nursery stage, few accessions viz., Pusa Nanha, TNAU papaya CO8, IGH1, IGH2 and the wild species yielded fruits. TSS, total sugar and reducing sugar decreased significantly in these fruits whereas, titrable acidity was increased notably, while no changes were recorded in ascorbic acid and carotene content. Similar observations were recorded when the accessions were subjected to infection at vegetative stage. However, the TSS and sugar content of the fruits were found to marginally increase as compared to control when the genotypes were infected at flowering stage. Among the accessions, minimum changes in fruit quality parameters were observed in wild species followed by papaya variety Pusa Nanha.

Keywords: total soluble solids, total sugars, reducing sugars, titrable acidity

Introduction
Papaya is a major fruit crop originated in Tropical America distributed throughout the tropics owing to its uniqueness in wide ecological adaptability, easiness in cultivation, high palatability, early fruiting, year round bearing, higher productivity, nutritive value and economic returns. The production of papaya is weakened by a serious viral disease caused by *Papaya Ring Spot Virus-P* (*PSRV*) transmitted through aphids. *PRSV* produces a range of symptoms such as leaf mosaic and chlorosis, water-soaked oily streaks on the petiole and upper parts of the trunk, distortion of young leaves leads to shoestring-like symptoms, stunting of infected plants and flower abortion. In addition to these symptoms, the infection is characterized by a typical ring spot symptom on fruits. When the plants are infected at nursery stage or early vegetative stage, infected trees do not produce fruits, whereas delayed infected plants which started fruiting will have reduced yields with altered fruit quality. Besides, the appearance of fruit deteriorates because of the ringspot and the quality of fruits tends to decline with the severity of the disease (Gonsalves, 1998) [7]. Plants that are susceptible to *PRSV* did not produce fruits, whereas tolerant plants produced reasonable quantity of fruits with mild symptoms (Gonsalves *et al*., 2010) [8].
Several attempts were made to screen and isolate resistant sources from papaya germplasm, no single variety was found to have resistance to *PRSV*. However, the closely related *Vasconcellea* species to cultivated papaya viz., *V. cundinamarcensis*, *V. candicans*, *V. stipulata*, *V. cauliflora* and *V. quercifolia* are reported to be resistant to *Papaya Ring Spot Virus*. Screening studies should also involve simultaneous evaluation of fruit quality

parameters since the edible good quality of *Carica papaya* gets compromised with *Papaya Ring Spot Virus*. Gonsalves (1998) [7] reported 50 % reduction in the sugar content of *PRSV* infected fruits having ring spot symptom. Change in the quality of fruits upon *PRSV* infection in *Carica papaya* germplasm along with *Vasconcellea* species can help to select the accessions with better quality traits and also to identify the stage at which the infection significantly reduced the quality of fruits. Thus an attempt was made to characterize the impact of changes on biochemical parameters of fruit quality subsequent to *PRSV* infection at different growth stages of the crop.

Materials and Methods

Plant material

The experiment was carried out at ICAR- Indian Institute of Horticultural Research, Bengaluru during the period from June, 2016 to August, 2017 under the insect proof net house. During the period of study, the maximum temperature ranged from 25.33 to 34.93°C with a mean of 29.03 °C while the minimum temperature range was 10.28 to 21.19 °C. The average relative humidity was 60.98%. Six cultivars of *Carica papaya* namely Arka Surya, Arka Prabhath, Red Lady, Pusa Dwarf, Pusa Nanha and TNAU papaya CO8, two intergeneric hybrids of Arka Surya and *Vasconcellea cauliflora* (IGH1 and IGH2) and three wild relatives *V. cauliflora, V. goudotiana and V. parviflora* were involved in this study. The fruits were collected from the trees inoculated by *PRSV* at nursery stage, vegetative stage and flowering stage, both in the control and infected plants. Twenty plants of each accession in two replicates were maintained according to the Randomized Block Design at each stage. Data on fruit quality attributes were recorded from five fruits randomly harvested at full maturity from each replication and allowed to ripen at room temperature and the means were calculated. The significance of difference in the quality attributes between control and inoculated trees were analyzed by t-test.

