


Effect of 6-BAP application on leaf yellowing of *Chrysanthemum morifolium* Ram cv. 'Shrek' and 'Bomber Green'

Efecto de la aplicación 6-BAP sobre el amarillamiento del follaje de *Chrysanthemum morifolium* Ram cv. 'Shrek' y 'Bomber Green'

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Chrysanthemum cultivar 'Shrek'.

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ABSTRACT

Growth regulators are used in commercial production of chrysanthemums without certainty about their effects on quality characteristics, particularly sensitivity to yellowing and leaf color loss. Cytokinin (CK) may contribute to the prevention and delay of chlorophyll degradation. This study aimed to quantify the response to the application of 6-benzylaminopurine (6-BAP) on delaying foliage yellowing associated with chlorophyll loss in the chrysanthemum cultivars 'Shrek' and 'Bomber Green.' 6-BAP was applied at concentrations of 33.3, 66.6, and 100 mg kg⁻¹, alongside a control without CK and a commercial control (TC) containing CK from an algae concentrate. Applications were made at 35, 43, 51, 59, 67, and 75 days after transplanting (DAT). The relative chlorophyll content was quantified using a SPAD-502 meter in leaves from the lower, middle, and upper strata. Additionally, the longevity of the foliage from harvested stems was evaluated under simulated travel conditions and subsequent exposure in vases. The chlorophyll content differed significantly ($P<0.05$) between cultivars and strata, increasing in the middle and upper strata while decreasing in the lower strata. Vase life was shorter in 'Shrek,' at 7 d, with a foliage yellowing severity of grade 1, the onset of which occurred between 5 and 7 days with the use of 66.6 and 100 mg kg⁻¹ of 6-BAP. Moreover, 'Shrek' plants exhibited yellowing of the foliage during growth; this physiological issue may be directly related to the plant material and its interaction with the environmental conditions of the cultivation area.

Keywords: growth regulator; Asteraceae; SPAD; chlorophylls; cut flower; vase life.

RESUMEN

La producción comercial de crisantemo emplea reguladores de crecimiento sin la certeza de su efecto sobre características de calidad, principalmente en la sensibilidad al amarillamiento y pérdida del color de las hojas. El aumento de CK (citoquinina) participaría en la prevención y retraso en el inicio de la degradación de clorofila. Con el objetivo de cuantificar la respuesta de la aplicación de 6-BAP sobre el retraso en el amarillamiento del follaje asociado con la pérdida de clorofila en crisantemo 'Shrek' y 'Bomber Green'; se empleó 6-BAP a 33,3, 66,6 y 100 mgkg⁻¹, un testigo absoluto sin adición de CK y testigo comercial con CK proveniente de un concentrado de algas. Con aplicaciones a los 35, 43, 51, 59, 67 y 75 días después del trasplante (DDT). Se cuantificó el contenido relativo de clorofilas con SPAD-502, en hojas de los estratos bajo, medio y superior. Así como la duración del follaje de los tallos cosechados en condiciones de viaje simulado y posterior exposición en florero. Se encontró que el contenido de las clorofilas fue diferente ($P<0,05$) en cada cultivar y estrato e incrementó en las hojas de los estratos medio y superior mientras que, en el inferior disminuyó. La vida de florero fue menor en 'Shrek' con una duración de 7 días, severidad en grado 1 e inicio del amarillamiento entre 5 y 7 días con uso de 66,6 y 100 mg kg⁻¹ de 6-BAP. Plantas de 'Shrek' presentaron amarillamiento del follaje durante el crecimiento, esta fisiopatía podría estar ligada directamente con el material vegetal y la interacción con las condiciones ambientales de la zona de cultivo.

Palabras clave: regulador de crecimiento; Asteraceae; SPAD; clorofilas; flor de corte; vida en florero.

INTRODUCTION

The chrysanthemum (*Chrysanthemum morifolium* Ram) belongs to the Asteraceae family (Shahrajabian *et al.*, 2019). Its flower size, long post-harvest life (Khaerunnisa *et al.*, 2021; Xia *et al.*, 2021), and the demand for it as a cut flower, pot, and garden plant (Hassanein and Alástrame, 2016) make it the third most economically important ornamental species worldwide (Xia *et al.*, 2021). Colombia has around 10,000 h dedicated

to the production of more than 520 species and 1,600 varieties of cut flowers and foliage, with chrysanthemums being the third-largest export flower, mainly to the United States (ICA, 2024).

