



Effect of Calcium Chloride, Cytokinin and Abscisic Acid on Growth of Rose Cut-flower (*Rosa hybrida*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Rose (*Rosa hybrida*) is a popular and extensively cultivated cut flower amongst commodities in the floriculture industry. However, there has been an increase in losses both in quantity and quality of cut roses which has significantly affected the production and return on investment. The highest loss reported has been at 18.87% at the producer level. These losses have been attributed to inadequate adaptability under circumstances involving humidity and temperature extremes, leading to high respiration, rapid cell disintegration and loss of aesthetic value of cut rose. This study therefore, investigated the effect of calcium chloride, cytokinin, and abscisic acid on the growth of tea hybrid rose, Rhodos variety. The study was cultivated over two flushes, August 2023-

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November 2023 and November 2023-January 2024 in Redlands Roses PLC, in Ruiru, Kiambu County. The field experiment was laid out in RCBD. There were 10 treatments including non-application (control); CaCl₂ (250, 500 and 750 mg/L), CKs (150, 250 and 350 mg/L), and ABA (5, 10 and 15 mg/L). Data was collected at seven days' interval throughout the growth period, starting from 3 weeks after initial bending in flush 1 and after pruning in flush 2. Data obtained was analyzed using SAS version 9.4 and significant means were separated using the Least Significant Difference at $\alpha=0.05$. The analysis of variance for flush 1 and 2 respectively, showed that CKs significantly ($p<0.05$) increased the number of shoots produced per plant (4.22 and 2.78 shoots), stem length (83.22 cm and 82.56 cm), leaf area (76.67 and 72.44 cm²), number of suckers (17.83 and 15.67), flush days (51.72 and 53.67 days), and chlorophyll content (72.95 and 70.77 SPADS) in cut flowers. On the other hand, the shortest stem length was recorded on plots treated with ABA (68.56 and 68.78 cm), flush days (49.06 and 50.67 days), chlorophyll content (64.74 and 65.13 SPADS) and leaf area (61 and 54.11 cm²). CaCl₂ reduced incidences of bent peduncles in flushes one and two (2.07 and 1.13). To improve on the growth qualities (number of shoots produced per plant, stem length, leaf area, flush days, and chlorophyll content) the growers should consider application of cytokinin at 250 mg/L and 350 mg/L. However, application of cytokinin comes with a risk of increased number of suckers. To reduce incidences of bent peduncles, growers should always apply 750 mg/L of CaCl₂ before the formation of the flower bud. To reduce the number of flush days in target of a specific market, growers can apply ABA (15 mg/L) at prebloom stage.

Keywords: Calcium chloride; abscisic acid; cytokinin; rose growth; flush days; chlorophyll content.

1. INTRODUCTION

Rose (*Rosa hybrida*) is a popular and extensively cultivated cut flower amongst commodities in the floriculture industry (Takahashi, 2025). It is grown because of its beauty, fragrance and long-lasting blooming season (Desta et al., 2022). It is primarily native to Asia, with a few native to Europe, North America, and Northwest Africa (Leghari et al., 2016). It presents as an erect or climbing shrub with stems that have sharp prickles, which are 1.5 feet to 6 feet tall. Rose may include floribunda, hybrid tea, Grandiflora, and miniature roses (Tkachenko, & Kapelian, 2021). The flower colour varies, some being scented and others unscented. According to Yu & Mingsan (2018), it may be grown for the production of cut flowers, for decoration purposes or as a raw material for the cosmetic and pharmaceutical industries. Currently China has overtaken Netherlands with a global production share of 19%, followed by the USA (12%), Netherlands (10%), Japan (8%) and Brazil (5%), respectively (Adebayo et al., 2020). According to Mwase (2015) and Bartilol et al. (2019), Kenya is the top African country exporting flowers to the EU, with an export proportion of 38%. Other nations in order of ranking consist of Ethiopia (15%), Zimbabwe (5%), Uganda (3%), South Africa (2%), Zambia (2%), and Tanzania (1%) [Mwase, 2015]. This indicates potential for further growth in the sector and an opportunity for businesses to capitalize on the increasing demand of roses. In Kenya, cut

rose is grown in greenhouses. The most important area in Kenya for producing cut flowers is Lake Naivasha, where about 70 farms span more than 3,000 hectares of greenhouse space and generate about 8,000 metric tons of flowers every month, mostly roses (Horticultural Crops Directorate, 2021).

The floriculture industry in Kenya has been a major contributor to the economy, resulting in increased Gross Domestic Product, job creation, and improved livelihoods for millions of people. According to Kamer (2022), the country exported 210 thousand metric tons of cut flowers in 2022 and contributed to up to 33% of the Agricultural Gross Domestic Product (Kogo et al., 2021). The floriculture business supports about 500,000 people, comprising over 100,000 workers at flower farms, and affects more than 2 million people's daily lives (Wangechi & Kariuki, 2022). Studies on the performance of the horticultural subsector show that increased horticultural exports led to increased Gross Domestic Product in Kenya (Horticultural Crops Directorate, 2021). The horticulture sector is expected to continuously contribute to the country's economy. Quality management at preharvest and postharvest stages of cut flowers is considered important and practical for supplying satisfying products to the market (Kaur, 2025). The quality of cut-roses after harvest is mainly affected by three major factors: Pre-harvest growth conditions; rapid respiration, which speeds up the aging of most flowers; and rapid

cell disintegration, thus reducing the vase life of cut flowers (Abdolmaleki et al., 2015). El-Beltagi et al. (2022) reported that continuous cold treatment and preservatives hardly improve the vase life and quality of roses as per the market requirements. This is due to the increased loss of cell wall integrity resulting from rapid respiration and depletion of food in the rose plant, thereby causing loss of commercial value of roses. Calcium chloride, cytokinin and abscisic acid (ABA) are inexpensive growth regulators and have great potential for commercial exploitation. These growth regulators are needed by plants for leaf emergence, healthy growth, flowering, fruiting and senescence (Chen & Zhang, 2018). They also help regulate the quantity of biomass generated, the plant's shape, and the capacity of the plant to resist environmental stress (Husen, 2022). In general, calcium chloride and the selected growth regulators can influence changes in the anatomical and metabolic processes associated with the growth of plants at low concentrations. Calcium chloride is an inorganic salt that functions as an endogenous signal molecule that controls plant growth factors (Maehara, 2020). It triggers the production of hormones and enzymes that regulate processes, including leaf and shoot development, cell wall formation, and root development (Kumar & Pandey, 2017). Additionally, it enhances the proper development of cell walls and preserving their integrity aids in maintaining the quality of fresh produce (Barman et al., 2018).