Method of inoculation

Infected leaves of the susceptible cultivar maintained in the insect proof net house were homogenized in 0.1M potassium phosphate buffer (1:5 w/v) (pH 7.0), two drops of □-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 of basal three leaves were mechanically inoculated on the upper surface of the leaves with the standard inoculum, using carborundum powder, 600 mesh as an abrasive. After 5 minutes of inoculation, the excess sap was washed off by distilled water.

Observations

Total soluble solids content in the pulp was determined using hand refractometer, make ERMA®, Japan readings and reported in ^{o} Brix units. Total sugars and reducing sugars were estimated by the method suggested by Somogyi (1952) [13] and expressed in percentage. Acidity was estimated as per the A.O.A.C method (2000) [2] and expressed as per cent citric acid equivalents. Ascorbic acid content was determined by 2,6–Dichlorophenol indophenol (DCPIP) method (AOAC, 2006) [2]. Total carotenoids content were analyzed by spectrophotometric method (Lichtenthaler, 1987) [11].
The statistical design used was a completely randomized design with 5 replicates. Results were analyzed by one-way analysis of variance (ANOVA) using SAS 9.3 package. Means were compared with Tukey's multiple comparison test ($P \leq 0.05$).

Result and Discussion

Fruit biochemical changes upon infection at nursery stage

When the accessions were infected at nursery stage, fruit set was observed in few accessions viz., Pusa Nanha, TNAU papaya CO8, IGH1, IGH2, *V. goudotiana*, *V. cauliflora* and *V. parviflora*. The accessions such as Arka Surya, Arka Prabhath, Red Lady and Pusa Dwarf did not set fruits. Highest reduction of total soluble solids were recorded in TNAU papaya CO8 (40.22 %) followed by IGH1 (34.05 %), IGH2 (29.89 %) and Pusa Nanha (12.68 %) (Table.1). The wild species showed non-significant difference in TSS when infected at nursery stage. Total sugars decreased notably when infected at nursery stage in TNAU papaya CO8 (35.39 %) succeeded by IGH1 (30.18 %), IGH2 (28.02 %) and Pusa Nanha (15.11 %) (Table.2). Significant decrease of reducing sugars were recorded in TNAU papaya CO8 (44.36 %) followed by IGH1 (41.77 %), IGH2 (33.56 %) and least in Pusa Nanha (18.42 %) (Table.2). Nursery stage infected plants recorded higher titrable acidity than the control plants and the highest per cent reduction was noted in IGH2 (26.98 %), which was on par with TNAU papaya CO8 (25.11 %) followed by Pusa Nanha and IGH1 (Fig.1). No significant difference was observed in ascorbic acid and carotene concentration.
Virus infection has significantly affected the physiology and growth of plants infected at the early stages of growth (Bertamini *et al*., 2005; Hanssen *et al*., 2011) [5, 10]. Accumulation of virus at the growing regions might be either in the fruit or growing points from the infection site were evident by the symptoms in fruits and the growing regions. The long distance transport of virus via the phloem vessels could be attributed to blockage of the nutrient uptake as well as carbohydrate flow towards the sink. In addition to the block in the transport of photoassimilates, production of photoassimilates were severely affected during virus infection through decomposition of photosynthetic related components viz., RuBisco, chloroplast structure, thylakoid membranes and PSII reaction centres (Baebler *et al*., 2009; Almasi *et al*., 2001) [3]. These factors rendered the plants unable to produce normal fruits (Hackel *et al*., 2006) [9]. Damage in the photosynthetic system lacks the photoassimilate production and supply to the sink. This might be the cause of lower sucrose accumulation in fruits which resulted in lower reducing sugars such as glucose and fructose. However, the increase in titrable acidity may be due to the increase in respiration rate of infected cells. Even though, the photosystem impaired because of the virus infection, no significant changes were noted in carotene content. It might be due to the accumulation of available carotene into the developing sink.