Chrysanthemums show diversity in height and growth habit (Vaghasia and Polara, 2015), so their final shape and commercial value are determined by axillary bud sprouting (Liu *et al.*, 2020). The use of plant growth regulators (PGRs) to modify growth processes, extend vase life, and delay senescence (Jamil *et al.*, 2015) is one of the main agronomic management factors in the maximization and regulation of horticultural production according to market needs (Shahrajabian *et al.*, 2019, 2021; Kentelky *et al.*, 2021; Xia *et al.*, 2021). PGRs, such as daminozide, that inhibit GA biosynthesis and alter growth by inhibiting cell elongation in the subapical meristem (Novita, 2022), could induce leaf senescence due to reduced GA3 concentration (Yu *et al.*, 2009). However, the mechanism of GA biosynthesis-mediated senescence remains unclear (Castro-Camba *et al.*, 2022; Ritonga *et al.*, 2023).

Some ornamental species are sensitive to foliage yellowing, a condition related to premature senescence (Geesta *et al.*, 2017). Increasing cytokinin (CK) concentration in plant tissues prevents the degradation of chloroplasts, nucleic acids, proteins, and other substances (Wu *et al.*, 2021; Sosnowsk *et al.*, 2023), delaying the loss of photosynthetic efficiency and contributing to the postponement of senescence (Mandal *et al.*, 2023). This is likely due to the conversion of chlorophyll *a* to chlorophyll *b*, which maintains the stability of photosynthetic complexes and prolongs the green color in the leaves (Talla *et al.*, 2016).

Foliar application of CK (6-BAP) could delay leaf senescence and ethylene biosynthesis (Iqbal *et al.*, 2017), promoting chloroplast photosynthetic activity (Hönig *et al.*, 2018; Amelia *et al.*, 2020), although the mechanisms behind this are unknown (Wu *et al.*, 2021). Nevertheless, the anti-senescence effect of CK helps to protect the photosynthetic machinery in the leaves of beans, broccoli, barley, maize, and wheat (Hönig *et al.*, 2018; Thomas & Ougham, 2014), as well as in chrysanthemum cultivars, where exogenous application of 6-benzyladenine (BA) effectively prevented foliar chlorosis related to premature senescence (Lailaty & Hartanto, 2021).

Currently, there are techniques for diagnosing photosynthetic pigments, which provide essential information on physiological responses to environmental factors and plant photosynthesis rates (Kamble *et al.*, 2015). Non-destructive methods to determine chlorophyll content allow rapid measurement and constitute a tool for improving plant production and productivity (Mielke *et al.*, 2010). The SPAD-502 chlorophyll meter generates an index for analyzing pigment amounts in leaves, calculating relative chlorophyll content (Cahyo *et al.*, 2020; Orjuela-Rodríguez *et al.*, 2024) accurately and reliably (Pinzón-Sandoval *et al.*, 2022).

The quality of a cut flower depends on external characteristics such as color and length, and on how perishable it is, which is defined by the longevity of the first two traits (Horibe, 2020). Some ornamental species show a decrease in quality a week after being harvested (Horibe, 2020), primarily due to leaf senescence, which starts with discoloration and is followed by leaf wilting. This may occur before the ornamental value of the flower heads is lost (Sedaghatthoor *et al.*, 2020), thus affecting the time that the flower stem remains in a vase, i.e., vase life (Vehniwal and Abbey, 2019; Nguyen and Lim, 2021). Factors that affect vase life quality and duration are of great interest and represent one of the most significant challenges for post-harvest producers (Geesta *et al.*, 2017; Nguyen and Lim, 2021), especially since each ornamental species has a different post-harvest behavior pattern (Nguyen and Lim, 2021).