According to Tavakkoli & Rudell (2016), cytokinin is important in regulating plant processes, such as root and shoot growth (Li et al., 2025), bud formation, cell division and differentiation, flowering and leaf aging (Mei et al., 2025). Abscisic acid is essential for the growth and maturation of plants (Wei et al., 2025). It is generated within the chloroplasts and then released in reaction to environmental conditions like extreme temperatures (Hao & Zhang, 2020). Plants use the carotenoid route, to synthesise abscisic acid (Chen et al., 2020). It is moved to other plant areas, attaches to receptors and starts several physiological processes. The processes may include seed dormancy and germination (Nguyen et al., 2025), stomata opening and closing (Gong et al., 2025), cuticle wax build-up, water uptake regulation, and stress response control (Hao & Zhang, 2020). There has been considerable growth in the global floriculture industry and this is anticipated to continue. However, there are increased losses in

the quantity and quality of cut roses. These losses have significantly affected the production and return on investment for cut roses (Bante et al., 2023). Omar et al. (2014) reported that the highest loss occurred in ranked order at the retail level (39.82%), wholesalers (27.52%), producers (18.87%) and local traders (13.78%). These losses have been attributed to high respiration and rapid cell disintegration resulting in rapid deterioration and loss of aesthetic value in cut rose. Additionally, most of the rose growers do not apply growth regulators during growth and postharvest, and efforts to minimize losses have been focused on manipulating the growing environment, using preservatives and cold treatment. Application of growth regulators can reduce preharvest and postharvest losses. However, there is limited knowledge on the effects of concentrations of growth regulators like calcium chloride, cytokinin and abscisic acid on rose growth. It is, therefore, necessary to determine suitable concentration levels that result in improved growth of rose cut-flowers.

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted in Redlands Roses PLC, Ruiru, Kiambu County, Kenya. The field experiment was conducted in greenhouse Delta installed with a 200-micron thick UV block and light diffusing polythene, automated ventilation, misters and fans, and nets. The first flush commenced in August 2023 and ended in November 2023 and the second flush commenced in November 2023 and ended in January 2024.

2.2 Experimental Design, Layout and Treatments

In the study was laid out in a randomised complete block design and replicated three times. The plot size was 2 m by 0.3 m and a spacing of 0.5 m between treatments and 1 m between blocks in hydroponic beds. There were 10 treatments including non-application (control); Calcium chloride (250, 500 and 750 mg/L), cytokinin (150, 250 and 350 mg/L), and abscisic acid (5, 10 and 15 mg/L). The first treatment was done 2 months after initial bending through foliar spraying on a sunny day after dehydrating the crop for 4 hours to improve the uptake. The second treatment application was done during the pre-bloom stage when the crop was at the pea size stage through foliar application.

2.3 Land Preparation, Propagation, Crop Establishment and Management

2.3.1 Land preparation

The closed hydroponic beds were raised from the ground using building stones to a 1% gradient (20 cm height from the beginning of the bed and 15 cm at the end of the bed) (Plate 1A). The troughs were laid in the dimensions of 50 m × 0.3 m × 0.3 m and a spacing of 1 m between beds (Plate 1B). The beds were filled with pumice 4.5 m³ in 50 m bed as planting media. Cleaning of the pumice with plain water to remove impurities was done (Plate 1C). The media was drenched with 0.04 ml/l phosphoric acid to attain a pH of 5.5 to 6.5 (Plate 1D). The driplines were laid 5cm from the end of the bed and 20 cm from each other. The driplines were

tested for dripper capacity discharge to ensure all drips were 1.6 L/hr.

2.3.2 Propagation and crop establishment

The propagules were established through top-grafting at Olij Kenya (2A). The scion was obtained from the Red Rhodos bud woods and the rootstalk was from natal briar bud woods. After 28 days the plants were taken to the hardening bay. On the 36th day plants were ready for transplanting (2B). Only disease and pest-free plants were selected for planting. The media was irrigated with plain water to help keep the beds wet during planting. Planting was done early in the morning using a spacing of 18.5 cm intra-row and 9.25 cm inter-row and 5 cm from the edge of the planting trough with each bed having 540 plants.



Plate 1. Raising of hydroponics planting beds (A), Reinforcing the Planting Troughs (B), Sterilizing the Media with Hydrogen Peroxide (C) and Stabilizing Planting Media pH with Phosphoric Acid (D)



Plate 2. Top grafts after establishment in Olij Kenya (A) and Top grafts after 36 days (B)

2.3.3 Crop management

Mulching was done using a black and white mulch paper of 25 microns' thickness (3A). watering was done based on the weather conditions and the stage of growth. Light watering of the young plants was done in the first week of planting. On hot days watering was also done on the paths between the beds on daily basis. Gapping was done 7 days after planting and it involved replacement of the plants that failed to establish after transplanting. Debudding was done after every 2 days from 14 days after planting up to 56 days when initial bending was done (3B). De-shooting was done after every 2 days from 28 days after planting up to the 56th day. Desuckering was done daily when the plants were at maroon stage. Initial bending of the entire shoot was done eight weeks after planting. Selective bending was done at an interval of 14 days after the initial bending to keep the bedding crop rejuvenated (3C). Weak, blind and short stems were selected and used as bedding plants. Sanitation involved weekly removal of any unwanted materials that could affect the overall survival and productivity of the plant through sweeping and weeding to keep the greenhouse clean (3C). Fertigation was through the closed hydroponic system using farm based fertilizer recipe. Scouting for pests and diseases was done three times a week. It involved visual checks for live pests, honey dew and pest damage on the crop. Monitoring for diseases involved checking for spores using a magnifying

lens. Identified pest and diseases were managed through the integrated pest management approach.

In the first trial, harvesting was done 110 days after planting and 55 days after cutting back in the second trial. The flowers were harvested in the morning and in the evening when the temperature was low and atmospheric humidity high. flowers were wrapped with nets in a conical shape and put in clean farm formulated postharvest solution in an egg tray bucket. The postharvest solution had a temperature of 8°C and a pH of 4.8. The harvested flowers were transported to the cold room for precooling.