Fruit biochemical changes upon infection at vegetative stage

When the infection was delayed till vegetative stage (one month after planting), fruit set was observed in Red Lady, TNAU papaya CO8, IGH1, IGH2, *V. cauliflora*, *V. goudotiana* and *V. parviflora* among the genotypes evaluated. No fruit set was recorded in Arka Surya, Arka Prabhath and Pusa Dwarf. The accessions inoculated at vegetative stage revealed highest reduction of TSS in Red Lady (36.57 %) followed by TNAU Papaya CO8 (33.34 %), IGH2 (28.28 %) and IGH1 (19.58 %). The least reduction was registered in

Pusa Nanha (13.42%). The wild relatives *viz*., *V. goudotiana*, *V. cauliflora* and *V. cundinamarcensis* recorded insignificant changes after inoculation (Table.1). Highest reduction in total sugars was registered in Red Lady (35.29 %) followed by IGH2 (26.23 %), TNAU papaya CO8 (25.61 %) and IGH1 (18.22 %) and the least reduction in Pusa Nanha (11.79 %). *V. goudotiana*, *V. cauliflora* and *V. cundinamarcensis* had shown non-significant difference as compared to control (Table.2). However, at vegetative stage infection, highest reduction of reducing sugars was observed in Red Lady (30.98%) followed by IGH2 (26.78 %) and TNAU papaya CO8 (16.56 %). The lowest decrease as compared to control was estimated in IGH1 (6.26 %) followed by Pusa Nanha (15.47 %) (Fig.1).When the plants were inoculated at vegetative stage highest reduction of titrable acidity was also observed in Red Lady (26.85 %) followed by TNAU papaya CO8 (16.74 %), Pusa Nanha (11.84 %) and IGH2, while the lowest reduction was in IGH1 (6.28 %) (Fig.2). Ascorbic acid and total carotene content remain unchanged when infection took place at vegetative stage.

As the virus is an obligate biotrophic pathogen, it can interfere in host primary metabolism. This alters the balance between source and sink since virus infected leaves and other plant parts function as sinks rather than as source. This carbohydrate reallocation within the plant system or competition between the sink sources, which directs towards the infected leaves deprives the carbohydrate flow to the fruits. This could be observed in the present study as similar to the plants infected at nursery stage, the plants infected at the vegetative stage in susceptible accessions also expressed the severe *PRSV* symptoms and arrested development of reproductive growth and as well imbalances in sugar and acid concentration. A sharp increase in respiration rate was reported in several instances due to viral infection (Shalitin and Wolf, 2000) [12] and this might be the cause for higher acidity levels in fruit pulp of the genotypes with high susceptibility in the present study.

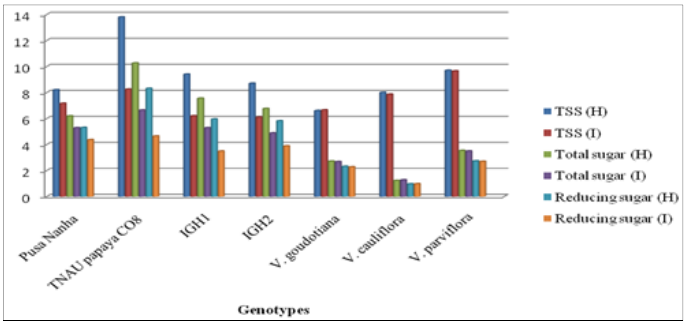
Fruit biochemical changes upon infection at flowering stage

When the infection is delayed till flowering stage (four to five months after planting), fruit set was observed in the accessions evaluated. Subsequent to *PRSV* inoculation at flowering stage, total soluble solids content showed significant variation in accessions as compared to control. Significant increase of TSS was recorded in Arka Prabhath (14.71 %), Arka Surya (14.19 %), TNAU papaya CO8 (7.97%) and Red Lady (6.42 %). However, there was decrease in TSS of IGH1 (7.77%), IGH2 (5.06 %), *V. goudotiana* (3.03 %) and Pusa Dwarf (3.02 %) (Table.1). In general there was low sugar content in the varieties that showed reduced incidence. Total sugar content also showed significant increase as compared to control in TNAU papaya CO8 (17.93 %) followed by Red Lady (10.17 %), Pusa Dwarf (7.77 %) and Arka Surya (7.02 %). No significant difference was noted in Arka Prabhath, Pusa Nanha, IGH1, IGH2, *V. cauliflora* and *V. goudotiana* (Table.2). Reducing sugar content of the fruit also varied notably when the accessions were infected at the flowering stage. Significant increase was observed in Red Lady (10.82 %), TNAU papaya CO8 (9.51 %), Pusa Dwarf (7.97%) and Arka Surya (5.41 %). IGH1 and IGH2 recorded decrease in reducing sugars as that of control fruits (Fig.1). Highest reduction of titrable acidity was recorded in Arka Surya, when infected at flowering stage as 18.06 %, which was on par with Arka Prabhath (13.22 %) and TNAU papaya CO8 (8.37 %) and the lowest reduction was accounted in Red Lady and IGH2 (Fig.3). In case of *V. cauliflora*, *V. goudotiana* and *V. cundinamarcensis* non-significant differences were observed in all stages of *PRSV* infection.

