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Original Article

Comparative study of the doses of cytokinin in the quality of caigua (*Cyclanthera pedata* L.) in Peru

Estudo comparativo das doses de citocinina na qualidade do caigua (*Cyclanthera pedata* L.) no Peru

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Abstract

Bioavailability of nutrients, the scarcity of synthetic fertilisers, and the rising cost of fuel have all contributed to an increase in production costs, which has in turn reduced crop productivity and led scientists to seek out new methods to ensure high-quality output. In this context, various cytokinins dosages were tested in Peru to see whether they affected the quality of caigua, in an effort to address these issues. To mitigate these problems, a pot experiment was carried out to check the effects of various doses of cytokinin in the quality of caigua in Peru. The experiment consisted of 5 treatments including (0, 50, 100, 150 and 200 mL of cytokinin) by using (Anthesis Plus per 200 L of water) as a source, each with three replicates and placed following a randomized complete block design (RCBD). Treatment with 100 mL of cytokinins foliar analysis resulted in a caigua length of 18.9 cm, an increase in diameter of 5.65 cm, and an improvement in pulp thickness of 7.60 millimeters. Physiological parameters of caigua plants taken after 45 days of sowing were considerably improved with the same treatment. Similarly, N, K and Zn concentration in leaf was higher in case of 100 mL of cytokinins foliar analysis. Therefore, policymakers must advise using the recommended quantity of cytokinins to bring about regime transition, and formers can gain by injecting 100 mL of cytokinins to boost production and the economy. It was concluded that the adequate dose of cytokinins is in treatment T3, which raised value of potassium concentration in leaves, this influenced optimal development, strengthening against environmental stress and therefore quality. For this reason, research was carried out on the comparative study of cytokinin doses in the quality of caigua in Peru; the objective was to determine the appropriate dose to obtain higher quality fruit. Likewise, it was underlined that the objective was to employ an ecological alternative of plant origin such as the usage of phytohormone that stimulates the growth of the plant and consequently the quality of the fruit. The obtained the results were served as a recommendation for farmers in the area.

Keywords: biostimulants, dosages, nutrient's availability, quality production.

Resumo

A biodisponibilidade de nutrientes, a escassez de fertilizantes sintéticos e o aumento do custo dos combustíveis contribuíram para um aumento nos custos de produção, o que, por sua vez, reduziu a produtividade das culturas e levou os cientistas a procurar novos métodos para garantir resultados de alta qualidade. Neste contexto, várias dosagens de citocininas foram testadas no Peru para examinar se afetavam a qualidade do caigua, num esforço para resolver estes problemas. Para mitigar esses problemas, foi realizado um experimento em vaso para verificar os efeitos de diversas doses de citocinina na qualidade do caigua no Peru. O experimento consistiu de cinco tratamentos, incluindo 0, 50, 100, 150 e 200 mL de citocinina utilizando Anthesis Plus por 200 L de água como fonte, cada um com três repetições e dispostos seguindo um delineamento em blocos completos casualizados (RCBD). O tratamento com 100 mL de análise foliar de citocininas resultou em comprimento de caigua de 18,9 cm, aumento de diâmetro de 5,65 cm e melhora na espessura da polpa de 7,60 mm. Os parâmetros fisiológicos das plantas de caigua colhidas após 45 dias da semeadura melhoraram consideravelmente com o mesmo tratamento. Da mesma forma, a concentração de N, K e Zn nas folhas foi maior no caso da análise foliar de 100 mL de citocininas. Portanto, os decisores políticos devem aconselhar a utilização da quantidade recomendada de citocininas para provocar a transição de regime, e os primeiros podem ganhar injetando 100 mL de citocininas para impulsionar a produção e a economia. Concluiu-se que a dose adequada de citocininas está no tratamento T3, que elevou o valor da concentração de potássio nas folhas, isso influenciou no desenvolvimento ideal, no fortalecimento contra o estresse ambiental e consequentemente na qualidade. Por esse motivo, foram realizadas pesquisas sobre o estudo comparativo de doses de citocinina na qualidade do caigua no Peru; o objetivo foi determinar a dose adequada para obter frutos de maior qualidade. Da mesma forma, foi sublinhado que o objetivo era empregar uma alternativa ecológica de origem vegetal, como a utilização de fito-hormona que estimulasse o crescimento da planta e, consequentemente, a qualidade do fruto. Os resultados obtidos serviram de recomendação para os agricultores da região.

