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Improving yield and quality of papaya (var. CO 8) through pre-harvest foliar application of plant growth regulators (PGRs)

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Abstract
Background: Potential improvement in quality and shelf life in general is determined by the physiological condition of the crop at harvest. Hence, the present study was aimed at improving the yield and quality of papaya fruit at harvest as influenced by pre-harvest spray of Plant Growth Regulators (PGRs).
Materials and Methods: Plant growth regulators (PGRs) were applied on papaya crop (var. CO 8) at 5th and 7th month after planting to investigate their influence on yield and postharvest fruit quality characters as well as shelf life. The PGRs (TIBA at 200 ppm, CPPU at 4 ppm, CCC at 500 ppm, MeJA at 100 pm, SA at 100 pm and BR at 0.5 ppm) used in the study were given as foliar sprays.
Results: The results revealed that exogenous application of growth regulators significantly decreased the days to flowering, days to first harvest and first bearing height. A significant reduction in the PRSV incidence of papaya was also observed with the application of MeJA and SA. Yield and yield attributes were improved by all sprayed PGRs, especially BR and TIBA increased the number of fruits/plant and fruit weight that resulted in higher yield. A positive increase in the fruit morphological and quality characters was also observed with the application of PGRs and resulted in higher shelf life.
Conclusion: The scientific results about the effects of pre-harvest application of growth regulators may contribute to improve papaya fruit yield with better quality, higher papain yield and reduced PRSV incidence.

Keywords: Tri-iodo benzoic acid, Cycocel, firmness, postharvest, shelf life

Introduction
Papaya (*Carica papaya* L.) is an important fruit crop grown in the tropics for its high nutritional and pharmacological attributes. Potential shelf life and quality in general is determined by the physiological condition at harvest. Hence, the present study was aimed at improving the yield and quality of papaya fruit at harvest as influenced by pre-harvest spray of Plant Growth Regulators (PGRs).
Tri-iodo benzoic acid (TIBA), a growth retardant, has been found to increase the yield of different crop plants at low concentrations. Studies have showed that application of TIBA in soybean induced greater growth and yield. (Chung and Kim, 1989 [4] and Ravichandran and Ramaswami, 1991) [29]. Cycocel (CCC) works as an anti-gibberellin (dwarfing agent), and its foliar application can retard growth by reducing stem elongation, regulating plant height and cell division. CCC has been reported to be helpful for proper partitioning of plant assimilates that leads to increase in fruit setting percentage as well as yield (Gardner *et al.*, 1985) [8].
Synthetic substituted phenylurea compound CPPU [N-(2-chloro-4-pyridyl)-N'-phenylurea], with cytokinin-like activity has been reported to hasten the import of assimilates into the fruit by expanding the sink size and by increasing sink activity, which are thought to be important factors in fruit growth (Elfving and Cline, 1993) [7]. In the recent years, CPPU has been employed to improve fruit set and fruit growth of several crops. Growth regulatory activity of Brassinosteroids (BRs) is due to their influence on the metabolic process associated with photosynthesis, nucleic acid and protein biosynthesis (Xiao *et al.*, 2009) [37]. Methyl Jasmonate (MeJA) and Salicylic acid (SA) are naturally occurring and have been reported to improve flowering and fruit set, influence quality characteristics and induce disease resistance or enhance stress responses in fruit crops (Ahmed *et al.*, 2015; Kucuker and Ozturk, 2014; Gonzalez-Aguilar *et al.*, 2003) [1, 20, 11]. Hongjie *et al.* (2004) [14] found that application of SA at 2mM and MeJA at 0.2mM showed best results in inhibiting the spore germination on fruits.

According to the above discussion, the present study was conducted to evaluate the effect of pre-harvest foliar application of PGRs on papaya crop (var. CO 8, a dioecious red pulped variety derived from CO 2.) growth, yield and fruit quality. An attempt was also made to evaluate the influence of these PGRs on PRSV disease incidence.