Chrysanthemums belong to the vase life group of 2 to 4 weeks (Sedaghatthoor *et al.*, 2020); however, it has been reported that they can last 20 to 30 d (Teixeira da Silva, 2003). Nevertheless, the primary cause of foliage yellowing in chrysanthemums has not been widely explained (Geesta *et al.*, 2017). Therefore, this study aimed to evaluate the response to exogenous application of 6-BAP in terms of delaying foliage yellowing associated with chlorophyll loss in the leaf blade of chrysanthemum 'Shrek' and 'Bomber Green' plants and cut stems.

MATERIALS AND METHODS

Location

The experiment was conducted in a cultivation system under plastic covering, located on a farm owned by Sunshine Bouquet SAS in the municipality of Madrid, Cundinamarca (Colombia). The climatic conditions for the experimental site are detailed in Table 1.

Table 1. Location and climatic conditions of the cultivation system during the experiment.

Location	Madrid
Departament	Cundinamarca
Altitude (msnm)	2,585
Minimum air temperature (°C)	4
Maximum air temperature (°C)	36
Minimum relative air humidity (%)	37
Maximum relative air humidity (%)	98
PAR radiation ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	385

Plant material

Chrysanthemum plants of the 'Shrek' and 'Bomber Green' varieties, measuring 14 cm in length, were transplanted into soil at a density of 67 plants per m². The plant material was selected based on the duration for which foliage maintained optimal conditions before yellowing began during vase exposure; 'Shrek' exhibited shorter duration than 'Bomber Green'. Agronomic management of the plants followed technical recommendations used by producers, with fertilization administered through fertigation with the following nutrient levels: N: 180 ppm, P: 62 ppm, K: 200 ppm, Ca: 130 ppm, Mg: 60 ppm, S: 43 ppm, Fe: 3.0 ppm, Mn: 3.0 ppm, Cu: 0.7 ppm, Zn: 0.6 ppm, B: 1.0 ppm, and Mo: 0.1 ppm for the entire cycle.

Experimental design and treatment application

A randomized complete block design with five treatments corresponding to different concentrations of 6-BAP was used (Tab. 2). Each treatment had four replicates, and each experimental unit consisted of 100 plants. CK applications were performed as foliar sprays in the early morning hours, using a motorized sprayer from the Maruyama brand. The

application of growth regulator treatments began 28 days after transplanting (DAT). CK applications were made as foliar sprays in the early morning at 38, 53, and 63 DAT. The timing of treatment applications was based on stem length magnitude change curves (Figure 1). The flower harvest was done at 75 DAT for 'Shrek' and 80 DAT for 'Bomber Green'.

Table 2. Treatments, 6-BAP concentration (mg kg⁻¹), and DAT for application in 'Shrek' and 'Bomber Green' chrysanthemum plants. TC = commercial control.

Treatment	Concentration of CK 6-BAP mg Kg ⁻¹	Days after transplanting (DAT)		
		38	53	63
Control	0	0	0	0
1	33.3	33.3	33.3	33.3
2	66.6	66.6	66.6	66.6
3	100	100	100	100
4	Farm TC	0	400	400