2.3.4 Greenhouse climate management

Weather monitoring was through an EL-USB-5 data logger. The data logger recorded greenhouse humidity, and temperature using a remote sensor and data collected daily. An automatic fogging system was installed in the greenhouse to manage in-house humidity. Fans were also installed in the greenhouse for enhancing air circulation. Greenhouse temperature was maintained between 23 °C - 30 °C during the day and 12 °C – 16 °C during the night. Humidity was maintained between 70 % and 80%. When greenhouse humidity was 65% and below, fogging was done for 6 seconds at an interval of 3 minutes until the appropriate greenhouse humidity was achieved.



Plate 3. Plastic mulch (A), First Bending (B) and Selective Bending and Sanitation Done in the Greenhouse (C)

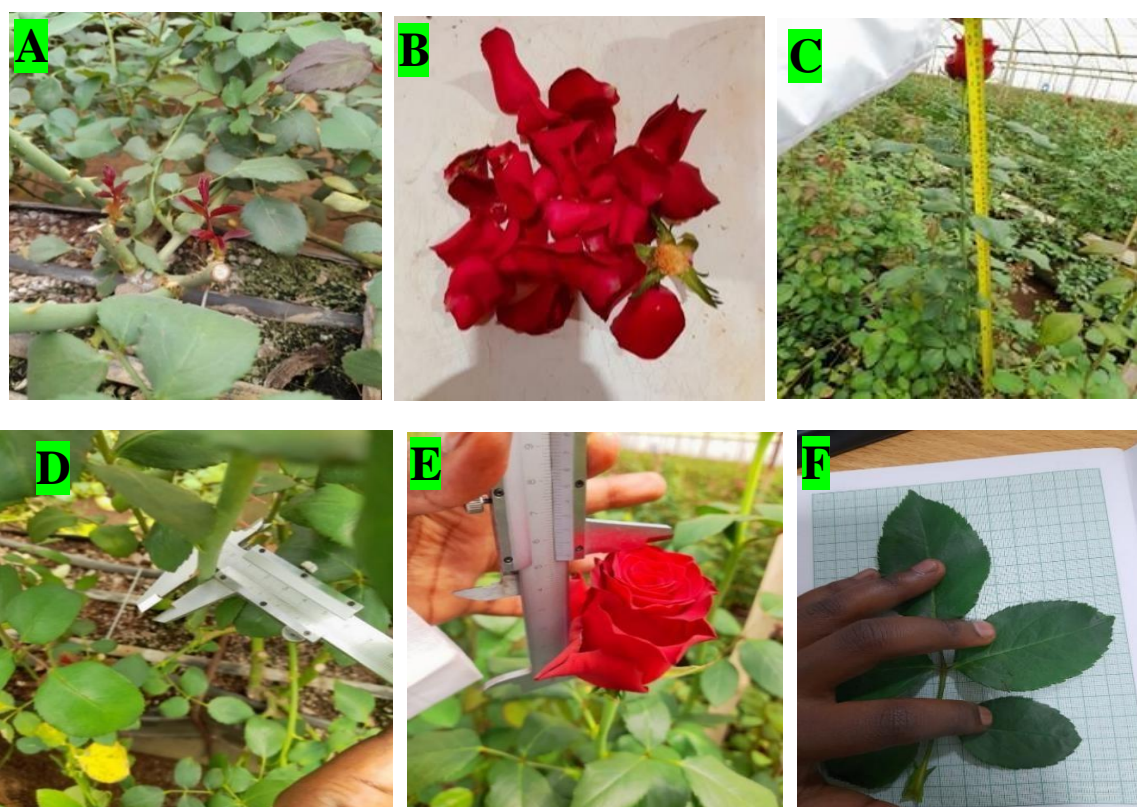


Plate 4. Lateral shoots (A), Number of Petals (B), Stem Length (C), Stem Thickness (D), Flower Head Size (E) and Leaf Area (F)

2.3.5 Data collection

After initial bending 3 stems in each experimental plot were tagged for data collection. The impacts of calcium chloride and growth regulators on growth of cut roses was assessed through counting the number shoots (4A) and petals produced (4B), measuring the stem length (4C), stem diameter (4D), flower bud size (4E), leaf area (4F), chlorophyll content, and number of days to flower maturation.

2.3.6 Data analysis

The collected data was analysed using analysis of variance (ANOVA) using SAS version 9.4. Significant means were separated using Least Significance Difference (LSD) at $\alpha=0.05$.

3. RESULTS AND DISCUSSION

The effect of calcium chloride, cytokinin and abscisic acid on growth of cut rose's cv. Red Rhodos was assessed at different rates. The analysis of variance showed that number of shoots, stem length, stem diameter, flower bud

size, leaf area, chlorophyll content, number of suckers, bent peduncles, flush days, and number of petals were significantly affected by the different rates of application of calcium chloride, cytokinin and abscisic acid. Moreover, Flush 1 had better results compared to flush 2. This is because, flowers in flush 1 emerged from the basal shoots which are always more vigorous while flowers in flush 2 emerged from lateral shoots which are always less vigorous compared to basal shoots.

Additionally, the greenhouse average temperature ranged between 23 °C and 27 °C. Relative humidity was maintained at an average of 70% to 80% (Table 1). The different growing temperatures and humidity caused varying morphological characteristics and harvest dates. Lower temperatures and higher humidity in the first flush (Table 1) yielded flowers with longer lengths (Table 3), bigger stem diameter, bigger flower bud size (Table 4), larger leaves (Table 5), more chlorophyll content (Table 6), and more flush days (Table 9) compared to flush 2. It is possible that lower temperatures slowed the growth causing the plant to consume less energy and store more sugar therefore improving the

plant morphological traits. Similar outcomes were reported by Younis et al. (2013) who observed that lower temperature than the recommended causes stems to elongate and flush intervals to lengthen.

3.1 Effect on Number of Shoots

The analysis of the treatment effect showed that the application of calcium chloride (250, 500, and 750 mg/L) had no significant effect on the number of shoots at different rates both in flush 1 and 2. Calcium chloride is a salt. When in excess, salts cause detrimental effects in plant growth. It could be that the amounts of calcium chloride applied in the current experiment were too low to affect the number of shoots produced in rose flower. It is also possible that at concentrations higher than 750 mg/L calcium chloride has a potential of causing detrimental impacts on shoot growth. According to Haque et al. (2017), salt treatment has a potential to decrease shoot emergence. Additionally, Pawłowska and Bach (2010) observed that rising concentrations of calcium chloride had a

detrimental impact on rose shoot regrowth and proliferation. Pessarakli (2021) also reported that a high salt level negatively affects shoot growth in plants.