Production of photoassimilates, transport of photoassimilates and utilization of photoassimilates in sink organ influences the proper development of fruit. Disturbances in any of these processes affected fruit set as well as slower growth rate (D'Aoust *et al*., 1999). Reduction in fruit growth can be a factor of both reduction in production and transport of photoassimilate, higher transpiration rates and disturbances in the vascular system which might negatively impact the water supply to the fruit. A study on effect of citrus decline suggested that fruit size and weight was proportional to TSS per fruit but inversely proportional to the ºBrix and TSS/box and acidity (Bassanezi *et al*., 2007). Thus the reduction in fruit size and weight upon *PRSV* infection concentrates the carbohydrates accumulated in the pulp and possibly resulted in marginally higher TSS, sugar and acidity in the infected trees. Higher acidity in fruit pulp could be related to higher respiration.

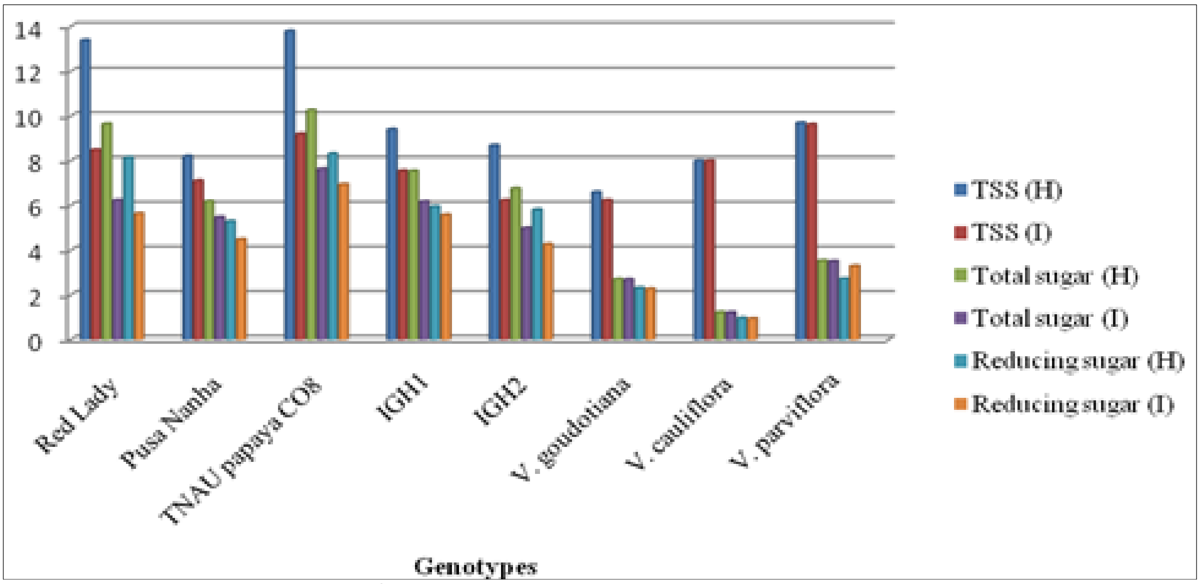
Conclusion

From the present study, it can be concluded that it is essential to prevent the early stage infection as it severely affects the fruit set. Although some fruit set was observed when the infection was at vegetative stage, the fruit quality was affected severely. Few accessions overcome the stress developed by virus and produced few fruits, but the fruit quality was very poor with low TSS, total sugars and reducing sugars plus high titrable acidity. However, higher number of fruits was produced when the infection occurred at later stages of plant development. Relatively lower impairment to fruit quality was observed in the wild relatives and Pusa Nanha as compared to other genotypes. The genes or gene products responsible for modulating these variations in quality attributes during *PRSV* infection in the different genotypes especially in the wild species require to be further explored.



