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Effect of Plant Growth Regulators on Physico-Chemical Properties of Sapota (*Manilkara zapota* L.) cv. Kalipatti

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ABSTRACT: A field experiment was conducted at Horticultural Research Station, Odisha University of Agriculture and Technology, Bhubaneswar during 2020-2022. The experiment was carried out with ten treatments involving different concentrations of plant growth regulators *viz*., Brassinolide (0.5 ppm and 1.5 ppm), Salicylic acid (150 ppm and 300 ppm), NAA (150 ppm), and their combinations were embedded in Randomized Block Design with three replications. Effect of plant growth regulators has significant effect on physio-chemical properties of sapota in relation to Total Soluble Solid, titratable acidity, reducing sugar, total sugars and ascorbic content of fruits. Among all Brassinolide alone 1.5 ppm and in combination with NAA 150 ppm has visible effect in increasing physio-chemical properties of sapota fruit.

Keywords: Sapota, Physio-chemical, NAA, SA, Brassinolide.

INTRODUCTION

Sapota (*Manilkara zapota* L.) is commonly known as 'Chiku' in India. It is an evergreen tree and belongs to the family Sapotaceae and is native of tropical America especially the South Mexico or central America. The chromosome number of sapota is 2n=26. It is the sixth important commercial fruit crop of India after mango, banana, citrus, apple and guava. In India, it was first introduced at Gholwad village of Dhanu Taluka in Thane District of Maharashtra State in 1898 (Chadha, 1992). It is a crop of tropical region, needs warm and humid climate. It can be grown on a wide range of soils. The most ideal soils are deep alluvium, sandy loams, red laterites and medium black. It requires 125- 250 cm annual rainfall and a temperature of 11-38°C. Sapota is mainly grown in India, Philippines, Malaysia, Indonesia, Florida, Guatemala, Mexico and Sri Lanka. India is the largest producer of sapota in the world. It commonly grows in Indian states like Maharashtra, Gujarat, Andhra Pradesh, Karnataka, Tamil Nadu, West Bengal and Odisha. In India the area under sapota cultivation is 78 thousand hectares with 783 thousand metric tonnes production (NHB, 2021-22) In Odisha sapota is mostly cultivated in Balasore, Cuttack, Kendrapara, Jagatsinghpur, Puri, Khurda and Ganjam.

The area under this fruit crop is increasing due to its high productivity, liking to Indian palate, continuous fruiting throughout the year and very little incidence of weeds, disease and pests. Besides, this is quite hardy and can tolerate salinity and water stress to a very great extent. Sapota fruit is a fleshy berry, which bears in axis of the leaves on the new growth. The fully ripe fruit is delicious and sweet, chiefly used for fresh table purpose. The fruit is good source of digestible sugar (12 to 18 percent). Sapota fruits are the source of energy 98 Kcal, moisture 74 g, protein 1 g, fat 1 g, fibre 3 g, carbohydrate 21 g, calcium 28 mg, phosphorus 27 mg, Iron 1 mg. Some another mineral (mg/100 g) like potassium 26 mg, magnesium 25 mg, iron 1.25 mg, sodium 5.9 mg, copper 0.08 mg and vitamins *viz.* carotene 97 mg, thiamine 0.02 mg. Riboflavin 0.03 mg, Naicin 0.2 mg, vitamin 'C' 6.0 mg per 100 g of fruit (Shanmugavelu and Shrinivasan 1973). The fruit gives a characteristic pleasant flavour when it is blended with milk. Therefore, its powder and

fresh form can be used in many milk products like milk shake, ice cream and many Indian sweets. In India, "chiku halwa" prepared from sapota shreads is a famous Indian sweet. Fruits are also used for preparing alcohol and liquor because of its richness in sugars. It has preventive properties against febrile and bilibous diseases. Most of the sapota fruit in India is consumed domestically and a minor percent is exported.

