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RESEARCH ARTICLE

Influence of plant growth regulators on qualitative attributes of acid lime Kuliana local in Odisha

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Abstract

Kuliana lime is an outstanding Odisha cultivar with significant potential to be included in future crop improvement operations due to its flavor and juice content. This research was conducted at the Horticultural Research Station, Department of Fruit Science and Horticultural Technology, Orissa University of Agriculture and Technology, Bhubaneswar during 2019–2020 and 2020–2021 to determine the optimal growth regulator in terms of quality metrics. certain growth regulators 2,4-D (2,4-D @ 10 ppm and 20 ppm) and GA_3 (GA_3 @ 50 ppm and 100 ppm) and NAA (NAA @ 100 ppm and 200 ppm) and SA (SA @ 100 ppm and 200 ppm) and SPMD (SPMD @ 0.01 ppm and 0.5 ppm) and PUT (PUT @ 0.01 ppm and 0.5 ppm) and BR (BR @ 0.1 ppm and 0.5 ppm) quality of Acid Lime kuliana local. The biometrical observations on various quality features were recorded in the same seasons over 2 years. The best was for fruit quality, which has been determined on the basis of growth regulators performances, as growth regulator variation has a significant effect on the quality of fruit. Brassinosteroids showed the best effects for TSS (7.17 °Brix), acidity (6.05 %), total sugar content (0.61 %), reducing sugar content (0.41 %), ascorbic acid (36.35 mg per 100 mL of juice), chlorophyll content (33.03 mg/100 g), flavonoid content (119.91 mg/100 g), protein (0.82 mg/100 g) and carbohydrate content (10.29 mg/100g) with the other treatments, including the untreated fruits in both years of the study. Our results revealed that the BR gave superior results in quality attributes for Kuliana lime in the East and Southeast Coastal Plain Zones of Odisha.

Keywords

brassinosteroids; lime; acidity; total sugar content; chlorophyll content

Introduction

Acid lime (*C. aurantifolia* swingle) belongs to the family Rutaceae. It originated in India and then expanded to the Middle East and other tropical and subtropical regions. The Mayurbhanj area of Odisha primarily grows Kuliana lime, a local elite renowned for its size and juice content. The traditional cultivation of Kuliana lime takes place in the village Kuliana, where the name originates, and it spans approximately 500 ha in the district, with widespread cultivation on both sides of the Budhabalanga river (1).

Numerous varieties of acid lime are developed in India, with variability in flavour and taste. Kuliana lime is a local elite landrace of the Mayurbhanj district of Odisha, where it is extensively grown as the hot summer and cold winter climate of the area is extremely conducive to its development.

Plant growth regulators are used in citrus production around the globe. They are used in orchards to promote or prevent vegetative growth, manipulate flowering, modify fruit set and fruit growth, improve internal fruit quality like TSS, juice, acidity and exterior rind quality traits such as firmness and colour etc.

In order to increase the production of high-quality fruits, the use of plant growth regulators has become a crucial component of modern agricultural practices. Plant growth regulators fall into one of 2 categories according to how long they have been a major part of the horticultural industry.

There are some plant growth regulators that are harnessed and used widely by farmers. These include Auxins, GA, cytokinins, ABA and ethylene. A part of these are plant growth regulators whose efficacy and efficiency are known to us, but due to a technological gap, they have not yet been harnessed at the grass root level. These include brassinosteroids, salicylic acid, polyamines and others.

Auxins increase the plasticity of plant cell walls and are involved in stem elongation. 2,4-D is used as an herbicide. It governs the differentiation of xylem and promotes cell division. NAA (Napthalene Acetic Acid) prevents fruit drop and promotes flowering and fruiting. GA_3 (Gibberellic acid) promotes cell division and elongation, shoot growth and is involved in regulating dormancy. It has been used to manipulate flowering and fruit development. SA is synthesized from phenylalanine. It is found in plants and plays roles in plant growth and development, photosynthesis, transpiration, ion uptake and transport. Polyamines are low molecular weight polycations found in all organisms (2). It is a new class of growth regulators that acts as a secondary hormonal messenger (3). Brassinosteroids (BRs) have been identified in many plant species since the discovery of brassinolide (4), a polyhydroxy-steroidal lactone, initially isolated from *Brassica napus* pollen (5). It is considered the 6th group of plant hormones (6). It is also suggested that BRs are natural, non-toxic, biosafe and economically viable phytohormone (7). Brassinosteroids are a class of plant polyhydroxy steroids that are recognized as new kinds of phytohormones that play critical roles in cell division, elongation, vascular differentiation, flowering, pollen growth and photo-morphogenesis.