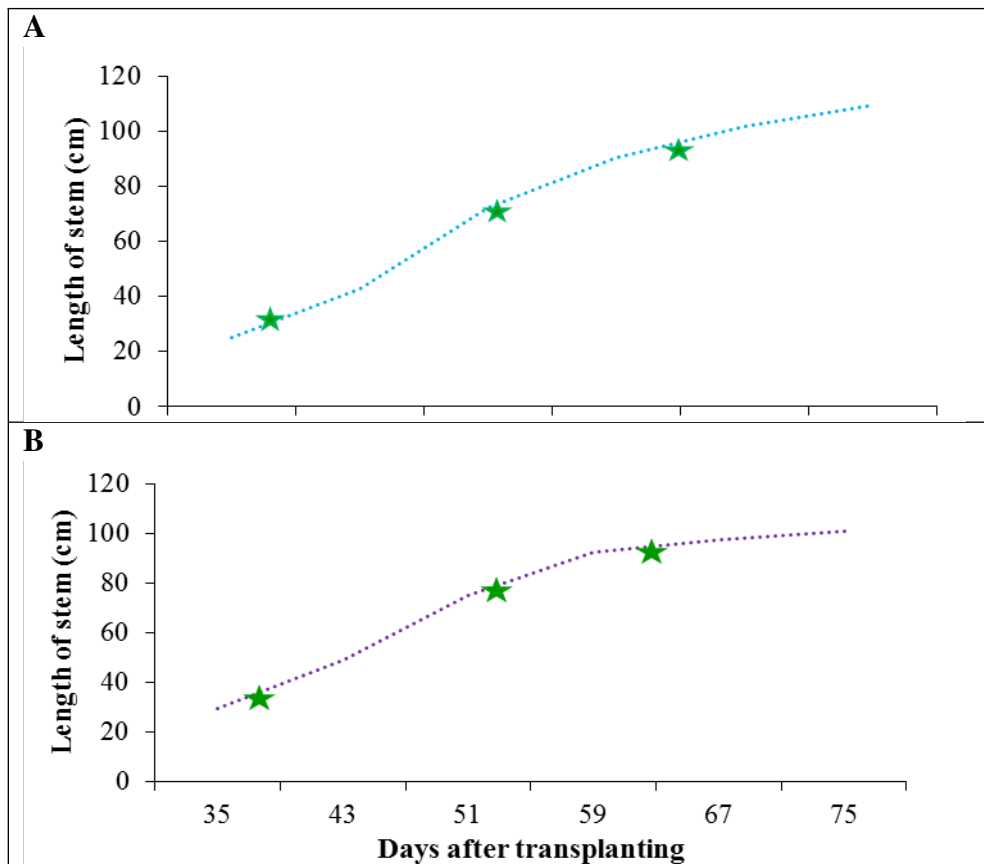


Figure 1. (A) Growth curve of chrysanthemum 'Shrek' and (B) 'Bomber Green,' with points indicating application times.

Determination of relative chlorophyll content in leaves


The chlorophyll index (relative content of chlorophyll) was determined using a portable chlorophyll meter (Chlorophyll Meter SPAD-502 Plus, Minolta Camera Co. Ltd., Japan) with the SPAD (Soil Plant Analysis Development) method (Yuan *et al.*, 2016). Three plants per treatment and chrysanthemum cultivar were used. Each plant was divided into three sections: upper, middle, and lower, and measurements were taken on fully expanded leaves. Measurements were conducted at 35, 43, 51, 59, 67, and 75 DAT, with an 8-d interval between samples.

Determination of vase life

Five stems from each of the 5 CK treatments with 4 replicates, totaling 100 stems, were taken. They were then subjected to simulated export travel conditions by being placed in darkness at 5°C for 8 d following the protocol recommended by producers. After the travel simulation phase, each bouquet was placed in a vase with tap water and mixture of the hydration solution used by producers at room temperature. Finally, foliage and flower color change were characterized, and the number of days each treatment and variety lasted without yellowing, foliage or spray burn, and leaf abscission, if these occurred, was recorded.

Severity scale for chrysanthemum foliage yellowing

A descriptive scale was developed based on the progression of leaf yellowing in chrysanthemum plants over the duration of exposure of stems in vases (Figure 2). The scale used in this trial to decide when to end the vase life evaluation was based on the producers' specifications.

Photo	Grade	Description
	0	Leaves with a solid and dark green color




	1	Leaves beginning to change color from intense green to yellow, starting at the edges. A lighter green hue is observed across the entire leaf blade.
	2	Leaves changing color from intense green to yellow, from the edge toward the center of the leaf blade. A lighter green hue is observed across the entire leaf blade.
	3	Leaves changing color from intense green to yellow over the entire leaf blade. Chlorosis begins at the leaf edge, where senescence starts to become visible.

Figure 2. Severity scale of leaf yellowing in cut stems of ‘Shrek’ and ‘Bomber Green’ chrysanthemum plants exposed to vase conditions.

Data analysis

An analysis of variance was performed and, when statistical differences were found, a Tukey's mean comparison test was conducted ($p < 0.05$). The data were analyzed using Stastix v 9.0 (Analytical Software, Tallahassee, FL, USA).