Brassinosteroids (BS) constitute a new group plant hormones that has been given different designations such as "New Class of plant hormones" (Clouse and Sasse 1998; Khripach *et al*., 1999), hormones of the twenty-first century, poly hydroxylated steroidal plant hormone (Fariduddin *et al*., 2014). It regulates many processes in plant growth and development, including cell elongation, cell division, vascular differentiation, reproduction, photomorphogenesis, germination of seeds, rhizogenesis, flowering, fruit ripening, tolerance response to various biotic and abiotic stresses, and senescence (Manoli *et al.,* 2018). Among all, brassinolide (BL), 24-epibrassinolide (EBL) and 28-homobrassinolide (HBL) are the most important derived from different plant parts and actively used in the physiological process, which promote growth, increases yield and improves the quality of fruits. They increase percentage of fruit setting and also promote fruit enlargement. They act in extremely low concentrations. Many results also suggest that brassinosteroids increase resistance against unfavourable environmental factors, stress and diseases (Khripach *et al.,* 2000).

Salicylic acid (SA) 'natural plant defender' is a phenolic phytohormone found in plants and plays an important role in plant growth and development. It stimulates flowering, increases flower life, improving flowering number or density and fruit set percentages (Kazemi, 2013; Mohammadi *et al.,* 2015) antioxidant activity (Ananieva *et al.,* 2004). Exogenous application of SA may influence a range of diverse physiological processes from regulatory signal in plants mediating defense against pathogen in plants to ion uptake and transport, photosynthesis, inhibition of ethylene biosynthesis, stomatal conductance fruit yield and quality (Raskin,1992). It is used as a food additive in harvested fruits to delay ripening processes as well as enhancing the tolerance of fruits against pathogens, particularly at the early maturity stage. It is also involved in plant responses to abiotic stress conditions such as salt and osmotic stresses. Furthermore, salicylic acid was reported to reduce fruit weight loss and softening.

NAA (1-Naphthalene Acetic Acid) is synthetic plant hormone in the Auxin family. It seems to be most effective, among the various tested synthetic auxins, in terms of fruit setting and fruit retention (Kaur *et al*., 2018). It is known to stimulate cell division, cell elongation, elongation of shoot, photosynthesis, RNA synthesis membrane permeability and water uptake also involved in many physiological processes like prevention of pre harvest fruit drop, flower induction, fruit set, delayed senescence and prevention of bud sprouting, leaf chlorophyll content, and increased yield in fruit crops.

MATERIALS AND METHODS

Plant material: Sapota (*Manilkara zapota* L.)

Experimental site. The present investigation was carried out Horticultural Research Station, Odisha University of Agriculture and Technology, Bhubaneswar during 2020-2022. The experiment was carried out with ten treatments involving different concentrations of Plant Growth Regulators *viz.*, Brassinolide (0.5 ppm and 1.5 ppm), Salicylic acid (150ppm and 300ppm), NAA(150ppm), and their combinations were embedded in Randomized Block Design with three replications.

The treatments involved Brassinolide 0.5ppm, brassinolide 1.5 ppm, Brassinolide 0.5 ppm along with NAA 150 ppm, Brassinolide 1.5 ppm alongwith NAA150 ppm, Salicylic acid 150 ppm, Salicylic acid 300ppm, Salicylic acid 150 ppm along with NAA 150 ppm, Salicylic acid 300ppm alongwith NAA 150ppm, NAA150ppm, control (water spray) without any growth regulators. There were mainly 3 basic stages of application of growth regulators first during vegetative stage, then during flowering stage and lastly during fruiting stage (pea stage of fruiting). For this analysis ten harvested fruits per plants were taken for analysis.

Biochemical analysis

Total soluble solids (TSS). The total soluble solids content of ripened fruit was measured with the help of a hand refractometer (Hanna, USA) and values expressed as °Brix at room temperature.

Titratable acidity (%). Five grams of pulp sample was ground to paste using mortar and pestle using distilled water. The samples were diluted and volume was made up to 50 ml using distilled water. A small amount of sample 10 ml was taken in a conical flask and was titrated against 0.1 N NaOH using phenolphthalein indicator. The titration process was carried out till the appearance of pink colour. The percent Titratable acidity was obtained by method described by (Ranganna, 1977). This was expressed in terms of percentage citric acid using following formula.

$$\text{Acidity (\%)} \frac{\text{Titre value} \times \text{Normality of alkali} \times \text{volume made up} \times 64 \times 100}{\text{Volume of the sample taken} \times \text{weight of sample taken} \times 100}$$