Fruit contains 51–53 % juice, 2.91–3 % TSS, 2.7 % titratable acidity and 28.97 mg/100 mL ascorbic acid. Acid lime juice is acidic in nature and rich in citric acid, malic acid and succinic acid concentrations. Vitamins C and B are the key vitamins present in the fruit. The flavedo of fruit contains pigments like chlorophyll and carotenoids. Albedo is rich in the content of cellulose, hemicelluloses, lignin and pectin. Flavedo also includes oil glands, from which essential oils are extracted for use in the cosmetics and perfume industries. The principal volatile chemicals present in the peel oil of fruit are limonene, citronellol, neral, γ-terpinene and β-pinene. Sugars, lipids, nitrogenous substances and phenols are present in lime juice. Flavonoids that are of dietary importance include flavones, flavanols and flavanones (8, 9).

Previous studies have explored the positive impacts of plant growth regulators on the quality characteristics of various fruits, but there has been limited research on lime. Because of this reason and attention to the risk of improper use of chemicals in pre- and post-harvest technology and consumers's demand for healthy products, this study was conducted to improve the quality of lime cv. Kuliana by spraying trees with different concentrations of plant growth regulators in the hot and humid agroclimatic zone of Odisha.

Materials and Methods

This section should be written well defined to understand the steps of the investigation carried out, allowing other researchers to reproduce the result. It can be listed in one heading or it can be listed in separate headings depending on the requirement and need of the author(s).

Plant material

The study was carried out on a 4-year-old air-layered acid lime cultivar, Kuliana lime, grown in 4 m×4 m spacings at the Horticultural Research Station, Department of Fruit and Horticultural Technology, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha for 7 growth regulators with control from 2019–20 to May 2020–21. The experimental site lies under the eighteenth agro-climatic area of the country, i.e., "Eastern Coastal. 6 years old lime cv. kuliana were chosen and sprayed at sun set to runoff with 2,4-D (2,4- D @ 10,20 ppm), GA_3 (GA_3 @ 50,100 ppm), NAA (NAA @ 100, 200 ppm), SA (SA @ 100, 200 ppm), PUT (PUT @ 0.01, 0.1 ppm), SPMD (SPMD @ 0.01, 0.1 ppm) and BR (BR @ 0.1, 0.5 ppm) in 3 different periods: the 1st was a month of September (pre-flowering), the 2nd spray was February (at the time of flowering) and the 3rd spray was March (fruit set stage). The present study was carried out in "Pooled RBD ANOVA software" with 2 years as treatments, each replicated twice in the same season. The juice from the fruit was extracted with a lemon press and measured by the measuring cylinder. The % of juice content was computed in relation to the volume of fruit. The total soluble solid was measured with the use of an Erma Hand Refractometer (0–30 °Brix) and expressed in °Brix. Titrable acidity, the amount of total sugar, reducing sugar in % and ascorbic acid content in mg/100 g of fruit juices were determined. Chlorophyll concentration, flavonoid content, CHO and protein were determined using spectrophotometry.

Juice content (ml)

The juice from the fruit was extracted by a lemon squeezer. The % of juice content was calculated in relation to the volume of fruit.

Total Soluble Soilds (oBrix)

The total soluble solids were estimated by using a hand refractometer (0-30° Brix). A drop of filtered juice was placed on the refractometer and the Brix (reading) was measured facing the light source. The data recorded was expressed as degrees Brix.

Acidity (%)

For measuring acidity 10 mL of acid lime juice was taken in a 100 mL conical flask and the volume was made up to 100 mL by adding 90 mL of distilled water, from the 100 mL of diluted acid lime juice, 10 mL was taken for titration. The content was titrated against 0.1 N NaOH after adding 1-2 drops of phenolphthalein indicator. The sample was titrated untill the colour of the sample changed to a light pink colour. The titration was repeated 3 times for confirmation of the result.