Palavras-chave: bioestimulantes, dosagens, disponibilidade de nutrientes, produção de qualidade.

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1. Introduction

A drop in yield and an increase in food prices in Peru may be attributed to factors such as a lack of synthetic fertilizers such as urea, diammonium phosphate, and potassium sulphate, as well as a scarcity of fruits and vegetables owing to the epidemic caused by Covid-19 (Vos et al., 2022; Balma et al., 2022; Gondal and Tayyiba, 2022). Well, in 2020 it was noted that imports of wheat, corn and other grains fell due to oil and fertilizer prices and it is expected that this 2022 will worsen due to the geopolitical conflict between Russia and Ukraine; therefore, it is necessary to discuss food security in Peru (Headey and Fan, 2008).

Products that promote optimal plant growth strengthen against environmental stressors, and boost production are important since fertilisers are expensive. For which, a natural alternative is the cytokinin-based bio stimulant that is obtained by plant extracts that contains nitrogen (N), phosphorus (P) and other elements, promote the development of shoots, flowers, roots and other organs that favor development (Grzegorczyk-Karolak et al., 2021; Góes et al., 2021). It is also necessary to highlight that the use of cytokinin influences in promoting the absorption of nutrients for the stimulation of the organs of the plant and intervene in the physiological process of photosynthesis, opening of stomata, resistance to environmental stress and other biochemical reactions that favor better development of the plant (Zaheer et al., 2022; Fan et al., 2022). For example, under N restriction, nitrate stimulated the expression of the IPT3 gene in the roots (Tsilimigka et al., 2022), which causes an increase in this phytohormone in the xylem and greater transport to the leaves.

Chemically, synthetic cytokinin has been shown to promote the plant's growth, which leads to a higher yield and quality of fruit, as well as an increase in production. However, the proper dosage of this phytohormone must be taken into consideration in order to maximize the absorption of nutrients and achieve positive effects (Buah et al., 2010).

The optimal amount of kinetin (cytokinin) was found to be 2.5 mg/L in study on the in vitro growth of stevia, a natural sweetener from nodal segments, which regenerates the shoots (Akhtar et al., 2020). By using growth hormones artificially in plants which are also produced by soil microbial community enhance the productivity of plants and also mitigate the effect of synthetic fertilizer. The biocontrollability of microbially generated opens up opportunities for integrated biological crop plant protection. In particular, the combination with other advantageous effects like the improvement of abiotic stress tolerance, or of the producing microbes, like the biofertilizer function of microalgae, seems to be appealing in environmentally friendly and sustainable approaches to reduce the use of chemical pesticides in climate change scenarios.

2. Materials and Methods

The experiment was conducted at the Lima, Barranca, Peru. It is situated at 10° 43' 42.62" latitude, 77° 43' 14.47" longitude at 142 meters above sea level (altitude) drop under semi-arid climate. The average day and night temperature during the study period was 18-25 °C.