Ascorbic acid. Ascorbic acid was estimated by volumetric method using 2,6-dichloro phenol indophenol dye according to the procedure suggested by Ranganna (1977) and expressed as mg/100 g pulp. To determine the ascorbic acid, 5 g fruit pulp was dissolved in 3 percent metaphosphoric acid and the volume was made up to 100 ml by adding distilled water. A five ml aliquot was titrated against standardized 2,6 dichlorophenolindophenol dye. The end point was marked by the appearance of a pink colour which persisted for at least a few seconds. The ascorbic acid

was expressed as mg of ascorbic acid per 100 g pulp of sample

$$\text{Ascorbic acid (mg/100g pulp)} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made up}}{\text{Aliquot of extract for estimation} \times \text{weight of sample}}$$

Sugars. Sugar was estimated by using Fehling 'A' and 'B' solutions by following Lane and Eynon Method as described by Ranganna (1977). Ten grams of fruit pulp was macerated with small amount of distilled water and filtered through a muslin cloth and the volume of the filtrate was made up to 100 ml by adding distilled water.

Reducing Sugars. Ten ml of filtered juice was taken in a 100 ml volumetric flask and the volume was made up to 100 ml by adding distilled water. The entire content was then transferred to a 100 ml burette. Then 5 ml of each Fehling's solution A and B were taken in a 250 ml conical flask together to which 40 ml of distilled water was added. The flask containing the solution was kept on an electric heater/gas flame for boiling. On the appearance of the first bubble 2- 3 drops of methylene blue indicator were added. The Fehling's solutions were then titrated against the fruit juice. The appearance of the brick red colour indicated the end point. The reducing sugar was calculated by following the Lane and Eynon method as described by Ranganna (1977)

$$\text{Reducing sugar (\%)} = \frac{0.05 \times \text{Volume}}{\text{Titre value} \times 10} \times 100$$

Total Sugar. Ten ml of juice was hydrolysed by adding 1N HCL and was transferred to a 250 ml volumetric flask followed by the addition of 30 ml distilled water to it. Then the entire content was heated for 4-5 min over a gas burner and was then cooled by placing it on the water bath. Then into a 250 ml conical flask, the entire content was transferred followed by the addition of 2-3 drops of phenolphthalein indicator. Then the entire solution was titrated against 1N NaOH solution taken in a burette. The appearance of light pink colour indicated the end point which signified the conversion of non-reducing sugars to reducing sugar present in the sample. Then the entire content was transferred to a burette and the same procedure was repeated as in case of estimation of reducing sugar. The total sugar was calculated by following Lane and Eynon Method as described by Ranganna (1977)

$$\text{Total sugar (\%)} = \frac{\text{Factor} \times \text{Dilution} \times 100}{\text{Titre value} \times \text{Volume of sample}}$$

Non-reducing sugar (%). The difference in percentage between total sugar and reducing sugar was taken for the estimation of non-reducing sugar.

Non reducing sugar % = (Total sugar – Reducing sugar) × 0.95

RESULTS AND DISCUSSION

The following results were obtained from the analysis

Table 1: Total Soluble Solids (TSS in °Brix).

TREATMENT	Mean (2020-21)	Mean (2021-22)	Mean (Pooled)
T_1- BR (0.5 ppm)	18.74	18.36	18.55
T_2- BR (1.5 ppm)	18.15	18.11	18.13
T_3- BR 0.5 ppm + NAA 150 ppm	20.02	19.20	19.61
T_4- BR 1.5 ppm + NAA 150 ppm	19.35	19.12	19.23
T_5- SA 150 ppm	17.05	17.13	17.09
T_6- SA 300 ppm	17.24	17.28	17.26
T_7- SA 150 ppm + NAA 150 ppm	18.11	18.14	18.13
T_8- SA 300 ppm +NAA 150 ppm	18.33	18.29	18.31
T_9- NAA 150 ppm	18.15	18.03	18.09
T_{10}- Control (water spray)	17.42	17.53	17.48
SEM	0.435	0.354	0.259
CD (0.05)	1.292	1.053	0.721

The data on TSS content of sapota fruit, as influenced by foliar sprays of plant growth regulators presented in the table: revealed that the maximum TSS contents was found in T_3 (BR 1.5 ppm along with NAA 150 ppm) i.e., 20.02 ^{0}Brix which was found at par with T_4 whereas minimum TSS was found in T_5 (17.05 °Brix). Similarly, in second year the TSS content ranged from 17.13 (T_5) to 19.20 °Brix (T_3).