Titre value x Normality of NaOH x volume makeup x 64 x 100

% Total acidity = Volume of titrate x weight of sample x 1000

Ascorbic acid (mg/100 mL of juice)

For the estimation of ascorbic acid, about 10 mL of extracted fruit juice was taken and the volume was made up to 100 mL with 3 % meta-phosphoric acid. 10 mL of this aliquot solution was taken and titrated with standard dye (2,6 – Dichlorophenol indophenol) till a pink end point was reached. The ascorbic acid content was estimated using the given formula and expressed as mg per 100 g of juice.

Titre value x Dye factor x volume formed x 100

Ascorbic acid = Aliquot taken x weight of sample.

Chlorophyll content (mg per g of FW)

The chlorophyll content of the leaf sample was quantified with acetone method.

Amount of chlorophyll-a, chlorophyll-b and total chlorophyll content in the extract was calculated in mg chlorophyll per g tissue by using following equations:

Chlorophyll A (mg g^{-1} of FW) = 12.7 x OD $_{663}$-2.69 X OD $_{645}$) x V (ml) / 1000 x W (g)

Chlorophyll B (mg g^{-1} of FW) = 22.9 x OD $_{645}$-4.68 X OD $_{663}$) x V (ml) / 1000 x W (g)

The O.D. values of the extracts were recorded in Spectronic-20 at 645 and 663 nm wavelengths against a Dimethyl Sulphoxide taken as blank. The total chlorophyll content was calculated by using the following formula:

Total chlorophyll content = 20.2 A645 + 8.02 A663/ A x 1000 x W x V

Where, V = Volume of the extract made, A = Length of the light path in cell (usually 1cm), W = Leaf fresh weigh (g), A 645 = Absorbance at 645 nm, A 663 = Absorbance at 663 nm

Flavonoid content (mg/100 g)

The total flavonoid content of the methanolic extracts was determined photometrically using an aluminium chloride ($AlCl_3$) assay. The diluted 0.5 mL of sample was mixed with 0.075 mL of 5 % $NaNO_2$ after 6 min and 0.15 % of 10 % $Alcl_3$ and allowed to stand for 5 min. Then, add 0.5 mL of NaOH and make up the volume to 2.5 mL with distilled water. Take the O.D value at 510 mL and calculate it using a standard graph. Catechol was used as a standard. 100 mg of catechol was dissolved in 100 mL of water, then diluted 10 times for a working standard and prepared 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL were used as standards. The total flavonoid content was expressed as the catechol equivalent in mg/100 g of dry extract weight.

Determination of Total Carbohydrates

A juice sample (100 mL) was taken in a test tube and hydrolyzed by keeping it in boilingwater bath with 5 mL of 2.5 N-HCl for 3 h and cooled to room temperature. The samples were neutralized by adding solid sodium carbonate until effervescence ceased. The volume was made up to 100 mL and centrifuged. The supernatant was collected and a 1mL aliquot was taken for analysis. Glucose was used as a standard. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard and '0' serves as blank. Volume was made up to 1 mL in all the tubes, including the samples, by adding distilled water. Then add 4 mL of anthrone reagent. A boiling water bath was used for heating for 8 min. Cool rapidly and read green to dark green at 630 nm. Draw a standard graph by plotting the concentration of the standard on the X-axis versus the absorbance on the Y-axis. From the graph, calculate the amount of carbohydrate present in the sample

Amount of carbohydrate present in 100 mg of the sample =

mg of glucose

tube.

Protein

The soluble protein was estimated using the Lowry method. The enzyme extract was precipitated by 20 % trichloro acetic acid, centrifuged and the residue dissolved in a 0.01 N sodium hydroxide (NaoH) solution.

The amount of protein was calculated by using a standard graph prepared from bovine serum albumin (200 µg mL^{-1}).

Data analysis

The experiment was conducted entirely according to the randomized block trial design. SAS and MSTAT-C were the computer programs used in all statistical analyses. An ANOVA (one-way analysis of variance) was used to analyze the data. The mean separation was performed using the least significant difference (LSD). Significant differences were indicated at $P < 0.05$.