Materials and Methods

Field experiments were conducted at the University Orchard, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India during two successive seasons; 2015- 2016. The soil type was a clayey loam with an available N: P: K of 221:18:250 kg/ha. Seedlings of 45 days old (var. CO 8) were transplanted in the field adopting a spacing of 1.8 x 1.8 m. One male tree for every ten female plants was allowed for facilitating an effective pollination. The experiment was laid out in a Randomized Completely Block Design (RCBD) with seven treatments. Four replications were maintained per treatment with nine plants/ replication. Recommended dose of fertilizers-50:50:50g NPK/plant was given as soil application from 3rd month after planting after thinning at bimonthly intervals.

The details of different treatments imposed in the present investigation are presented in the below:

Details of the treatments imposed during the experiment

S. No.	Pre-harvest treatment details
T_1	Control
T_2	T_1 + TIBA (Triiodobenzoic acid) (200 ppm)
T_3	T_1 + CPPU (Chloro pyridyl phenylurea) (4 ppm)
T_4	T_1 + CCC (Chlormequat chloride) (500 ppm)
T_5	T_1+ MeJA (Methyl Jasmonate) (100 ppm)
T_6	T_1 + SA (Salicylic acid) (100 ppm)
T_7	T_1+ BR (Brassinolide) (0.5 ppm)

*Control refers to the standard recommended dose of micronutrients (($ZnSO_4$ (0.5%) + Boric acid (0.2%) + $CaNO_3$ (1.0%)) for papaya fruit (Jeyakumar *et al.*, 2001) [16]. The nutrient and PGR spray was given through foliar application using a hand sprayer at 5th and 7th month after planting. Tween- 20 was used as the surfactant to allow better penetration and retention of the PGRs applied.

Ten plants were selected at random in each experimental unit to record the yield and yield attributes. The observations on growth parameters like days to flowering (days taken for the first flower to appear from the date of planting), days to first harvest (days taken from the date of planting to the date of first harvest of fruit at colour break stage) and first fruit bearing height (height of the plant from the ground level to the height at which first mature fruit appeared was measured as first bearing height); yield attributes like number of fruits per plant, papain wet yield, fruit weight and fruit yield per plant, fruit length, fruit circumference, cavity index and number of seeds/fruit were recorded. The fruit morpho-physiological characters *viz.* firmness, chrome ‘b’ value, rind thickness, pulp thickness, physiological loss in weight (PLW) were recorded in randomly selected fruits at the time of peak harvest. The fruit quality attributes *viz.* TSS, total sugars, reducing sugars, titrable acidity, ascorbic acid, carotenoid content; lycopene content and shelf life of fruit were also recorded. The morphological and quality parameters were recorded after the fruit achieved the edible stage.

Papain activity was assessed by estimating the tyrosine enzyme activity following the procedure suggested by Moore (1984) [26] and the enzyme activity was expressed in tyrosine units (TU) per milligram of papain. Calcium and magnesium content in the fruit pulp was estimated by using versenate titration method (Jackson, 1973) [15]. Papaya Ring Spot Virus (PRSV) disease incidence was recorded using the score chart ranging from 0-9. 0- No symptoms, 1- Mild mosaic or oily spots, streaks on petioles or stem, oily spots on fruit, 3- Mild mosaic and oily streaks / spots on petiole or stem and ring spots on fruit, 5- Oily spots /streaks on petiole (or) stem (or) ring spots on fruit, 7- Oily spots/streaks on petioles, stem, (or) on fruit, (ring spots), severe mosaic or blistering on leaves and leaf deformation and severe leaf reduction or mild fruit deformation with ring spots, 9- Oily spots/streaks on petiole or stem and shoe string formation or severe fruit deformation with ring spots and stunted plants. Disease severity was calculated using the following formula:

$$\text{PRSV \%} = \frac{\text{Total score of all plants}}{\text{Total number of plants}} \times \frac{100}{\text{maximum score}}$$

For disease rating, the scores were transformed to arc sine values for estimating the analysis of variance (ANOVA).

The experiment was laid out in a randomized block design. All data were tested for the effects of treatments on analyzed parameters by one-way analysis of variance (ANOVA) technique. Treatments means were compared using Duncan`s multiple range test ($p \leq 0.05$).

Results and Discussion

The observations on growth and yield attributes, fruit quality and shelf life were significantly influenced by the application of PGRs.