The study also showed that application of cytokinin (150, 250, and 350 mg/L) had a significant effect on the number of shoots at different rates both in Flush 1 and 2 compared to the control (Table 2). It could be that the application of cytokinin in Rhodos variety accelerated cell division in the meristem encouraging development of more shoots. It has been reported that cytokinin enhances growth of more shoots because it encourages cell division and the development of plant tissues especially in meristematic parts responsible for new shoot emergence (Cammarata et al., 2019). The findings of this study were in agreement with Roy et al. (2017) who did research on the effect of pre-plant soaking of corms in cytokinin on sprouting, vegetative growth and corm formation in gladiolus (*Gladiolus grandiflorus* L.). They observed that at 300 ppm cytokinin increased the most shoots per corm in variety Jessica.

Table 1. Greenhouse environment weather condition in flush 1 and flush 2

Flush	Month	Temperature (°C)			Relative Humidity (%)		
		Minimum	Max	Mean	Minimum	Max	Mean
Flush 1	August	13.0	29.6	23.43	48	93.9	81.8
	September	14.3	33.2	24.66	43	89.2	77.6
	October	13.8	32.9	23.2	45	91	79.3
	November	14.9	33.4	25.67	41	90	80.4
Flush 2	December	16.9	35.8	26.54	39	81	73.5
	January	17.1	36.4	27.31	36	84	71

Table 2. Effect of calcium chloride, cytokinin and abscisic acid on the number of lateral shoots in flush 1 and 2

Treatment	Rate of Concentration in mg/L	Number of lateral shoots in Flush 1	Number of lateral shoots in Flush 2
Calcium chloride	250	3.22b	1.78b
Calcium chloride	500	3.22b	1.89b
Calcium chloride	750	3.11b	1.78b
Cytokinin	150	3.89a	2.56a
Cytokinin	250	4.11a	2.67a
Cytokinin	350	4.22a	2.78a
Abscisic acid	5	2.89b	1.67b
Abscisic acid	10	2.89b	1.33b
Abscisic acid	15	2.78b	1.33b
Control	0	3.22b	1.78b
	LSD	0.85	0.61
	CV	27.50	33.15

Means followed by the same letter(s) along the column are significantly different at 5% probability level.

The application of abscisic acid (5, 10, and 15 mg/L) had no significant effect on the number of shoots at different rates both in flush 1 and 2 (Table 2). Abscisic acid is a growth retardant but at low concentrations, it may have no impact on plant growth. Therefore, it is likely that the amounts of abscisic acid applied in the current experiment were too low to affect the number of shoots in rose flower, and at concentrations higher than 15 mg/L abscisic acid has a potential of causing detrimental impacts on shoot growth. According to Vysotskaya et al. (2018), ABA inhibits growth. This observation was supported by Brookbank et al. (2021) who reported that large concentrations of exogenously administered ABA restricted growth.

3.2 Effect on Stem Length

Calcium chloride application at 250, 500, and 750 mg/L had no significant effect on stem length (Table 3). The impact of calcium chloride on the growth and elongation of a plant can be affected by multiple physiological, and environmental variables. It could be that the uptake of exogenously applied calcium was affected by the lower temperatures observed during the vegetative growth. This therefore, affected physiological activity of calcium chloride causing it to have no significant effect on stem length. Moreover, Aldon et al. (2018) explained that the amount of calcium that plants require varies depending on the stage of development and the species of the plant. Therefore, if a plant is not lacking in calcium, then it could be unresponsive to more of it. This may also explain

why the application of calcium chloride had no effect on the stem length of the Rhodos variety in the current study. Contrary to the findings of this study, Mehdi et al. (2015) observed that the stem length of cut rose cv. "Dolce Vita" increased with exogenous application of calcium chloride. However, at high concentration of calcium chloride the stem length reduced.

This study revealed that applying cytokinin at 150, 250, and 350 mg/L positively affected the lengthening of stems compared to control at 0 mg/L. The longest stems were observed in 350 mg/L with 83.22 cm and 82.56 cm in flush 1 and 2 respectively (Table 3). It may be that the active role of cytokinin in cell division and elongation led to longer stems of cut rose flowers in this study. Jacquard et al. (2019) also reported that application of cytokinin caused the cells in the stem tissues to divide and elongate more in plants. Gabrel et al. (2018) reported a similar pattern of findings using the Chrysanthemum morifolium cv. "Zambla White" reached the highest plant height when exposed to high concentrations of cytokinin (200 ppm).

Application of abscisic acid was applied at 5, 10, and 15 mg/L significantly reduced the length of the stems. The shortest stems at 68.56 and 68.78 cm were noted with application of abscisic acid at 15 mg/L in flush 1 and 2, respectively (Table 3). Based on the findings of this study, abscisic acid probably reduced the stem length of Rhodos variety by inhibiting stem elongation because of its ability to retard growth.

Table 3. Effect of calcium chloride, cytokinin and abscisic acid on stem length produced in flush 1 and 2

Treatment	Rate of Concentration in mg/L	Stem length in Flush 1 (cm)	Stem length in Flush 2 (cm)
Calcium chloride	250	79.22b	78.33b
Calcium chloride	500	79.67b	78.44b
Calcium chloride	750	77.89b	76.11b
Cytokinin	150	82.44a	81.44a
Cytokinin	250	82.67a	82.44a
Cytokinin	350	83.22a	82.56a
Abscisic acid	5	69.22c	69.33c
Abscisic acid	10	68.89c	69.00c
Abscisic acid	15	68.56c	68.78c
Control	0	78.67b	77.33b
	LSD	3.93	4.21
	CV	5.43	6.14

Means followed by the same letter(s) along the column are not significantly different at 5% probability level within each flush.

According to Chen et al. (2020), abscisic acid stimulates stem cell dormancy and inhibition of differentiation in the principal root meristem. Kishor et al. (2022) indicated that in stressful conditions, elevated levels of abscisic acid leads to restricted stem development as the plants adapted to preserve resources and water. This is achieved by abscisic acid through controlling the expression of genes related to cell division and elongation (Sun et al., 2018).

3.3 Effect on the Stem Diameter and Flower Bud Size

Analysis of treatment effect revealed that the application of calcium chloride at 250, 500, and 750 mg/L had no significant effect on the diameter of the stem and the flower bud size compared to the control at 0 mg/L (Table 4). These observations imply that, in this specific variety, there is no direct relationship between the administration of calcium chloride and stem and flower diameter. The findings of the study on the effect of calcium chloride on stem and flower diameter was similar to the findings of Sabah et al. (2019) in snapdragon plants (*Antirrhinum majus* cv. butterfly). They reported that when applied independently calcium chloride had no discernible effect. Similarly, on a research in *Rosa hybrida* cultivars and calcium application, Oloo-Abucheli (2018) reported that field treatment of cut roses with calcium had no effect on stem and flower diameter. According to Ali and Abd Asal (2023) calcium is necessary for many physiological functions in plants, such as the creation and integrity of cell walls, however calcium chloride by itself usually has no direct impact on stem diameter.