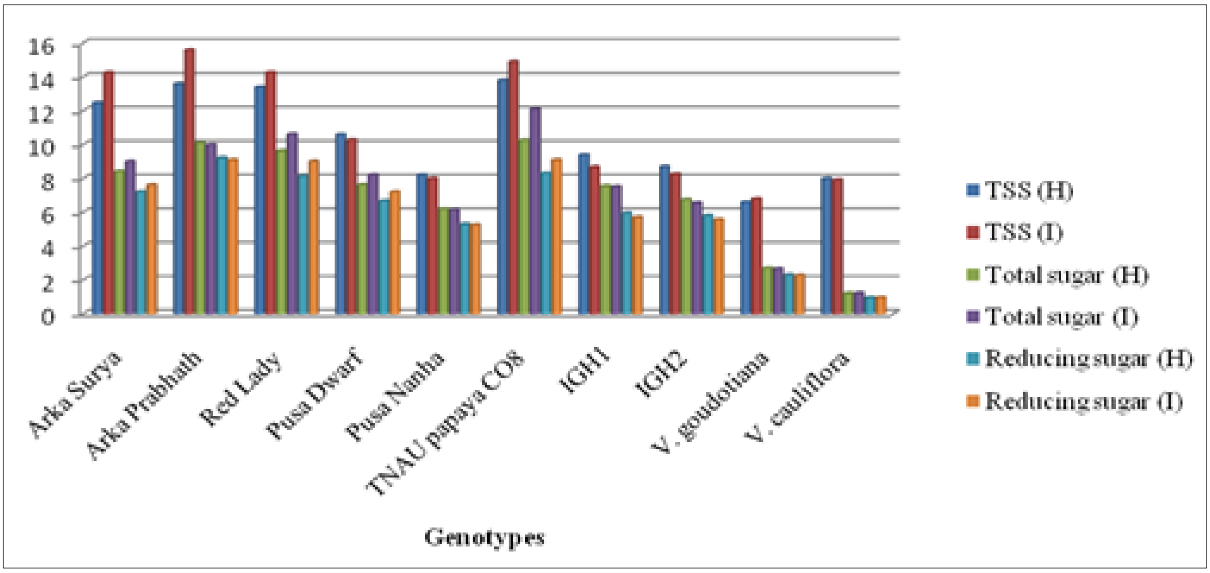
H represents healthy and I represents infected

Fig 1: Alterations in TSS, total sugars and reducing sugars in the fruit pulp of control and PRSV infected fruits



H represents healthy and I represents infected

Fig 2: Alterations in TSS, total sugars and reducing sugars in the fruit pulp of control and PRSV infected fruits



H represents healthy and I represents infected

Fig 3: Alterations in TSS, total sugars and reducing sugars due to PRSV

Table 1: TSS of fruit pulp in control and *PRSV* infected plants

Varieties/ Wild species	TSS (ºBrix)								
	Infected at nursery stage		Percent reduction	Infected at vegetative stage		Percent reduction	Infected at flowering stage		Percent increase / decrease
	Control	Infected		Control	Infected		Control	Infected	
Arka Surya	12.48	No fruit set		12.48	No fruit set		12.48	14.25	+14.19[b]
ArkaPrabhath	13.60	No fruit set		13.60	fruit set		13.60	15.60	+14.71[b]
Red Lady	13.40	No fruit set		13.40	8.50	36.57[a]	13.40	14.26	+6.42[e]
Pusa Dwarf	10.60	No fruit set		10.60	No fruitset		10.60	10.28	-3.02[g]
PusaNanha	8.20	7.16	12.68[d]	8.20	7.10	13.42[e]	8.20	8.00	NS
TNAU papaya CO8	13.80	8.25	40.22[a]	13.80	9.20	33.34[b]	13.80	14.90	+7.97[d]
IGH1	9.40	6.20	34.05[b]	9.40	7.56	19.58[d]	9.40	8.67	-7.77[d]
IGH2	8.70	6.10	29.89[c]	8.70	6.24	28.28[c]	8.70	8.26	-5.06[f]
V. goudotiana	6.60	6.65	NS	6.60	6.24	NS	6.60	6.80	NS
V. cauliflora	8.00	7.86	NS	8.00	8.00	NS	8.00	7.92	-3.03[g]
V. parviflora	9.70	9.65	NS	9.70	9.62	NS	**	**	
Mean			29.21			22.77			7.98
CV(%)			1.76			1.59			1.70
SE (d)			0.420			0.295			0.111
Tukey HSD at 1%			2.089			1.340			0.486