Growth attributes

The foliar application of PGRs have resulted in decrease in the days to flowering, days to first harvest and fruit bearing height compared to the control plants (Table 1.). The treatments T_7- T_1 + BR and T_2- T_1 + TIBA depicted early flowering (22 and 17 days earlier respectively) and attained early harvest (25-26 days earlier) than the control plants. This trend was followed by the treatments T_4- T_1 + CCC and T_5- T_1 + MeJA which have shown a 14 days early flowering and 20 days early harvest compared to the control plants. The first bearing height in papaya is an important biometric trait, as it directly contributes for the ultimate yield in terms of total number and weight of fruits in unit time. First bearing height was observed to be lowest with the application of TIBA and CCC. Padmalatha *et al.* (2013) [27] reported that TIBA positively influences the days to 50 per cent flowering, days to flowering, spike length, spike weight, number of florets per spike and spike longevity. Similar effects of BR as obtained in this study in improving flowering performance of several cultivars of vegetables, including lettuce, radish, pepper and bush beans was reported by Meudt *et al.* (1984) [24]. The effect of BR in inducing early flowering reported by Alvarez *et al.* (2005) [2] and Eleiwa *et al.* (2011) [6] suggest that BR has a synergistic response with auxins and an additive effect with gibberellins (Mandava *et al.*, 1981) [23].

The growth-promoting activity of BRs can be attributed to enhanced photosynthesis by positively regulating the synthesis and activation of a variety of photosynthetic enzymes including Rubisco, and also increasing CO_2 assimilation and quantum yield of PSII in cucumber (Xiao *et* *al.*, 2009) [37]. Singh and Jindal (1972) [34], reported that foliar application of TIBA as well CCC has been reported to positively influence the femaleness, flowering, sex expression and improved fruit yield with superior quality of the fruits in cucurbits. The reduction in vegetative growth due to TIBA treatments might be due its anti-auxin character by preventing the transport of naturally produced auxins thereby reducing cell elongation.

Employing jasmonic acid and its derivatives (MeJA and (PDJ) n-Propyl dihydrojasmonates) as growth regulators have demonstrated improvement in flowering, plant growth and development, senescence, fruit ripening in apple and plums by several researchers (Rohwer and Erwin, 2008, Kondo *et al.*, 2001 [30, 19] and Zapata *et al.*, 2014) [39]. PRSV disease incidence recorded at flowering and first harvest stage showed that preharvest application of T_5- T_1 + MeJA greatly reduced the disease incidence. The experiment also revealed the effect of T_6- T_1 + SA in reducing the PRSV disease of papaya crop compared to control plants (Table 1.).

Similar to the finding from this study, a single preharvest spray (undertaken three days before harvest) with 0.2 mM MeJA or 2 mM SA, was highly effective in enhancing resistance to infection (*Monilinia fructicola*), with reduced lesion diameters in comparison with the postharvest applications in sweet cherry (Yao and Tian, 2005) [38]. The study reported that the pre-harvest sprays increased activities of defense-related enzymes such as β-1,3-glucanase, phenylalanine ammonia lyase and peroxidase, showing a good potential as a strategy for the control of postharvest decay. Jasmonic acid and its methyl ester, methyl jasmonate, function as a basic signaling compound in metabolic reactions of plants (Rudell *et al.*, 2002) [31]. Several studies (Rudell *et al.*, 2002; Larrondo *et al.*, 2003; Saniewski *et al.*, 2004; Jin *et al.*, 2009; Gong *et al.*, 2013) [31, 21, 32, 18, 10] showed that MeJA stimulated secondary metabolites in grapevine, strawberry, apple, plum, peach and mango fruits.

Table 1: Effect of PGRs on growth attributes and Papaya Ring Spot Virus (PRSV) disease incidence of papaya (var. CO 8)