Application of cytokinin at 150, 250, and 350 mg/L and abscisic acid at 5, 10, and 15 mg/L had no significant effect on the diameter of the stem and the flower bud size compared to the control at 0 mg/L (Table 4). Cytokinins mainly promote the formation of shoots by stimulating cell division in the meristem. Even though more cell division can lengthen shoots overall, this does not always translate into a corresponding stem or flower diameter increase. Aside from cytokinin regulation, additional variables that affect stem diameter include; physiological factors (development of vascular bundles), environmental factors (temperature and humidity), and cultural factors (feeding, and crop balancing) (Xiang et al., 2019).

Additionally, it is very likely that in the variety used in this study, there was no direct

relationship between the administration of abscisic acid and stem and flower diameter. It is also possible that the effect of the varying concentrations of abscisic acid were overshadowed by the activity of other hormones in the plant. This is because abscisic acid is most effective in stressful conditions but during the growth of Rhodos variety the growing conditions were favourable. In research on Short-and long-term responses of pepper seedlings to abscisic acid exposure Ban et al. (2017) observed that while stem diameter increased in pepper, it was not influenced by abscisic acid. According to Skubacz & Daszkowska-Golec (2017), a complicated interaction between variables exists where the effects of abscisic acid are obscured by other physiological functions or outside stimuli, underscoring the complex interactions between abscisic acid signalling mechanisms and biological processes in plants.

3.4 Effect on Leaf Area

During the study it was observed that different concentrations of calcium chloride (250, 500 or 750 mg/L) had a no effect on the leaf area of cut roses "Rhodos variety" (Table 5). These outcomes imply that, in this specific variety, there is no direct relationship between the administration of calcium chloride and leaf area. According to El Habbasha and Ibrahim (2015), the impact of calcium on the growth of a plants can be affected by multiple physiological, and environmental variables. Moreover, Aldon et al. (2018) explained that the amount of calcium that plants require varies depending on the stage of development and the species of the plant. This explains why the application of different concentrations of calcium chloride applied at different timings had no effect on the leaf area of cut roses "Rhodos."

In this study leaf area was significantly influenced by cytokinin application. The biggest leaf size of 76.67 and 72.44 cm² was observed with application of 350 mg/L Cytokinin in flush 1 and 2, respectively (Table 5). It is very likely that cytokinin increased the leaf area of the Rhodos variety because of its ability to promote cell division and enhance cell expansion during the developmental stage of the leaf. According to Wu et al. (2021), cytokinin enables shoot apical meristems to continue growing and produce stem cells that eventually generate leaves during the early stages of leaf production. The findings of this study are in agreement with those of Mondal & Sarkar (2018) studied on the Hybrid tea rose

cv. Bugatti during spring-summer months. They observed that maximum leaf area was obtained from plants with cytokinin treatment compared to the control experiment that had the least leaf area. Similar outcomes were also noted by Sardoei (2014) who reported that leaf area increases with increasing concentration of growth regulators (Gibberellic Acid and Benzyl Adenine).

During the study, abscisic acid treatment decreased the leaf area of the Rhodos variety compared to the control. It is possible that exogenous application of abscisic acid induced stomata closure. This affected the gaseous exchange in the crop causing a reduction in photosynthesis which affected the growth and enlargement of leaves. Chen et al. (2020) indicated that when abscisic acid simulated the impacts of water stress it causes reactions that are comparable to those brought on by internally produced abscisic acid. Additionally, applying abscisic acid cause stomatal closure, which lowers the amount of carbon dioxide that enters the leaf during photosynthesis (Negin et al., 2019). This therefore causes a limitation in the synthesis of carbohydrate which is crucial for cell division and proliferation therefore reducing the leaf area. Similarly, Khaleghnezhad et al. (2021) reported that under all moisture levels in the experiment, the application of abscisic acid exhibited a declining trend in both photosynthetic rate and leaf area in *Dracocephalum moldavica* L. under drought stress. They also observed that leaf area was drastically decreased by the

decline in leaf growth in increasing concentrations of abscisic acid.

3.5 Effect on Chlorophyll Content

Although calcium chloride at 250, 500 and 750 mg/L, had varying means, the treatments had no statistically significant effect on chlorophyll content 3 weeks after first application of treatments (after initial bending and pruning) both in flush 1 and 2. After the second application of treatments (at maroon stage) 500 and 750 mg/L of calcium chloride significantly influenced chlorophyll content. Despite the application of treatments 2 times during pre-harvest of the Rhodos variety, 250 mg/L of calcium chloride had no significant effect on chlorophyll content. The effects of 500 and 750 mg/L of calcium chloride on chlorophyll content was observed after a second application of the treatments. It conceivable that the uptake and translocation of the calcium ions by the plant was slow (Pathak et al., 2021) and also, the effectiveness of calcium chloride on chlorophyll content could have been based on the amount of calcium accumulated by the plant (Guo et al., 2023). Therefore, this explains why the effect of calcium chloride was only visible in Rhodos variety after more than one time of application at high concentrations. The observations were in agreement with Zomorodi et al (2022) who observed that applications of calcium chloride significantly increased the amount of chlorophyll in Periwinkle, particularly at the highest concentrations.

Table 4. Effect of calcium chloride, cytokinin and abscisic acid on the stem diameter and flower bud size produced in flush 1 and 2

Treatment	Rate of Concentration in mg/L	Stem diameter (mm)		Flower bud size(cm)	
		Flush 1	Flush 2	Flush 1	Flush 2
Calcium chloride	250	8.44a	7.11a	5.30a	5.23a
Calcium chloride	500	8.83a	7.56a	5.34a	5.23a
Calcium chloride	750	8.89a	7.89a	5.4a	5.44a
Cytokinin	150	8.06a	6.83a	5.3a	5.16a
Cytokinin	250	8.56a	7.17a	5.13a	5.15a
Cytokinin	350	8.61a	7.33a	5.38a	5.36a
Abscisic acid	5	8.78a	7.39a	5.45a	5.51a
Abscisic acid	10	8.06a	6.81a	5.39a	5.42a
Abscisic acid	15	8.44a	7.06a	5.26a	5.16a
Control	0	8.72a	7.39a	5.35a	5.30a
	LSD	1.08	1.36	0.33	0.36
	CV	13.53	19.92	6.41	7.22

Means followed by the same letter(s) along the column are not significantly different at 5% probability level for each flush.