2.1. Methodology

A field experiment was conducted at Lima Research Farm area, Barranca, Peru in order to check the adequate dose of cytokinins to obtain a higher quality of caigua. For this purpose, soil was prepared and divided into plots and sub plots by using suitable instruments. The randomized complete block design (RCBD) was used for this research. The plot's net size was 5 m × 1.2 m. A total of five main plots and a total of twenty sub plots were employed as treatment plots and replication plots, respectively. After that, the soil was leveled by using a laser and leveler. In the research, cytokinin concentrations of 0, 50, 100, 150, and 200 mL were used as treatment doses (Anthesis Plus per 200 L of water) (See detail in Table S1, Supplementary Material). Before seed sowing, physiochemical characteristics of soil were determined by using the procedure mentioned in ICARDA manual (See detail in Table S2, Supplementary Material). Seeds rate was 2 kg per hac and were sown by using drill machine (Mario et al., 2009). The standard dosages of cytokinin (100 mL/200 liters of water) were applied 20 days and 60 days after planting under the agro ecological conditions of the area by using the source Anthesis plus. The recommended doses of nitrogen (N), potassium (K) and phosphorus (P) fertilizers (100 kg NPK/ha) were applied for the maximum growth after calculation according to the soil present in pot. The sources of fertilizer were urea, triple super phosphate (P_2O_5), and potassium sulphate (K_2O) (Mario et al., 2009). Fertilizer was applied with water. The N dose split into three parts, first before sowing while other amounts were applied after first and second irrigation. Three irrigations were used during the vegetative growth period to capsule formation.

2.1.1. Chemical composition of Anthesis plus (contains cytokinin)

Anthesis plus is the source of cytokinin. It is chemically composed of inorganic nutrients, growth regulator cytokinin. Its synthetic chemical prepared in the lab obtained after the hydrolysis of the vegetable protein. Corresponding to the chemical composition of Anthesis Plus that contains nutrients, cytokinin obtained from the hydrolysis of proteins of vegetable origin, favors the development of the plant.

2.1.2. Harvesting of crop

Crop was harvested at maturity and possible parameters were taken during and at the end of experiment. Plants were taken from the central rows in all plots, which were marked with a tape for evaluations. This was done in order to avoid the edge effect. When the harvest arrived, 20 fruits per treatment were taken and the evaluations of fruit size, equatorial diameter (the measurement was made in the wide part of the fruit), pulp thickness were made. For data collection, observation, measurement and quantification techniques were used. This process was done in all the demonstration plots. Leaf analysis was then carried out at National Institute for Agricultural Innovation INIA -Huaral to evaluate how much nutrients were present in caigua leaves based on yield. Analysis of variance and Duncan's test were used to analyse the data, and the findings were evaluated and analyzed.

2.1.3. Physiological parameters of leaves of caigua

The physiological parameters such as chlorophyll contents were determined by using the SPAD meter (Zhang et al., 2022) while electron transport reaction (ETR), quantum yield (YII), photosynthetic active radiation (PAR), fluorescence yield (Ft) was noted by using instrument MINI-PAM-II (WALZ, Germany) (Bagheri et al., 2022).

2.1.4. Chemical parameters of soil of caigua?

Soil saturated extract was obtained by applying pressure on chamber in which soil paste was added and then extract was obtained after filtration. Soil extract was preserved by adding one drop sodium hexametaphosphate in 25 mL extract and this was done to minimize the precipitation of salts. NPK were determined in soil samples by adopting the procedure mentioned in ICARDA manual (Ryan et al., 2001).

2.1.5. Statistical analysis

Obtained data were processed by analysis of variance and the results were compared with the data of the Fisher table at 5% error, to determine the significance. After performing the analysis of variance, the operation was performed with Duncan's test at 5% error, which allowed determining which treatment stood out in relation to the others and if there was homogeneity or differentiation qualified by letters.

3. Results and Discussion

3.1. Agronomic parameters

Regarding the evaluations of the physical characteristics of the caigua in post-harvest detailed in Table 1. It was determined that at an adequate dose of cytokinin, which was T3, improved the physical parameters significantly. Therefore, it was interpreted that at this dose the absorption of nutrients promoted for the development of the plant's organs, which favors the quality of the fruit.