Results and discussion

Juice content (mL)

Juice is an incredibly important characteristic for its industrial processing, being also related to size, which in turn, although governed by the genetic traits of each cultivar, is modified by cultural techniques such as the administration of plant growth regulators. The juice content of the Acid Lime cv. Kuliana fruit was significantly impacted by several growth regulators.

It was found from pooling Table 1 that growth regulator use raised the juice weight as compared to control. Due to the application of growth regulators, the largest quantity of juice content (13.52 mL) was determined in the treatment T_{14} (0.5 ppm BR), which was followed by T_{13} (13.24 mL) and the lowest amount of juice content (11.28 mL) was examined in the treatment T_{15} (control) in the investigated year 2019–21. It may be believed that improving water balance in fruits might boost juice content. Similar findings were reported in Washington navel orange (10).

Table 1. Effect of growth regulators on juice content, TSS and acidity of Acid lime cv. Kuliana local.

Treatments	Juice content (mL)			TSS (Brix)			Acidity (%)		
	Season 1	Season 2	Pooled	Season 1	Season 2	Pooled	Season 1	Season 2	Pooled
2,4-D @ 10 ppm	11.63	11.82	11.73	7.08	7.12	7.10	5.74	5.71	5.73
2.4-D @ 20 ppm	11.74	11.93	11.84	6.86	7.07	6.96	5.78	5.68	5.73
GA_3 @ 50 ppm	12.11	12.31	12.21	6.74	7.05	6.90	5.96	5.94	5.95
GA_3 @ 100 ppm	12.32	12.56	12.44	6.62	6.89	6.76	6.08	6.01	6.05
NAA @100 ppm	11.89	12.04	11.96	6.57	6.77	6.67	5.87	5.78	5.83
NAA @ 200 ppm	12.05	12.15	12.10	6.53	6.64	6.59	5.81	5.75	5.78
SA @ 100 ppm	12.46	12.68	12.57	6.41	6.47	6.44	6.16	6.11	6.13
SA @ 200 ppm	12.53	12.77	12.65	6.38	6.42	6.40	6.26	6.17	6.22
SPMD @ 0.01 mM	12.78	12.85	12.82	6.49	6.49	6.53	6.37	6.25	6.31
SPMD @ 0.1 mM	12.87	12.95	12.91	6.42	6.53	6.48	6.49	6.36	6.43
PUT @ 0.01 mM	13.00	13.04	13.02	6.34	6.38	6.36	6.53	6.49	6.51
PUT @ 0. 1 mM	13.05	13.15	13.10	6.28	6.31	6.29	6.61	6.55	6.58
BR @ 0.1 mM	13.11	13.36	13.24	6.24	6.17	6.23	6.68	6.66	6.67
BR @ 0.5 mM	13.15	13.89	13.52	6.01	6.07	6.05	6.88	6.79	6.83
Control	10.97	11.58	11.28	7.15	7.19	7.17	5.63	5.68	5.65
Mean	12.37	12.60	12.49	6.61	6.71	6.66	6.19	6.20	6.19
CD @ 5 %	0.083	0.071	0.07	-	-	-	0.108	0.22	0.16
SE (m)±	0.028	0.024	0.02	0.026	0.02	0.02	0.037	0.07	0.06

Note: **SPMD** -Spermidine, **PUT**-Putrescine, **BR**- Brassinosteroid.

TSS (Brix)

During the years 2019–21, there was no significant variation among the fruit TSS in acid lime cv. Kuliana in response to different degrees of plant growth regulator treatments. From the pooled data Table 1, it was suggested that the minimum fruit TSS (6.05 °Brix) was found in treatment T_{14} (0.5 ppm BR), which was followed by treatment T_{13} (0.1 ppm BR), i.e., 6.11 °Brix and the highest fruit TSS (7.17 °Brix) was obtained in treatment T_{15} (control).

Acidity (%)

Table 1 displays the results related to the acidity content of acid lime fruits as impacted by various concentrations of growth regulators applied at different levels. In both the years 2019 and 2021, the treatment T14 (0.5 ppm BR) had the highest acidity content (6.83 %), followed by T13 (0.1 ppm BR) (6.67 %) and the treatment T15 (control) had the lowest acidity (5.65 %). Brassinosteroid-induced cell development may increase osmotic pressure, leading to an accumulation of organic acids, potentially causing the increase in acidity.