Table 5. Effect of calcium chloride, cytokinin and abscisic acid on the leaf area produced in flush 1 and 2

Treatment	Rate of Concentration in mg/L	Leaf Area in Flush 1 (cm ²)	Leaf Area in Flush 2 (cm ²)
Calcium chloride	250	65.89b	63.17b
Calcium chloride	500	66.94b	63.78b
Calcium chloride	750	68.94b	63.89b
Cytokinin	150	75.00a	71.06a
Cytokinin	250	76.11a	71.44a
Cytokinin	350	76.67a	72.44a
Abscisic acid	5	61.00c	54.11c
Abscisic acid	10	61.11c	54.94c
Abscisic acid	15	61.89c	57.22c
Control	0	66.06b	63.28b
	LSD	5.96	9.37
	CV	9.33	15.82

Means followed by the same letter(s) along the column are not significantly different at 5% probability level within each flush.

Treatment analysis indicated that cytokinin application at 250 and 350 mg/L had a significant effect on chlorophyll content 3 weeks after first application of treatments (after initial bending and pruning) both in flush 1 and 2. Application of 350mg/L had the highest means of 53.81 SPADS and 46.8 SPADS in flush 1 and 2 (Table 6). Cytokinin at 150 mg/ had no statistically significant effect on chlorophyll content. After the second application of treatments (at maroon stage), 150, 250, and 350mg/L of cytokinin significantly influenced chlorophyll content. While application of 350 mg/L of cytokinin was not significantly different from application of 250 mg/L, where 350mg/L was applied higher chlorophyll content of 72.95 and 70.77 SPADS was observed in flush 1 and 2, respectively (Table 6). The effect of 150mg/L of cytokinin on chlorophyll content was observed after a second application of the treatments. These findings indicate that cytokinin substantially influenced the concentration of chlorophyll content in the leaves of Rhodos variety 3 weeks after first application and at week seven after second application in flush 1 and 2, respectively. Therefore, based on these findings It very likely that the effects of cytokinin on chlorophyll content are more evident on higher concentration (250 and 350 mg/L) than on lower concentration (150 mg/L). This explains why 150 mg/L had no significant effect on chlorophyll content even after 3 weeks of first application of treatments and was less significant than 250 and 350 mg/L at seven weeks after second application of treatments.

Hormones gradually build up in target tissues and become more effective following 3 or more

weeks of treatment application (Dobránszki & Mandler-Drienyovszki, 2014). This probably explains why the impact of cytokinin on chlorophyll content was evident after 3 weeks. Sosnowski et al. (2023) reported that floral induction caused fluctuations in endogenous cytokinin levels which affected the amount of chlorophyll in the leaves of plants. This therefore justified the need for a second application of cytokinin treatment in Rhodos variety when the plants were at maroon vegetative stage of growth. Similarly, Cavusoglu et al. (2021) observed that the highest concentrations of cytokinin application substantially increased the amounts of Chlorophyll a and b on pepper (*Capsicum annum* L.). They also reported that when cytokinin was applied externally to the pepper plant, it promoted the growth of chloroplasts and increased chlorophyll production, decreased chlorophyll degradation, and maintained photosynthetic activity.

Although abscisic acid at 5, 10, and 15 mg/L, had varying means, the treatments had no statistically significant effect on chlorophyll content 3 weeks after first application of treatments (after initial bending and pruning) both in flush 1 and 2. Despite the application of treatments 2 times, 5 mg/L abscisic acid had no significant effect on chlorophyll content. Compared to the control, application of 10 and 15 mg/L of abscisic acid significantly reduced the chlorophyll content with application of 15 mg/L having the lowest chlorophyll content of 64.74 and 65.13 SPADS in flush 1 and 2, respectively (Table 5). The effects of 10 and 15 mg/L of abscisic acid on chlorophyll content was observed after a second application of the

treatments application. It is likely that endogenous level of abscisic acid increased with continued exogenous application of higher concentration of treatments 10 and 15 mg/L which reduced the chlorophyll content in the Rhodos variety. It is also possible that the increase in endogenous abscisic acid stimulated the aging process in plants therefore causing degeneration of chlorophyll within the leaves. According to Yang et al. (2014), genes responsible for degradation of chlorophyll are strongly expressed when the concentration of abscisic acid is high. This possibly explains why the chlorophyll content was significantly reduced after the second application of 10 and 15 mg/L and why 5 mg/L had no effect on the chlorophyll content. Wang et al. (2018) in a study in Arabidopsis observed similar outcomes as the current study.

3.6 Effect on the Number of Suckers

Compared to the control (0 mg/L), calcium chloride application at 250, 500, and 750 mg/L and abscisic acid application at 5, 10, and 15 mg/L on Cut roses “Rhodos variety” were observed to have no effect on the number of suckers produced per plant (Table 7). Calcium chloride is primarily responsible of strengthening cell wall while abscisic acid responsible of plant response to stress. It could be that abscisic acid

influenced the response of Rhodos variety to stresses resulting from environmental fluctuations while calcium chloride strengthened the cell walls while playing no active role in regulating the emergence and development of suckers. According to Muller and Leyser (2011), hormones including cytokinins, and auxin control the shoot branching and development of auxiliary shoots. Moreover, Demidchik et al. (2018) reported that calcium is definitively attributed to the strengthening and stabilization of the cell wall. Lamers et al. (2025) also reported that abscisic acid is majorly characterised with stress response in plants. The development of suckers, which includes branching from the primary stem, is primarily associated with growth processes instead of stress reactions (Salama & Elsherbeny, 2016) and rigidity of cell walls (Zhang et al., 2021, Ali, & Abd Asal 2023).