According to the evaluations of the physical characteristics of the caigua that can be seen in above result section, it is indicated that T3 stood out in caigua length, equatorial diameter and pulp thickness. Therefore, this dose of cytokinin promoted the absorption of nutrients that influenced the development of lateral roots, shoots, flowers, thus obtaining higher yields. This is due to the fact that cytokinin induces root initiation and elongation, activates leaf senescence, stimulates plant photomorphogenic development, and stimulates the generation of axillary shoots at the plant level. Therefore, the application of these growth regulators based on cytokinins with other compounds such as Cyt-hor, improved the yield of 29.9 t/ha of red onion bulb in comparison to control (Alvarez et al., 2020).

3.2. Soil analysis

The result of this analysis showed Anthesis Plus significantly affected soil chemical attributes (Table 2). In soil, after harvesting, soil organic carbon, organic matter, Zn, N, and K and P contents significantly responded to the treatments. Soil organic matter contents were significantly improved from 0.50% to 0.65%, highest being from application 150 of cytokinin by using (Anthesis Plus per 200 L of water) as a source, whereas the lowest value was obtained from the unfertilized (control) treatment (Table 2). Same trend was observed in all the chemical parameters as mentioned in Table 3. The significant ($P<0.05$) relationship was observed in all these soil characteristics at different level of treatments.

Table 1. Impact of cytokinin application on caigua agronomic parameters.

Treatment	Cytokinin dose (mL/200 l)	Caigua length (cm)	Diameter (cm)	pulp thickness (mm)
T3	100	18.91 a	5.65 a	7.6 a
T4	150	17.82 ab	5.42 a	7.3 a
T2	50	16.42 cb	5.22 a	7.1 a
T5	200	15.81 cb	5,12 a	7.0 a
T1	0	14.70 c	4.97 a	6.8 a
Significance		*	**	**
Variance coefficient		9.01	10.20	4.3

Note: Significant (*), Not significant (**), equal letters are interpreted to be statistically homogeneous. T1: Control (0 mL of cytokinins); T2: 50 mL of cytokinins; T3: 100 mL of cytokinins; T4: 150 mL of cytokinins; T5: 200 mL of cytokinins.

Table 2. Soil analysis of the experimental area for caigua cultivation.

Treatments	Organic carbon (%)	Organic matter (%)	Zn in soil (mg/kg)	K in soil (mg/kg)	P in soil (mg/kg)
T1	0.58 ± 0.012d	0.51 ± 0.053e	0.24 ± 0.02c	90.3 ± 1.69e	5.1 ± 0.08e
T2	0.63 ± 0.009b	0.58 ± 0.035b	0.26 ± 0.01ab	101 ± *b*	5.9 ± 0.04b
T3	0.60 ± 0.016c	0.54 ± 0.024c	0.26 ± 0.04ab	98.0 ± 0.81c	5.7 ± 0.05c
T4	0.65 ± 0.061a	0.61 ± 0.012a	0.29 ± 0.03a	105 ± 1.24a	6.4 ± 0.32a
T5	0.59 ± 0.032d	0.53 ± 0.043d	0.25 ± 0.00c	95.0 ± 0.81d	5.4 ± 0.06d

The Duncon test ($P<0.05$) reveals that means with different letters are substantially different. Means values represents ± standard deviation. T1: Control (0 mL of cytokinins); T2: 50 mL of cytokinins; T3: 100 mL of cytokinins; T4: 150 mL of cytokinins; T5: 200 mL of cytokinins.

The organic supplements substantially improved soil characteristics and enhanced the organic carbon and organic nitrogen as well as electrical conductivity as shown in Table 3. According to the results of soil analysis carried out at INIA (National Institute of Agrarian Innovation) – Huaral after crop harvesting, it was determined that the pH was slightly lower, the electrical conductivity of the soil have excessive salt concentration, higher percentage of organic matter, nitrogen, phosphorus and average concentration of potassium.