Total sugars (%)

Pooled Table 2 demonstrated the changes in total sugar content of fruits. The total sugar rose with the rise in concentration. The statistical analysis of the data indicates that acid lime cv. Kuliana, which received T14 (0.5 ppm BR) foliar application of plant growth regulators, showed the highest total sugars (0.61 %), statistically on par with T13 (0.1 ppm BR) (0.60 %). On the other hand, T15 (control) yielded the lowest total sugar (0.43 %) in both years.

It might be due to the foliar application of brassinosteroids in lime plants, which can potentially impact the total sugar content of the fruits. Brassinosteroids have been observed to stimulate photosynthesis and the activity of enzymes involved in sugar metabolism. As a result, this can lead to an increased accumulation of sugars like glucose, fructose and sucrose in lime fruits. Similar results were reported earlier indicating that brassinosteroid increased the sugar content of oranges (11).

Reducing sugar content (%)

Table 2. Effect of growth regulators on Total sugars, Reducing sugars and Ascorbic acid of Acid lime cv. Kuliana local.

Treatments	Total sugars (%)			Reducing sugars (%)			Ascorbic acid (mg/100 g)		
	Season 1	Season 2	Pooled	Season 1	Season 2	Pooled	Season 1	Season 2	Pooled
2,4-D @ 10 ppm	0.45	0.45	0.45	0.25	0.27	0.26	31.23	32.51	31.87
2.4-D @ 20 ppm	0.47	0.46	0.47	0.26	0.28	0.27	31.53	32.74	32.14
GA_3 @ 50 ppm	0.49	0.48	0.49	0.29	0.30	0.30	32.46	33.23	32.85
GA_3 @ 100 ppm	0.50	0.49	0.49	0.30	0.31	0.31	32.74	33.42	33.08
NAA @100 ppm	0.47	0.47	0.47	0.27	0.28	0.28	31.66	33.05	32.36
NAA @ 200 ppm	0.49	0.47	0.48	0.29	0.29	0.29	31.92	33.11	32.52
SA @ 100 ppm	0.51	0.49	0.50	0.31	0.34	0.33	32.89	33.71	33.30
SA @ 200 ppm	0.50	0.50	0.50	0.32	0.35	0.34	33.06	34.45	33.75
SPMD @ 0.01 mM	0.53	0.51	0.52	0.33	0.36	0.35	33.24	34.86	34.05
SPMD @ 0.1 mM	0.54	0.55	0.54	0.35	0.38	0.37	33.69	35.37	34.53
PUT @ 0.01 mM	0.57	0.57	0.57	0.38	0.36	0.37	34.38	35.85	35.12
PUT @ 0. 1 mM	0.59	0.59	0.59	0.38	0.39	0.38	34.71	36.31	35.51
BR @ 0.1 mM	0.60	0.60	0.60	0.39	0.41	0.40	35.64	36.64	36.14
BR @ 0.5 mM	0.60	0.61	0.61	0.39	0.43	0.41	35.82	36.88	36.35
Control	0.42	0.44	0.43	0.24	0.23	0.24	30.71	31.57	31.14
Mean	0.51	0.52	0.55	0.31	0.33	0.32	33.04	34.25	33.64
CD @ 5%	0.020	0.022	0.023	0.007	0.021	0.015	0.058	0.051	0.05
SE (m)±	0.007	0.007	0.007	0.002	0.007	0.005	0.02	0.017	0.02

Note: **SPMD** -Spermidine, **PUT**-Putrescine, **BR**- Brassinosteroid.

The outcomes of various amounts of growth regulators with varying concentrations on lowering the sugar content of fruit have been provided in pooled Table 2. The maximum reducing sugar % (0.41 %) was recorded for T_{14} (0.5 ppm BR), which was on par with T_{13} (0.1 ppm BR) (0.40 %) and the lowest reducing sugar % (0.24 %) was reported for the treatment T_{15} (control) in both the years 2019–20 and 2020–21. It might be due to the fact that Brassinosteroid can stimulate the activity of enzymes involved in sugar metabolism pathways, leading to increased production and accumulation of reducing sugars in fruits. This enhancement in reducing sugar content can contribute to improved fruit taste, sweetness and overall quality.