RESULTS

Chlorophyll relative content for ‘Shrek’ in the three thirds of the plants

The relative chlorophyll content in the leaves of the lower third of ‘Shrek’ chrysanthemum showed a general trend of decrease, with significant differences observed ($P < 0.05$) at 43 and 75 DAT. The application of 33.3 mg kg^{-1} of 6-BAP resulted in the highest values, while the lowest values occurred in the leaves of the control plants without CK addition (Figure 3A).

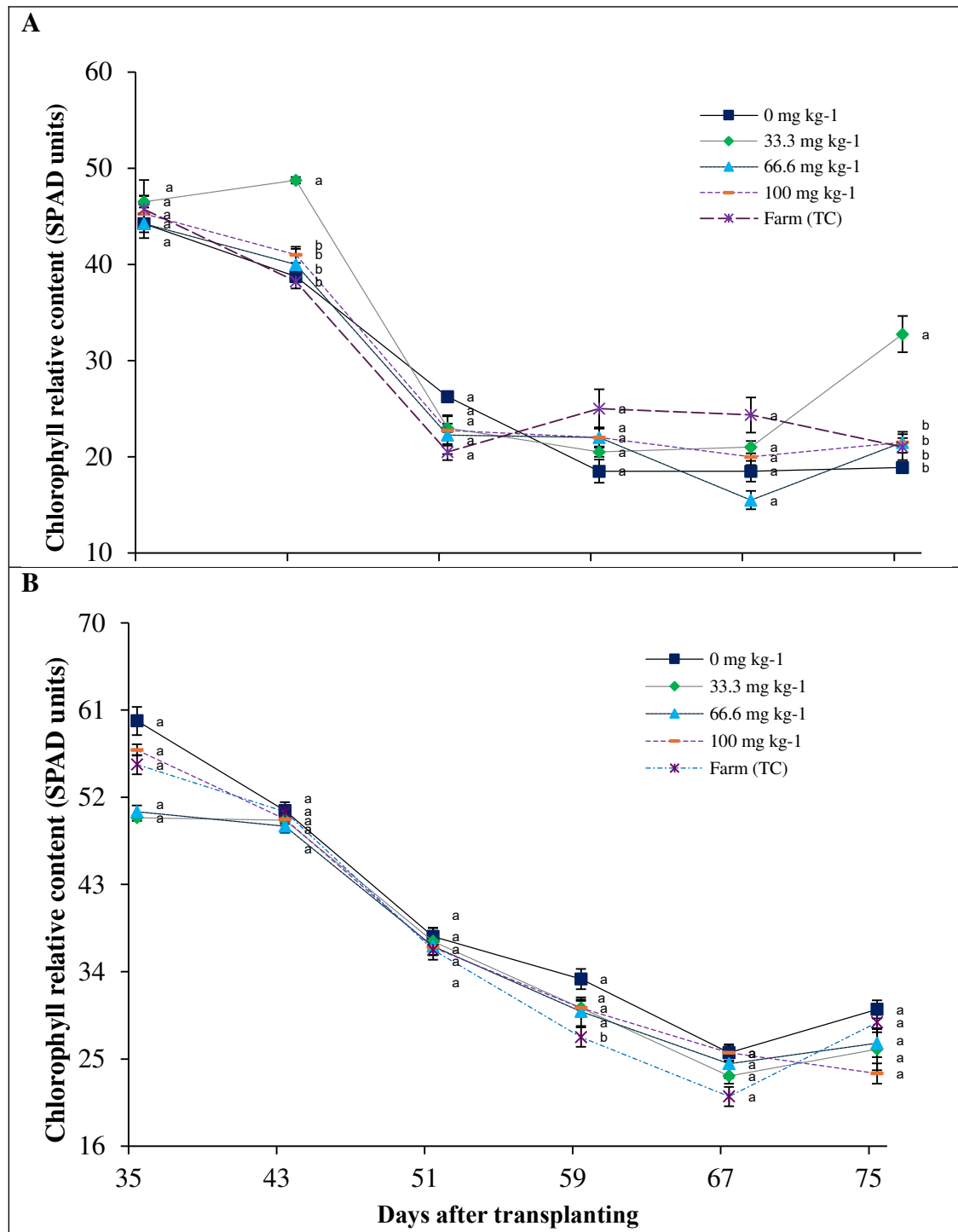
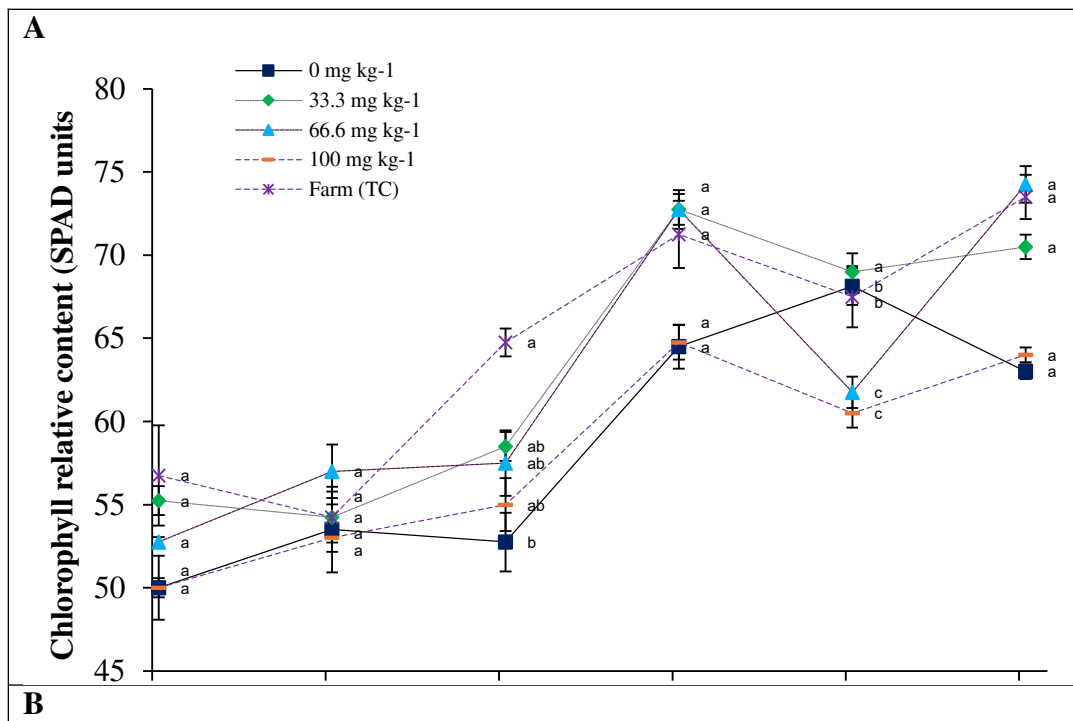