Table 2: Total sugar of fruit pulp in control and *PRSV* infected plants

Varieties/ Wild species	Total sugars (g/100g)								
	Infected at nursery stage		Percent reduction	Infected at vegetative stage		Percent reduction	Infected at flowering stage		Percent increase/ decrease
	Control	Infected		Control	Infected		Control	Infected	
Arka Surya	8.42	No fruit set		8.42	No fruit set		8.42	9.01	+7.02[c]
ArkaPrabhath	10.12	No fruit set		10.12	No fruit set		10.12	10.00	NS
Red Lady	9.64	No fruit set		9.64	6.24	35.29[a]	9.64	10.62	+10.17[b]
Pusa Dwarf	7.62	No fruit set		7.62	No fruit set		7.62	8.21	+7.77[bc]
PusaNanha	6.19	5.28	15.11[d]	6.19	5.48	11.79[d]	6.19	6.15	NS
TNAU papaya CO 8	10.26	6.64	35.39[a]	10.26	7.64	25.61[b]	10.26	12.10	+17.93[a]
IGH1	7.55	5.28	30.18[b]	7.55	6.18	18.22[c]	7.55	7.51	NS
IGH2	6.76	4.88	28.02[c]	6.76	5.00	26.23[b]	6.76	6.56	NS
V. goudotiana	2.71	2.67	NS	2.71	2.70	NS	2.71	2.68	NS
V. cauliflora	1.22	1.27	NS	1.22	1.21	NS	1.22	1.24	NS
V. parviflora	3.53	3.49	NS	3.53	3.50	NS	**	**	
Mean			27.18			23.43			10.72
CV(%)			1.59			3.10			5.97
SE (d)			0.354			0.594			0.543
Tukey HSD at 1%			1.759			2.782			2.543

Table 3: Titrable acidity of fruit pulp in control and *PRSV* infected plants

Varieties/ Wild species	Titrable Acidity (%)								
	Infected at nursery stage		Percent increase/ decrease	Infected at vegetative stage		Percent increase / decrease	Infected at flowering stage		Percent increase/ decrease
	Control	Infected		Control	Infected		Control	Infected	
Arka Surya	0.06	No fruit set		0.06	No fruit set		0.06	0.07	18.06[a]
Arka Prabhath	0.10	No fruit set		0.10	No fruit set		0.10	0.09	13.22[ab]
Red Lady	0.15	No fruit set		0.15	0.21	26.85[a]	0.15	0.16	6.71[b]
Pusa Dwarf	0.12	No fruit set		0.12	No fruit set		0.12	0.12	NS
Pusa Nanha	0.17	0.19	11.87[b]	0.17	0.19	11.84[c]	0.17	0.16	NS
TNAU Papaya CO8	0.12	0.18	25.11[a]	0.12	0.19	16.74[b]	0.12	0.13	8.37[ab]
IGH1	0.16	0.18	12.57[b]	0.16	0.15	6.28[d]	0.16	0.16	NS
IGH2	0.15	0.19	26.98[a]	0.15	0.17	13.49[c]	0.15	0.16	6.74[b]
V. goudotiana	0.20	0.19	NS	0.20	0.20	NS	0.20	0.20	NS
V. cauliflora	0.18	0.17	NS	0.18	0.19	NS	0.18	0.17	NS
V. parviflora	0.17	0.17	NS	0.17	0.18	NS	**	**	
Mean			19.14			15.05			10.62
CV(%)			5.77			5.09			27.54
SE (d)			0.902			0.625			2.39
Tukey HSD at 1%			4.487			2.929			11.187

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