Treatments	Days to flowering	Days to first harvest	First bearing height	PRSV disease incidence (%)	
				At flowering	At first harvest
T_1	141.00^g	268.67^g	103.44^g	41.14 (0.70)^g	43.22 (0.72)^g
T_2	124.08^b	243.00^a	92.38^a	37.56 (0.66)^f	32.20 (0.60)^f
T_3	133.17^e	267.00^f	97.03^d	36.11(0.64)^e	31.15(0.59)^e
T_4	126.58^c	250.00^d	93.42^b	34.02 (0.62)^d	30.06 (0.58)^d
T_5	135.50^f	248.50^c	100.62^f	25.51(0.53)^a	21.92 (0.49)^a
T_6	129.50^d	266.00^e	99.83^e	29.87(0.58)^b	22.55 (0.49)^b
T_7	119.17^a	244.00^b	96.79^c	30.56(0.59)^c	27.86 (0.56)^c
Mean	129.86	255.31	97.64	33.54 (0.62)	29.85(0.58)
S. Ed.	1.98**	3.50**	1.45**	0.008**	0.007**
LSD (0.05)	4.13	7.27	3.01	0.016	0.015

*Significant difference among the treatments is indicated by DMRT, Arcsine transformed value is given in parenthesis

T1: Control
T2: T1 + TIBA (200 ppm)
T3: T1 + CPPU (4 ppm)
T4: T1 + CCC (500 ppm)
T5: T1+ MeJA (100 ppm)
T6: T1 + SA (100 ppm)
T7: T1+ BR (0.5 ppm)
Control refers to the standard recommended dose of nutrients [$ZnSO_4$ (0.5%) + Boric acid (0.2 %) + $CaNO_3$ (1.0 %)].
RDF - 50:50:50g NPK/plant was given as soil application from 3rd month after planting at bimonthly intervals to all the treatments

Yield and yield characteristics

The data on yield parameters (Table 2.) depicted that application of T_7- T_1 + BR had a positive influence in significantly improving the number of fruits per plant (45.21), fruit weight (1.52 kg), fruit yield/plant (68.72 kg/plant), fruit length (29.96 cm), fruit circumference (40.94 cm) and cavity index (13.57). The results were similar to the findings of Vardhini and Rao (2004) [36] who reported that the growth promotion in tomato due to BR application was associated with elevated levels of nucleic acids, soluble proteins, reducing sugars, non-reducing sugars and starch. Foliar application of 0.01 or 0.05 ppm brassinolide as two or three sprays increased the photosynthesis and leaf chlorophyll content in tobacco (Han *et al.*, 1988) [13]. Jeyakumar *et al.* (2008) [17] also found that foliar application of brassinolide @ 0.1 ppm in fenugreek significantly increased the specific leaf weight.

Results also revealed the pre-harvest application of TIBA significantly improved the number of fruits per plant, fruit weight and yield/plant next to BR. The increase in cavity index (22) of the fruit treated with TIBA was attributed to the greater fruit circumference (41.34 cm). Fruit length was also found to be influenced by CPPU, with the lowest cavity index. CPPU has been reported to stimulate both cell division and cell elongation in grapes resulting in berry size increase when applied shortly after fruit set (Dokoozlain, 2000) [5]. CPPU is highly immobile (Basi *et al.*, 1993) [3] and so increases in cell division on fruit are restricted to the immediate areas where the droplet is applied. The increase in fruit size was because of an increase in the volume of "small" isodiametric parenchyma cells in the outer pericarp (Patterson and Mason, 1993) [28].

Table 2: Effect of PGRs on yield and yield attributes of papaya (var. CO 8)

Treatments	No. of fruits/plant	Weight of a single fruit (kg)	Fruit yield (kg/plant)	Fruit length (cm)	Fruit circumference (cm)	Fruit volume (cc)	Cavity volume (cc)	Cavity index
T_1	36.96^g	1.14^f	42.13^g	25.39^g	38.50^g	590	110	18.64^f
T_2	42.08^b	1.42^b	59.75^b	26.45^e	41.34^a	1000	220	22.00^g
T_3	40.18^c	1.28^e	51.43^e	27.98^d	40.48^d	1090	130	11.93^a
T_4	39.32^f	1.36^d	53.48^d	26.04^f	38.98^f	650	100	15.38^d
T_5	39.45^e	1.27^e	50.10^f	28.04^c	39.16^e	930	170	18.28^e
T_6	40.14^d	1.39^c	55.79^c	28.62^b	40.59^c	950	120	12.63^b
T_7	45.21^a	1.52^a	68.72^a	29.96^a	40.94^b	1400	190	13.57^c
Mean	40.48	1.34	54.49	27.50	40.00	944	149	16.06
S. Ed.	0.50**	0.01**	0.54**	0.37**	0.45**	15.4**	1.9**	0.21**
LSD (0.05)	1.04	0.03	1.13	0.77	0.95	32.09	4.09	0.44