Figure 3. Chlorophyll relative content in the lower third of 'Shrek' (A) and 'Bomber Green' (B) chrysanthemum plants in response to CK application (0, 33.3, 66.6, 100 mg kg⁻¹ 6-BAP, and Farm (TC)). Different letters indicate significant differences between treatments at each sampling point according to Tukey's test ($p < 0.05$). Vertical bars on each mean indicate the standard error ($n=4$).

The relative chlorophyll content in the leaves of the middle third increased across the six evaluation points, with the highest accumulation of chlorophyll in this stratum observed at the end of 75 DAT. Significant statistical differences ($P < 0.05$) were found at 67 DAT with the use of 6-BAP at 33.3 mg kg⁻¹, yielding 69.00 SPAD units, which was a higher chlorophyll content compared to leaves without CK application. Conversely, the application of 66.6 and 100 mg kg⁻¹ of 6-BAP resulted in lower relative chlorophyll content at 61.00 SPAD units compared to the leaves of plants without the addition of an exogenous CK source, which had 68.13 SPAD units' (Figure 4A).



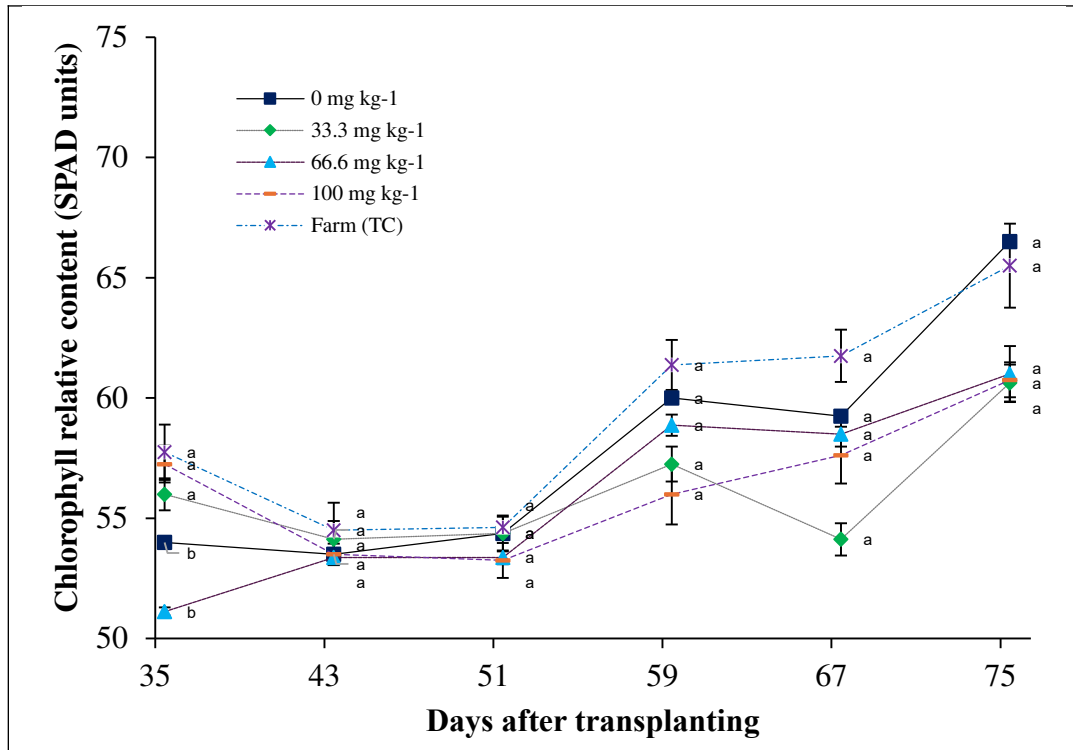


Figure 4. Chlorophyll relative content in the middle third of 'Shrek' (A) and 'Bomber Green' (B) chrysanthemum plants in response to CK application (0, 33.3, 66.6, 100 mg kg⁻¹ 6-BAP, and Farm (TC)). Different letters indicate significant differences between treatments at each sampling point according to Tukey's test ($p < 0.05$). Vertical bars on each mean indicate the standard error.

The relative chlorophyll content in the upper third increased across the six evaluation points, reaching the highest chlorophyll concentration at 75 DAT. Statistical differences ($P < 0.05$) were observed only at 67 DAT with the use of 6-BAP at concentrations of 66.6 and 100 mg kg⁻¹, yielding 86.75 and 80.75 SPAD units, respectively, which were the lowest chlorophyll concentrations at this evaluation point (Figure 5A).