Ascorbic acid (mg/100 g)

Vitamin C is a potent antioxidant and is an important part of the human diet. It helps save the person from many dangerous diseases and scavenges the reactive oxygen species (ROS) produced in the body. Vitamin-C (ascorbic acid) contents in fruits vary in concentration for various citrus species; vitamin-C is affected by environmental conditions, time of fruit harvesting, plant vigour, age of the plant and the application of growth regulators. So, vitamin C was investigated as a quality parameter for the plants treated with various growth regulators. The ascorbic acid concentration in the fruit was considerably impacted by different levels of growth regulators. From the pooled data in Table 2, it is noticed that treatments were found to be statistically significant for ascorbic acid content in fruit. The maximal ascorbic acid concentration (36.35 mg/100 g) was estimated in treatment T_{14} (0.5 ppm BR), which was followed by treatment T_{13} (0.1 ppm BR) (36.14 mg/100 g). The lowest value of ascorbic acid (31.14 mg/100 g of pulp) was estimated in treatment T_{15} (control). Similar results were found in Washington navel orange (10, 14).

Chlorophyll content (mg/100 g)

The chlorophyll concentration in the acid lime CV. Kuliana fruit was considerably impacted by several growth regulators used in the study. It was found from the pooled Table 3 that growth regulator application raised the fruit chlorophyll concentration as compared to control. Due to the application of growth regulators, the maximum quantity of chlorophyll content (33.03 mg/100 g) was estimated in treatment T_{14} (0.5 ppm BR), which was followed by treatment T_{13} (0.1 ppm BR) (32.78 mg/100 g) and the lowest amount of chlorophyll content (27.54 mg/100 g) was estimated in treatment T_{15} (control) in the studied years 2019–21. BR's increased transcription and translocation processes of the enzymes that are involved in chlorophyll synthesis in connection with a lower level of catabolozing enzymes might be the explanation for the higher chlorophyll content. BRs increased transcription and translation processes of the enzymes that are involved in chlorophyll synthesis in connection with a reduced number of catabolizing enzymes might be a key explanation for enhanced chlorophyll content. In support of this observation, an increase in chlorophyll content caused by Homobrassinosteroid spray was also observed in tomato, green gram and chickpea crops respectively (1, 12, 13).

Table 3. Effect of growth regulators on chlorophyll content, Flavonoid content and Protein content of Acid lime cv. Kuliana local.

Treatments	Chlorophyll content (mg/100 g)			Flavonoid content (mg/100 g)			Protein content (%)			Carbohydrate content (mg/100 g)		
	Sea son 1	Sea son2	Pooled	Sea son 1	Sea son 2	Pooled	Sea son 1	Sea son 2	Pooled	Sea son 1	Sea son 2	Pooled
2,4-D @ 10 ppm	29.52	29.63	29.57	112.21	113.72	112.97	0.56	0.58	0.57	8.18	8.40	8.29
2.4-D @ 20 ppm	29.88	29.96	29.91	112.64	114.85	113.75	0.59	0.57	0.58	8.35	8.56	8.46
GA_3 @ 50 ppm	30.71	30.84	30.77	114.38	116.75	115.57	0.69	0.69	0.69	8.59	8.76	8.68
GA_3 @ 100 ppm	30.86	30.97	30.92	114.74	116.93	115.84	0.72	0.70	0.71	8.61	8.81	8.71
NAA @100 ppm	30.41	30.61	30.51	113.57	115.25	114.41	0.63	0.61	0.62	8.47	8.59	8.53
NAA @ 200 ppm	30.52	30.72	30.62	113.75	115.73	114.74	0.66	0.69	0.67	8.53	8.65	8.59
SA @ 100 ppm	31.45	31.65	31.55	115.63	117.73	116.68	0.71	0.71	0.71	8.72	8.82	8.77
SA @ 200 ppm	31.62	31.77	31.69	115.85	118.64	117.25	0.75	0.75	0.75	8.80	8.88	8.84
SPMD @ 0.01 mM	31.74	31.85	31.79	116.59	118.85	117.72	0.76	0.77	0.76	9.13	9.12	9.13
SPMD @ 0.1 mM	31.87	32.43	32.15	116.89	119.53	118.19	0.77	0.78	0.78	9.32	9.47	9.40
PUT @ 0.01 mM	32.00	32.62	32.31	117.43	120.73	119.08	0.80	0.79	0.80	9.57	9.59	9.58
PUT @ 0. 1 mM	32.14	32.86	32.50	117.62	120.85	119.24	0.82	0.82	0.82	9.67	9.73	9.70
BR @ 0.1 mM	32.25	33.31	32.78	117.84	121.73	119.78	0.81	0.83	0.82	9.74	9.86	9.80
BR @ 0.5 mM	32.43	33.62	33.03	117.93	121.88	119.91	0.83	0.84	0.83	10.17	10.40	10.29
Control	27.06	28.02	27.54	111.75	112.72	112.24	0.55	0.51	0.53	8.04	8.05	8.05
Mean	30.96	31.39	31.18	115.25	117.72	116.49	0.71	0.70	0.70	8.92	9.04	8.99
CD @ 5 %	0.054	0.068	0.06	0.064	0.064	0.06	0.03	0.04	0.04	0.115	0.142	0.169
SE (m) ±	0.018	0.023	0.02	0.022	0.022	0.02	0.01	0.01	0.01	0.039	0.049	0.044