It may be due to the higher levels of the elements of N, P, Ca, Mg, Cu, Fe and Zn; however, at this dose, no better fruit quality was obtained, being T3 with 100 mL of cytokinin (Anthesis Plus)/200 L of water, which stood out in relation to the other treatments. This is because most of the nutrients are found under normal conditions with the exception of K, which is in high concentration; because this element intervenes in many biochemical and hormonal reactions that promotes the development of the plant's organs, especially in carbohydrate formation and translation, which results in fruit quality (Haberer and Kieber, 2002). Well, it has been determined in other investigations that the application of cytokinin influenced the nutritional characteristics of the gherkin, increasing the N, P and K levels, and generating in turn a higher yield, better fruit sizes and ensuring their quality (Kieber and Schaller, 2014).

3.3. Physiological parameters

Results revealed significant differences ($P < 0.05$) of caigua plant in response to different cytokinins concentrations compared with the control. All the concentration of cytokinins significantly improved the physiological attributes of caigua crop. For example, the maximum fluorescence yield (29%) and quantum yield (38%) of caigua plants were observed in 150 mL of cytokinins concentartion application followed by 100 mL of cytokinins, 50 mL of cytokinins and 200 mL of cytokinins showing 23.3% and 29.0%, 17.2% and 23.3%, 13.5% and 18.2% higher fluorescence yield and quantum yield respectively than that of control (Figure 1).

Similarly, in other physiological parámeters, significant enhancement was noted. The maximum number of electron transport reaction (39.7%) and photosynthetically active radiation (41.8%) were recorded in the application of cytokinin 150 mL of treatment. In comparison, cytokinin (100 mL) treatment showed a 31.6% and 39.0% increase in the electron transport reaction and photosynthetically active radiation respectively, compared to control as shown in Figure 2. The highest chlorophyll contents were observed in 150 mL of cytokinins application that increased (34.5%), followed by cytokinins (100 mL), cytokinins (50 mL) and cytokinins (200 mL), showing that 27.7%, 17.7% and 13.2% increase in chlorophyll contents respectively than that of control (Figure 2).

Similarly, all the physiological parameters were improved with application of cytokinin suggesting that normal dose could be significant in promoting caigua plants that may be due to improvement in soil properties, or amount of nutrients that cytokinin contains (Sakakibara, 2006). Our results support the findings of Antaurco et al. (2019). Likewise, improved chemical parameters were observed and our findings matched with Antaurco et al. (2019).

3.4. Leaf analysis by treatment

According to the foliar analysis by treatment shown in Table 4, the values of elements such as N, P, Ca, Mg, Cu, Fe, Zn, and K were raised at a greater dosage of cytokinin (Anthesis plus), but the T3 dose that stood out in the post-harvest characteristics had normal and high K values. As a result, the quality of caigua is thought to be influenced by variations in this nutrient (See Table 4).

Table 3. Soil analysis of the experimental area for caigua cultivation.

Treatments	pH	EC dS m^{-1}	Ca^{+2}+Mg^{2+} mmolc L^{-1}	N in soil (mg/kg)
T1	8.01 ± 0.18d	2.91 ± 0.02d	10.3 ± 1.69cde	0.29 ± 0.08bcd
T2	7.99 ± 0.16bc	2.73 ± 0.07d	10.4 ± 0.8cd	0.31 ± 0.04bc
T3	7.6 ± 0. 11a	2.81 ± 0.04c	10.6 ± 0.8c	0.32 ± 0.05c
T4	7.06 ± 0.09a	3.91 ± 0.09a	11.8 ± 0.73a	0.36±0.01a
T5	7.48 ± 0.10b	3.11 ± 0.03b	10.9 ± 0.83b	0.35 ± 0.06b

The Duncon test ($P<0.05$) reveals that means with different letters are substantially different. Means values represents ± standard deviation. T1: Control (0 mL of cytokinins); T2: 50 mL of cytokinins; T3: 100 mL of cytokinins; T4: 150 mL of cytokinins; T5: 200 mL of cytokinins.

Table 4. Foliar analysis according to the doses of cytokinin (Anthesis plus).