On pooled data observation maximum TSS content was found in T_3(19.61) and minimum was found in T_5 (17.09). By the pooled analysis of data revealed that the combination of growth regulators had a significant effect on TSS content of fruits in both the year of experimentation. Different concentrations of brassinosteroid alone and in combination with NAA had significant effect on TSS over control during both the seasons. The findings of Shireen *et al*. (2018) ;Yan wang *et al.* (2019) are in conformation with the present investigation.

Table 2: Acidity (%).

TREATMENT	Mean (2020-21)	Mean (2021-22)	Mean (Pooled)
T_1- BR (0.5 ppm)	0.168	0.167	0.168
T_2- BR (1.5 ppm)	0.167	0.173	0.170
T_3- BR 0.5 ppm + NAA 150 ppm	0.168	0.168	0.168
T_4- BR 1.5 ppm + NAA 150 ppm	0.160	0.164	0.162
T_5- SA 150 ppm	0.162	0.170	0.166
T_6- SA 300 ppm	0.164	0.166	0.165
T_7- SA 150 ppm + NAA 150 ppm	0.165	0.167	0.166
T_8- SA 300 ppm +NAA 150 ppm	0.163	0.165	0.164
T_9- NAA 150 ppm	0.184	0.183	0.184
T_{10}- Control (water spray)	0.199	0.188	0.193
SEM	0.004	0.003	0.003
CD (0.05)	0.011	0.010	0.007

The titratable acidity content was significantly influenced by various treatments tested and their combinations. The data on titratable acidity content of sapota fruit at harvest (Table 2) showed the minimum acidity percentage was found in T_4 (0.160 and 0.164) in first and second year respectively. While maximum acidity was found in control (0.199 and 0.188) during first and second year respectively.

The pooled mean of two years data revealed that maximum acidity percentage was found in control (0.193) whereas minimum was found in case of T4 (0.162). Whereas, application of different concentration of salicylic acid and their combination with NAA had significantly reduced the titratable acidity value over control, a parallel finding was reported by Kanwaljit *et al.* (2017); Yan wang *et al.* (2019).

It is indicated from the (Table 3) that different treatments had pronounced effect on the reducing sugar during both the years, the fruits from untreated sapota trees (T_{10}) recorded minimum (9.04%) reducing sugars while brassinolide 1.5 ppm in combination with NAA 150 ppm (T_3) recorded maximum amount of reducing sugar (9.78) which was found at par with treatments T_2 and T_4. A Similar trend was noticed in second year where maximum was found in T_3 (9.31) and minimum was in case of control (8.81).

On perusal of pooled data observation maximum reducing sugar content was found in T_3 (9.55) and minimum was found in control (8.92). Different concentrations of brassinosteroid alone and in combination with NAA had significant effect on TSS over control during both the seasons.

Table 3: Reducing Sugar.

TREATMENT	Mean (2020-21)	Mean (2021-22)	Mean (Pooled)
T_1- BR (0.5 ppm)	9.51	8.98	9.25
T_2- BR (1.5 ppm)	9.62	9.04	9.33
T_3- BR 0.5 ppm + NAA 150 ppm	9.78	9.31	9.55
T_4- BR 1.5 ppm + NAA 150 ppm	9.69	9.14	9.42
T_5- SA 150 ppm	9.20	8.83	9.02
T_6- SA 300 ppm	9.26	9.18	9.22
T_7- SA 150 ppm + NAA 150 ppm	9.39	9.08	9.23
T_8- SA 300 ppm +NAA 150 ppm	9.43	9.22	9.32
T_9- NAA 150 ppm	9.29	9.10	9.19
T_{10}- Control (water spray)	9.04	8.81	8.92
SEM	0.103	0.170	0.099
CD (0.05)	0.305	0.506	0.277

Table 4: Total Sugar.