Analysis of Variance revealed that cytokinin application at 150, 250, and 350 mg/L significantly ($p < 0.05$) increased the number of suckers on Rhodos variety during production. Application of 350 mg/L had the highest number of suckers at 17.83 and 15.67 both in flush 1 and 2 (Table 7). While treatment with 150 and 250 mg/L cytokinin had varying means, they expressed no statistical difference. Cytokinin has the ability to enhance lateral growth and suppress apical dominance. This explains why

Table 6. Effect of calcium chloride, cytokinin and abscisic acid on the chlorophyll content in flush 1 and 2

Treatment	Rate of Concentration in mg/L	Flush 1		Flush 2	
		At week 3	At week 7	At week 3	At week 7
Calcium chloride	250	47.27b	66.57d	41.24b	66.37d
Calcium chloride	500	47.57b	68.17c	41.88b	67.44c
Calcium chloride	750	48.74b	68.37c	42.42b	67.54c
Cytokinin	150	48.18b	70.15b	42.29b	68.33b
Cytokinin	250	52.48a	71.84a	46.36a	70.66a
Cytokinin	350	53.81a	72.93a	46.80a	70.77a
Abscisic acid	5	47.17b	65.09d	40.51b	66.15d
Abscisic acid	10	46.73b	64.75e	40.27b	65.58e
Abscisic acid	15	47.48b	64.74f	41.34b	65.13f
Control	0	48.34b	66.34d	42.39b	66.15d
	LSD	2.90	2.66	3.68	2.34
	CV	6.33	7.21	9.35	3.77

Means followed by the same letter(s) along the column are not significantly different at 5% probability level within each flush.

Table 7. Effect of calcium chloride, cytokinin and abscisic acid on the number of suckers produced per treatment in flush 1 and 2

Treatment	Rate of Concentration in mg/L	Suckers per treatment in Flush 1	Suckers per treatment in Flush 2
Calcium chloride	250	8.00c	7.67c
Calcium chloride	500	7.83c	7.67c
Calcium chloride	750	8.17c	8.00c
Cytokinin	150	13.17b	11.00b
Cytokinin	250	15.17b	13.00b
Cytokinin	350	17.83a	15.67a
Absciscic acid	5	7.67c	7.00c
Absciscic acid	10	8.33c	8.00c
Absciscic acid	15	8.17c	8.00c
Control	0	8.33c	8.00c
	LSD	2.64	3.020
	CV	22.16	18.73

Means followed by the same letter(s) along the column are not significantly different at 5% probability level within each flush.

Table 8. Effect of calcium chloride, cytokinin and abscisic acid on the number of bent peduncles produced in flush 1 and 2

Treatment	Rate of Concentration in mg/L	Bent Peduncles in Flush 1	Bent Peduncles in Flush 2
Calcium chloride	250	2.83a	2.67a
Calcium chloride	500	2.83a	2.67a
Calcium chloride	750	2.07b	1.13b
Cytokinin	150	3.83a	3.33a
Cytokinin	250	3.83a	3.33a
Cytokinin	350	4.00a	4.00a
Absciscic acid	5	4.17a	4.33a
Absciscic acid	10	4.00a	4.00a
Absciscic acid	15	4.17a	4.67a
Control	0	4.17a	4.33a
	LSD	2.10	3.30
	CV	50.42	59.96

Means followed by the same letter(s) along the column are not significantly different at 5% probability level within each flush.

cytokinin application increased the number of suckers in the Rhodos variety in the current study. Wang et al. (2017) reported that cell division in the meristematic tissues is activated when cytokinin levels are high in a specific region of the plant, such as close to the nodes or along the stem. It is certain that because of the accelerated cell division, branching is enhanced and therefore more suckers are formed. This explains the reason for more suckers in the highest concentration of cytokinin (350 mg/L) in this study. Ragini et al. (2019) conducted a study to find out the effect of cytokinins on bulbous flower crops and observed that corms treated with cytokinin produced the maximum number of suckers per corm. While sucker's development in

bulbous crop because of cytokinin application is favourable, in tea hybrid cut roses they are not favourable (Ragini et al., 2019). This is because they grow rapidly and run the risk of starving the primary part of the plant off its nutrients and water. Consequently, the quality of the cut rose is compromised and the cost of labor increased.

3.7 Effect on Bent Peduncle

Analysis of treatment effects showed that application of calcium chloride at 750 mg/L significantly ($p < 0.05$) reduced the number of bent peduncle formation in the Rhodos variety, which promoted erect posture of the stems. 750 mg/L had the lowest number of bent peduncles

of 2.07 and 1.13 in flush 1 and 2, respectively (Table 8). Application of calcium chloride at 250 and 500 mg/L had no significant influence on the number of bent peduncles. It is conceivable that the high application of calcium chloride (750 mg/L) increased the endogenous calcium within the crop, creating strong cellular structure which countered the effect of auxins on altering plant wall plasticity. This therefore reduced the incidences of bent peduncles. Abdolmaleki et al. (2015) reported that plants suffering from a calcium deficit may exhibit physiological problems such as weakening stems and heightened vulnerability to bending of their necks. Therefore, application of calcium chloride application of calcium can reduce stem weakening and bending. Further, El-Beltagi et al. (2022) and Ahmad et al. (2025) studied that at high concentrations of calcium cut roses can create more robust and resilient cellular structures of the cell wall in their stems. Based on the findings of this study, it is doubtless that optimum stem rigidity and structural integrity with reduced bent peduncles can be ensured by preventing the signs of calcium deficiency in cut roses. The findings of this study are in agreement with those of Elshawa et al. (2023) who indicated that using calcium chloride pre-harvest treatments reduced neck bending in gerbera cut flowers.

During the entire growing, application of 150, 250, and 350 mg/L of cytokinin; 5, 10, and 15 mg/L of abscisic acid had no significant influence on the number of bent peduncles (Table 8). Auxin alters the plant wall plasticity and therefore causing cell differential growth. It is very likely that the involvement of auxins in differential growth in Rhodos variety overshadowed the activity of cytokinin and abscisic acid making them to have no effect on the bent peduncles. Prior research by Jing et al. (2020) has demonstrated that the bending is caused by relative variations in cell expansion and division on either side of the bud. Zaccai et al. (2009) also reported that the prevalence of bent peduncles is higher when plants are young and develop more quickly, primarily in the warmer months. Additionally, Philosoph-Hadas et al. (2005) reported that auxin concentrations in plants have the ability to drive differential growth, which causes bending. This is because auxin regulate plant growth, development, and reaction to external stimuli (Muday, 2001; Caumon & Vernoux, 2023, Li et al., 2025).