Parameter	0 mL/200 l H_2O	50 mL/200 L H_2O	100 mL/200 L H_2O	150 mL/200 L H_2O	200 mL/200 L H_2O
N %	2.80	3.60	4.80	5.80	6.54
Ca %	1.15	1.20	4.25	5.20	5.10
Mg%	0.70	0.65	1.48	1.80	1.55
Na %	0.01	0.05	0.12	0.21	0.15
Cu ppm	27	15	22	25	33
Fe ppm	85	45	140	190	216
Zn ppm	25	32	51	65	75
B ppm	10	23	78	20	62

Average values were taken from three readings.

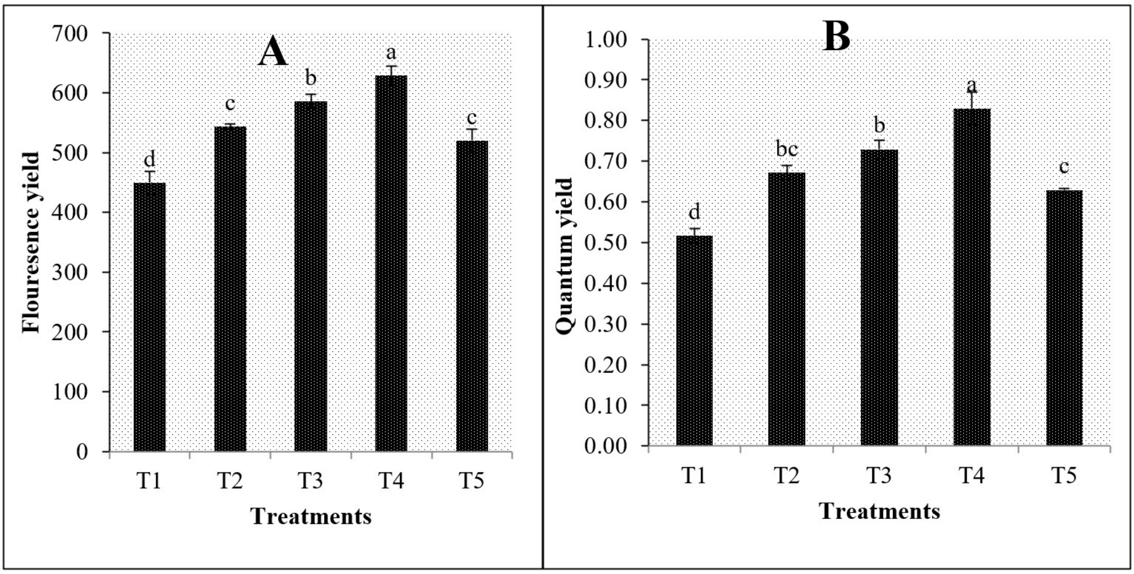

Figure 1. Impact of foliar applied cytokinins on fluorescence yield (A) and quantum yield (B) of Caigua crop plants (means ± STD, n = 3). The crop was harvested at maturity. The Duncon test (P<0.05) reveals that means with different letters are substantially different. Means values represents ± standard deviation. T1: Control (0 mL of cytokinins); T2: 50 mL of cytokinins; T3: 100 mL of cytokinins; T4: 150 mL of cytokinins; T5: 200 mL of cytokinins.