TREATMENT	Mean (2020-21)	Mean (2021-22)	Mean (Pooled)
T_1- BR (0.5 ppm)	15.85	15.91	15.88
T_2- BR (1.5 ppm)	15.90	15.83	15.86
T_3- BR 0.5 ppm + NAA 150 ppm	16.30	16.22	16.26
T_4- BR 1.5 ppm + NAA 150 ppm	16.15	16.17	16.16
T_5- SA 150 ppm	15.34	15.20	15.27
T_6- SA 300 ppm	15.44	15.37	15.40
T_7- SA 150 ppm + NAA 150 ppm	15.65	15.57	15.61
T_8- SA 300 ppm +NAA 150 ppm	15.72	15.74	15.73
T_9- NAA 150 ppm	15.48	15.68	15.58
T_{10}- Control (water spray)	15.43	15.61	15.52
SEM	0.137	0.170	0.101
CD (0.05)	0.406	0.506	0.282

The total sugar content was significantly influenced by various treatments tested and their combinations. Total sugar content of sapota fruit at harvest (Table 4) showed the minimum total sugar percentage was found in control (15.34% and 15.21%) in first and second year respectively. While maximum total sugar content was found in T_3 (16.30% and 16.22%) during first and second year respectively.

The pooled mean of two years data revealed that minimum total sugar percentage was found in control (15.27) whereas maximum was found in case of T_3 (16.26) which was found at par with T_4 (16.16). Whereas, application of different concentration of salicylic acid and their combination with NAA had significantly increase the total sugar percentage over control.

Table 5: Ascorbic Acid.

TREATMENT	Mean (2020-21)	Mean (2021-22)	Mean (Pooled)
T_1- BR (0.5 ppm)	11.05	10.94	10.99
T_2- BR (1.5 ppm)	11.62	11.11	11.37
T_3- BR 0.5 ppm + NAA 150 ppm	11.34	11.06	11.20
T_4- BR 1.5 ppm + NAA 150 ppm	11.72	11.39	11.56
T_5- SA 150 ppm	8.82	9.03	8.93
T_6- SA 300 ppm	9.66	9.51	9.59
T_7- SA 150 ppm + NAA 150 ppm	10.01	9.80	9.91
T_8- SA 300 ppm +NAA 150 ppm	10.64	10.57	10.61
T_9- NAA 150 ppm	9.40	9.57	9.48
T_{10}- Control (water spray)	8.55	8.57	8.56
SEM	0.507	0.328	0.274
CD (0.05)	1.505	0.976	0.765

Data presented in the (Table 5) indicated that significant variation was observed among the treatments on ascorbic acid contents of the fruits. The maximum (11.72) ascorbic acid content was found in T_4 which were at par with T_2 and T_3. Whereas minimum (8.55) ascorbic acid content was observed in control (T_{10}). During second year application of BR 1.5 ppm along with NAA 150 ppm (T_4) significantly showed a maximum (11.39) ascorbic acid content whereas minimum (8.57) was found in control (T_{10}).

From the pooled mean of two years data, it was observed that maximum (11.56) ascorbic acid content was found in T_4 which was at par with T_2 and T_3. Similarly minimum (8.56) was recorded in control (T_{10}). Similar findings have given by Laila *et al*. (2018); Harindra *et al*. (2015).

CONCLUSIONS

Application of plant growth regulators has significantly promoted the physio-chemical properties of sapota in relation to total soluble solid (TSS), Acidity, Total sugars, reducing sugars and ascorbic content of fruits. Brassinolide alone 1.5 ppm and in combination with NAA 150 ppm has visible effect in increasing physio-chemical properties of sapota fruit. As the application of brassinolide stimulate the source sink relationship via improving the mobilization and accumulation pf assimilates to the growing fruits, which ultimately improves the physiochemical properties of fruits.

FUTURE SCOPE

There is need to be conduct in depth studies about the crosstalk of BR with other new generation plant growth hormones are need to be studied. Moreover, a better understanding of BR biosynthetic pathways and molecular level characterization will facilitate metabolic engineering of BRs for targeted applications.

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Conflict of Interest. None.

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