3.8 Effect on Flush Days

Compared to the control (0 mg/L), calcium chloride at 250, 500, and 750 mg/L, had no effect on numbers of days taken by the flowers to mature (Table 9). It is very likely that calcium chloride in the current study increased the rigidity of the cell wall and was not directly involved in influencing the growth and development of cut roses "Rhodos variety". Demidchik et al. (2018) also observed that calcium is definitively attributed to the strengthening and stabilization of the cell wall. Analysis of variance indicated that cytokinin application at 350mg/L significantly ($p < 0.05$) influenced the number of days taken by the Rhodos variety to mature. Flowers treated with 350 mg/L of cytokinin took 51.72 and 53.67 days. Compared to the control (0 mg/L), cytokinin at 150 and 250 mg/L, had no statistically significant effect on numbers of days taken by the flowers to mature (Table 9). Cytokinin promotes cell division and increase cell expansion. It is possible that at high concentration cytokinin enhanced more cell division and expansion, lengthening the vegetative phase which delayed the plant from moving to the reproductive phase. This therefore increased the number of days taken to harvest flowers treated with cytokinin. It has been reported that cytokinin essentially encourages cell division and vegetative development (Werner et al., 2021; Hussain et al., 2025), delay senescence (Mantilla et al., 2021), and change nutrient distribution (Mok, 2019) therefore lengthening the time needed to harvest.

Abscisic acid significantly ($p < 0.05$) reduced the number of days taken by the Rhodos variety to mature (Table 9). Flowers treated with 15 mg/L of abscisic acid took 49.06 and 50.67 days to mature in flush 1 and flush 2, respectively (Table 9). Abscisic acid at 5 and 10 mg/L had no effect on numbers of days taken by the flowers to mature. It is conceivable that abscisic acid accelerated the transition to the floral phase and caused a dormant-like state in the vegetative tissues to begin earlier. Because of its critical involvement in stress reactions, plants respond to environmental challenges like drought and cold by increasing their levels of abscisic acid, which signals them to preserve resources and speed up reproductive growth (Vishwakarma et al., 2017; Shu et al., 2018, Mèmonsso et al., 2023). Furthermore, abscisic acid controls stomatal closure, which aids in water management and further communicates to the

Table 9. Effect of calcium chloride, cytokinin and abscisic acid on the number of maturation days taken in flush 1 and 2

Treatment	Rate of Concentration in mg/L	Maturation Days Flush 1	Maturation Days Flush 2
Calcium chloride	250	49.78b	52.44b
Calcium chloride	500	49.89b	52.67b
Calcium chloride	750	49.67b	52.33b
Cytokinin	150	50.44b	53.00b
Cytokinin	250	50.22b	53.00b
Cytokinin	350	51.72a	53.67a
Abscisic acid	5	49.56b	52.22b
Abscisic acid	10	49.56b	52.22b
Abscisic acid	15	49.06c	50.67c
Control	0	49.78b	52.33b
	LSD	1.47	1.55
	CV	3.13	3.14

Means followed by the same letter(s) along the column are not significantly different at 5% probability level within each flush.

Table 10. Effect of calcium chloride, cytokinin and abscisic acid on the number of petals produced in flush 1 and 2

Treatment	Rate of Concentration in mg/L	Number of Petals in Flush 1	Number of Petals in Flush 2
Calcium chloride	250	35.00a	34.22a
Calcium chloride	500	35.78a	34.78a
Calcium chloride	750	34.89a	34.00a
Cytokinin	150	35.00a	34.11a
Cytokinin	250	35.11a	34.30a
Cytokinin	350	35.89a	34.89a
Abscisic acid	5	35.33a	34.33a
Abscisic acid	10	35.67a	34.67a
Abscisic acid	15	35.44a	34.44a
Control	0	35.33a	34.33a
	LSD	1.94	1.87
	CV	5.85	5.78

Means during Flush 1 and 2 were not significantly different at 5% probability level

plant that it must swiftly finish its life cycle (Abhilasha & Roy Choudhury, 2021). It is possible that the exogenous application of abscisic acid in the current study caused a dormant-like state in the vegetative tissues of the Rhodos variety and accelerated the transition to floral phase. Therefore, this reduced the number of days needed to harvest cut roses.

3.9 Effect on the Number of Petals

Analysis of treatment effects showed that the application of calcium chloride, cytokinin, and abscisic acid at all the tested rates had no significant ($p < 0.05$) effect on the number of petals (Table 10). These outcomes imply that, in

the Rhodos variety, there is no relationship between the administration of calcium chloride, cytokinin, abscisic acid treatments and the number of petals per flower bud. Additionally, genetic variables are primarily responsible of the development of petals (Zhou et al., 2025). Han et al. (2018) reported that in contrast to the impacts of the growth regulators, genetic variables regulate the expression of genes involved in petal formation, growth and pattern development. According to Hong et al., (2021) Petal specification is primarily determined by the A and B genes based on the ABC paradigm for blooming. The interaction of these genes results in the formation of the floral organs (Vasisth & Sharma, 2022). Additionally, Bowman & Moyroud

(2024) reported that the development of sepals and carpels is controlled by one class C gene and a mixture of class A gene, respectively. Together, class B and class A genes determine the development of the petals, and class B and class C genes encourage the growth of the stamens (Bowman & Moyroud, 2024).

4. CONCLUSION

From this study, it can be concluded that application of cytokinin increased the number of shoots produced per plant, stem length, leaf area, and number of suckers in cut flowers. On the other hand, Abscisic acid reduced the stem length, and leaf area. At high rates cytokinin increased the levels of chlorophyll content after first application while lower rates increased chlorophyll content after the second application. High rates of calcium chloride also increased the chlorophyll content after second application of treatment. At high rates of application, abscisic acid, significantly reduced the chlorophyll content and especially after second treatment application. It was also observed that at high rates of application, Calcium chloride reduced incidences of bent peduncles. Additionally, high rate of cytokinin increased flush days while high rate of abscisic acid reduced the flush days of cut roses. However, application of cytokinin, abscisic acid, and calcium chloride on Rhodos variety at preharvest had no effect on stem diameter, flower bud size, and number of petals.

5. RECOMMENDATION

To achieve optimal growth and quality in cut flowers, growers should adopt the application of cytokinin (250 and 350 mg/L) and calcium chloride (500 and 750 mg/L) during growth while avoiding use of abscisic acid. However, high concentration of cytokinin may increase the number of suckers present on individual stems. Additionally, the results showed that cytokinin had better growth performance than calcium chloride and abscisic acid but this cannot be fully concluded since soilless media, one variety, one type of rose, and a control was involved during the study. As a result, further study is recommended on soil media and other types of roses for a more extended duration of two years, as roses are perennial plants and longer data collection periods yield superior results.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) declare that NO generative AI technologies such as Large Language Models

(ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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