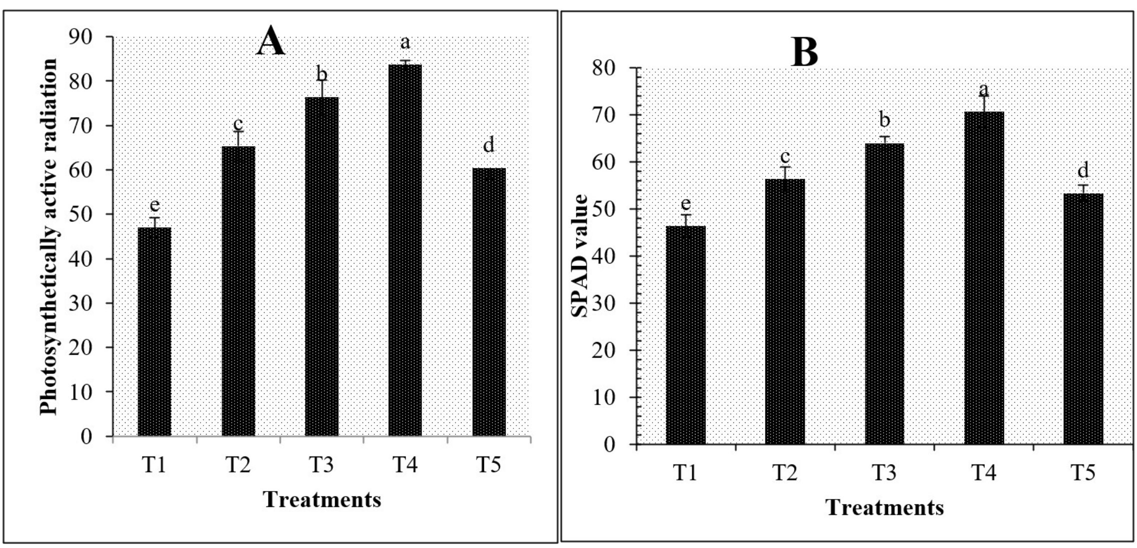

Figure 2. Impact of foliar applied cytokinins on photosynthetically active radiation (A) and SPAD value (B) of Caigua crop plants (means ± STD, n = 3). The crop was harvested at maturity. The Duncon test (P<0.05) reveals that means with different letters are substantially different. Means values represents ± standard deviation. T1: Control (0 mL of cytokinins); T2: 50 mL of cytokinins; T3: 100 mL of cytokinins; T4: 150 mL of cytokinins; T5: 200 mL of cytokinins.

It was highlighted that at a higher dose of cytokinin that was T5 with 200 mL of cytokinin (Anthesis Plus) / 200 L of water, quality of caigua plants were not considerably improved that may be due to toxicity of nutrients present in plant root surroundings. It may be due to the higher levels of the elements of N, P, Ca, Mg, Cu, Fe and Zn; however, at this dose, no better fruit quality was obtained, being T3 with 100 mL of cytokinin (Anthesis Plus)/200 L of water, which stood out in relation to the other treatments (Bajguz and Piotrowska, 2009; Gondal et al., 2021a, b, c). This is because most of the nutrients are found under normal conditions with the exception of K, which is in high concentration; because this element intervenes in many biochemical and hormonal reactions that promotes the development of the plant's organs, especially in carbohydrate formation and translation, which results in fruit quality (Qauod et al., 2022).

4. Conclusions

It was determined that at an adequate dose that was T3 with 100 mL of cytokinin (Anthesis plus)/200 L of water, it stood out in fruit length, diameter and pulp thickness. This dose promoted the absorption of nutrients that influenced the development of plant organs and strengthening, which influenced the yield and quality of caigua. Policy makers should make the set the current researched level for former for quality production. Almost nothing is known about how environmental cues or signals from plants affect how much cytokinin are produced by bacteria. Therefore, it is still unclear if plant-derived substances differentially affect the production of cytokinin in helpful vs pathogenic microbes, and whether the inhibition of cytokinin production in pathogens represents a form of plant defence. It will be necessary to take into account the composition of synthetic communities or the potential attractive applications of cytokinin producing bacteria in crop plant management while developing screening procedures for novel beneficial microbes. In contrast to cytokinin produced by pathogenic organisms, which has the opposite effect on plant growth and defence, cytokinin is derived from sources of beneficial microorganisms. To understand the kind and structure of cytokinin molecules produced by both beneficial and pathogenic species, as well as their positive and harmful effects on plants, more research is necessary.

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Supplementary Material

Supplementary material accompanies this paper.
Table S1: Treatment detail of the study
Table S2: Physiochemical properties of soil used in experiment
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