*Significant difference among the treatments is indicated by DMRT

Effect of PGRs on fruit morpho-physiological characters and shelf life

The effect of different treatments on fruit morphological characters is presented in Table 3. The results indicated that application of PGRs have positive influence in improving the fruit characters and shelf life. BR application resulted in higher fruit firmness and rind thickness with a shelf life of 6 days that is correlated by the least chrome ‘b’ value. The chrome ‘b’ values indicate the yellowness of the fruit *i.e.* higher the “b’ value faster is the colour change. Number of seeds/ fruit have also been significantly improved with the application of BR. TIBA has recorded the highest pulp thickness (2.48 cm) while physiological loss in weight was observed to lesser in MeJA and SA treated fruits. This indicates that compounds such as jasmonates and salicylic acid play key roles in activating the signal transduction pathways which lead to the biosynthesis of defense compounds such as phenolics and alkaloids. The findings of Shafiee *et al.,* (2010) [33] also showed that strawberry fruits showed less fruit weight loss than control when salicylic acid was supplied along with nutrients. Therefore, exogenous application of salicylic acid has been reported to reduce decay, delay ripening and extend postharvest life of various fruits including bananas (Srivastava and Dwivedi, 2000) [35], Kiwi (Zhang *et al.*, 2003) [40], apples (Mo *et al.*, 2008) [25] and cherries (Gholami *et al.*, 2010) [9].

Shelf life of the papaya fruit in the present study was also significantly improved with the application of T_7- T_1 + BR, T_5- T_1 + MeJA and T_3- T_1 + CPPU. This effect could be attributed to maintenance of pulp integrity and slowing down water loss with improved disease resistance (Lester and Grusak, 2004; Gong *et al.*, 2013) [22, 10].

From the mentioned results, an overall enhancement in the fruit quality characteristics was obtained by the different sprayed substances as given in Table 4. Since the variety ‘CO 8’ is suitable for both dessert purpose and for papain extraction, fruit and papain quality attributes were examined in this variety. BR increased the soluble solids content, total and reducing sugars, higher ascorbic acid, carotenoid and lycopene content while titrable acidity was not affected. Possibly, the higher total soluble solids in BR treated fruits could be due to more efficient translocation of photosynthates to fruit juice. BR might have aided in the biosynthesis of metabolites and rapid translocation of photosynthetic products, minerals from other plant parts into developing fruits. CPPU was also observed to improve the total sugars, reducing sugars with increased level carotenoid content and fruit calcium levels while CCC recorded higher magnesium content in the fruit. This could be attributed to its cytokinin like effect in promoting cell division and elongation at initial fruit development phase might reduce the intercellular space and thus increase the fruit density of apples (Greene, 2001) [12].

Papain wet yield was found to be higher in T_7- T_1 + BR and T_5- T_1 + MeJA treated fruits while the papain enzyme activity was found to be higher in T_5- T_1 + MeJA and T_6- T_1 + SA sprayed fruits. Although there are no reports on the effect of MeJA, BR and SA in improving the papain yield, the effect of these growth regulators in increasing the papain yield could be attributed to the increase in antioxidant levels and defense related compounds in the fruit.

Table 3. Effect of PGRs on morphological characters, PLW and shelf life of papaya fruit (var. CO 8)

Treatments	Firmness (Newton)	Chrome ‘b’ value	Rind thickness (mm)	Pulp thickness (cm)	No. of seeds/fruit	PLW (%)		Shelf life (days)
						3rd day	5th day	
T_1	16.3^g	53.2^a	1.21^g	2.17^g	190.00^g	7.16 (0.27)	9.26 0.31)	3.50^f
T_2	18.0^e	52.4^c	1.31^e	2.48^a	690.00^d	6.86 (0.27)	8.35 0.29)	5.00^d
T_3	18.3^d	51.8^e	1.35^d	2.31^e	717.00^c	6.35(0.25)	8.30 0.29)	5.81^b
T_4	17.9^f	52.6^b	1.27^f	2.21^f	374.00^f	7.00(0.27)	8.40 0.29)	4.01^e
T_5	19.0^b	51.9^d	1.39^b	2.26^d	581.00^e	5.23^a (0.23)	7.28 0.27)	6.00^a
T_6	18.8^c	51.2^f	1.38^c	2.36^c	750.00^b	6.05^b (0.25)	7.25 (0.27)	5.42^c
T_7	20.9^a	51.1^g	1.42^a	2.41^b	775.00^a	6.15^c (0.25)	7.67 (0.28)	6.00^a
Mean	18.46	52.04	1.33	2.31	582.44	6.40(0.26)	8.07(0.29)	5.11
S. Ed.	0.17**	0.61*	0.02**	0.03**	9.37**	0.005**	0.003**	0.06**
LSD (0.05)	0.35	1.27	0.04	0.06	19.68	0.010	0.007	0.13