Note: **SPMD** -Spermidine, **PUT**-Putrescine, **BR**- Brassinosteroid.

Flavonoid content (mg/100 g)

Flavonoids are important compounds contributing to fruit quality. Brassinosteroid can increase flavonoid content in fruit crops by stimulating the expression of genes involved in flavonoid biosynthesis. This augmentation in flavonoid content might enhance fruit colour, taste and nutritional value. The varying degrees of growth regulators significantly affected the flavonoid concentration in the fruit. From the pooled data in Table 3, it is seen that treatments were found to be statistically significant for flavonoid content in fruit. The highest flavonoid concentration (119.91 mg/100 g) was obtained in treatment T_{14} (0.5 ppm BR), which was followed by treatment T_{13} (0.1 ppm BR) (119.78 mg/100 g). The lowest amount of flavonoid content (112.24 mg/100 g) was estimated in treatment T_{15} (control). In support of this observation, an increase in flavonoid content caused by Homobrassinosteroid spray was also observed in Opuntia (14).

Protein content (mg/100 g)

Protein content in the fruit was considerably impacted by varying levels of growth regulators. From the pooled data in Table 3, it is noticed that treatments were found to be statistically significant for protein content in fruit. The highest protein content (0.83 mg/g) was measured in the treatment T_{14} (0.5 ppm BR), which was on par with the treatments T_{13} (0.1 ppm BR) (0.82 mg/g) and T_{12} (Put @ 0.1 mM). The low value of protein content (0.53 mg/g) was estimated in treatment T_{15} (control). Similar results were found in opuntia, which increased protein content with the application of Brassinosteroid (14).

Carbohydrate (mg/100 g)

The carbohydrate content of the fruit was significantly influenced by different levels of growth regulators. From the pooled data in Table 3, it is noticed that treatments were found to be statistically significant for carbohydrate content in fruit. The maximum carbohydrate content (10.28) was estimated in treatment T_{14} (0.5 ppm BR), followed by treatments T_{13} (0.1 ppm) (9.80). The minimum value of carbohydrate content (8.04) was estimated in treatment T_{15} (control). It might be due to the application of brassinosteroids in lime plants, which can potentially enhance carbohydrate accumulation in fruits. Brassinosteroids have been observed to stimulate photosynthesis and the activity of enzymes involved in carbohydrate metabolism. Consequently, this can lead to increased levels of sugars such as glucose, fructose and sucrose in lime fruits.

Conclusion

Based on the study, it can be concluded that Plant growth regulators have become powerful tools to modify several physiological process in plants which are extensively and profitably used in horticultural crops. Among the growth regulators, Brassinosteroid @ 0.5 ppm showed best results interms of highest percent of juice content, TSS, total sugars, reducing sugars, ascorbic acid, chlorophyll content, flavonoid content, protein content and acidity.

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Authors' contributions

All authors contribute equally to performing research, data analysis, and manuscript preparation.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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