*Significant difference among treatments is indicated by DMRT. PLW- Physiological Loss in Weight, Arc sine transformed value is given in parenthesis

Table 4: Effect of PGRs on fruit quality characters of papaya (var. CO 8)

Treatments	TSS (°Brix)	Total sugars (%)	Reducing sugars (%)	Titrable acidity (%)	Ascorbic acid (mg/100g)	β-carotene content (mg/100g)	Lycopene content (mg/100g)	Papain wet yield (g)	Papain Enzyme activity (TU Activity/mg)	Ca (mg/100g)	Mg (mg/100g)
T_1	13.04^{a}	10.95^{g}	9.60^{g}	0.125	44.99^{f}	2.65^{g}	2.16^{g}	2.21^{g}	262.33^{g}	21.34^{g}	10.40^{g}
T_2	13.10^{b}	11.52^{d}	10.26^{e}	0.129	45.18^{e}	2.85^{d}	2.25^{d}	2.43^{d}	288.27^{e}	22.71^{f}	11.20^{f}
T_3	12.42^{d}	12.24^{a}	10.28^{d}	0.128	45.31^{d}	3.17^{b}	2.29^{b}	2.39^{e}	289.79^{d}	34.73^{a}	12.21^{e}
T_4	11.98^{f}	11.20^{f}	9.85^{f}	0.118	45.81^{d}	2.68^{f}	2.19^{f}	2.31^{f}	275.55^{f}	22.71^{e}	13.60^{a}
T_5	11.84^{g}	11.36^{e}	10.37^{c}	0.125	46.08^{c}	2.92^{c}	2.25^{e}	2.52^{b}	299.16^{a}	30.78^{d}	13.11^{b}
T_6	12.26^{e}	11.54^{c}	10.46^{b}	0.126	47.88^{b}	2.83^{e}	2.27^{c}	2.46^{c}	298.24^{b}	31.34^{c}	11.88^{d}
T_7	13.12^{c}	11.78^{b}	10.61^{a}	0.128	48.02^{a}	3.28^{a}	2.36^{a}	2.56^{a}	297.70^{c}	32.22^{b}	12.80^{c}
Mean	12.54	11.51	10.20	0.13	46.18	2.91	2.25	2.41	287.29	27.98	12.17
S. Ed.	0.18**	0.14**	0.15**	NS	0.42**	0.04**	0.04**	0.03**	4.41**	0.41**	0.18**
LSD (0.05)	0.38	0.29	0.31	NS	0.87	0.09	0.08	0.06	9.18	0.84	0.38

*Significant difference among the treatments is indicated by DMRT

Conclusion

It might be concluded that pre-harvest application of T_7- T_1 + BR and T_2- T_1 + TIBA had a positive influence in increasing the growth attributes and yield characteristics, while the PRSV disease incidence could be considerably reduced with the application of T_5- T_1 + MeJA and T_6- T_1 + SA after two sprays. Also, the fruit quality characters were significantly improved with the application of T_7- T_1 + BR and T_3- T_1 + CPPU while application of T_7- T_1 + BR and T_5- T_1 + MeJA had higher papain yield, together contributing for improved shelf life. Hence, the application of PGRs has favorably influenced the metabolic activities possibly due to their increased endogenous level following external application. These may have enhanced the process of synthesis, translocation and accumulation of quality constituents like TSS, sugars, ascorbic acid, carotenoid content following strong source sink relationship and disease resistance.

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