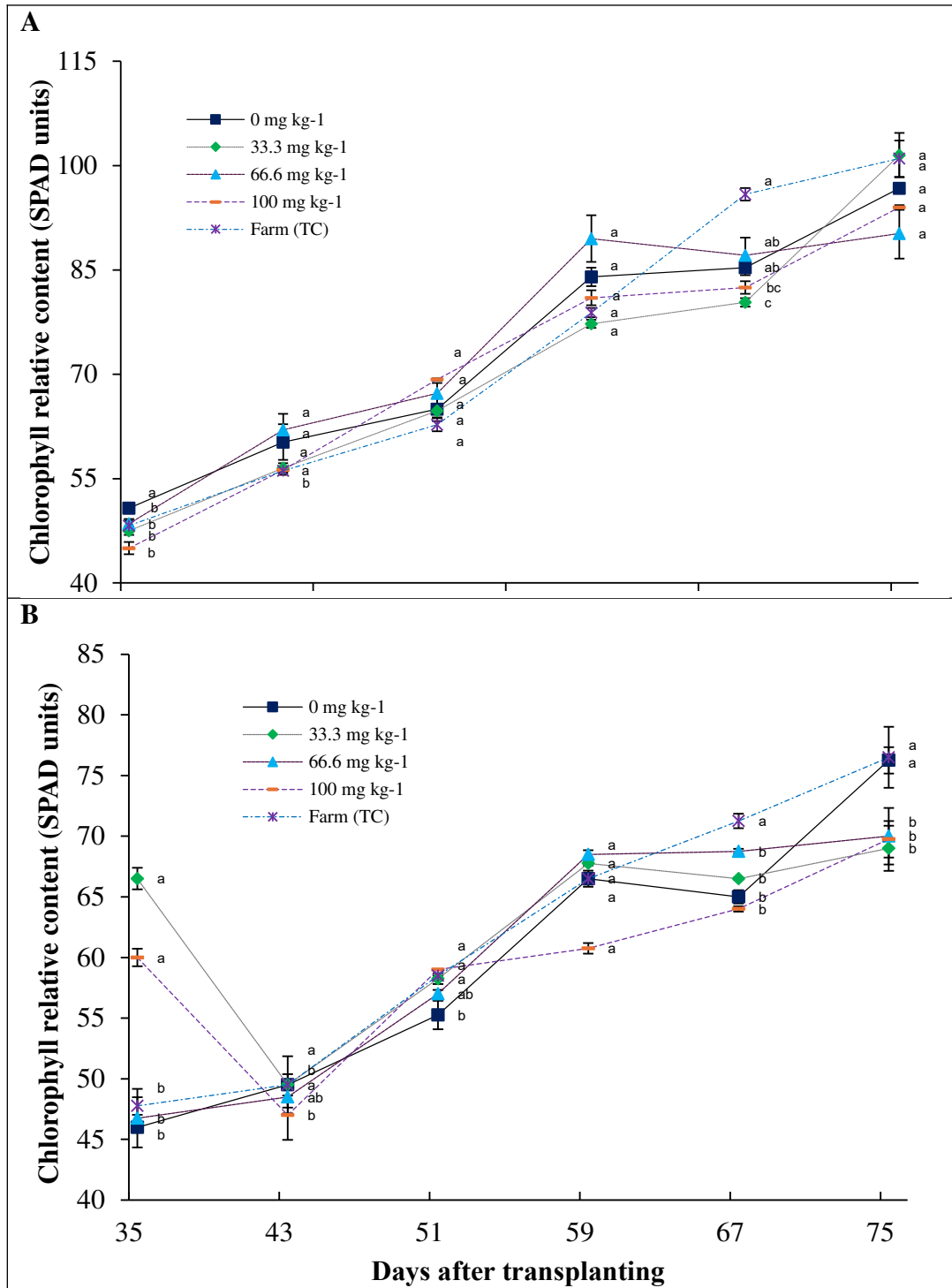


Figure 5. Chlorophyll relative content in the upper third of 'Shrek' (A) and 'Bomber Green' (B) chrysanthemum plants in response to CK application (0, 33.3, 66.6, 100 mg kg⁻¹ 6-BAP, and Farm (TC)). Different letters indicate significant differences between treatments at each sampling point according to Tukey's test (p < 0.05). Vertical bars on each mean indicate the standard error.

Chlorophyll relative content for 'Bomber Green' in the three thirds of the plants

In the leaves located in the lower third of 'Bomber Green' chrysanthemum plants, the relative chlorophyll content showed no statistical differences ($P < 0.05$) at any of the six evaluation points when using exogenous CK sources compared to control plants (without CK addition) (Figure 3B). In the leaves of the middle third of these plants, the relative chlorophyll content increased with and without CK addition, reaching the highest value for this variable at 75 DAT; however, there were no statistical differences ($P < 0.05$) in chlorophyll content between the plants subjected to treatments and the control plants without CK (Figure 4B).

At 43 DAT, statistical differences ($P < 0.05$) were found in the relative chlorophyll content in the upper third of the leaves in response to CK use and chlorophyll concentration with 6-BAP at 100 mg kg⁻¹. Meanwhile, at 51 DAT, there were statistical differences ($P < 0.05$) with the application of 6-BAP at concentrations of 33.3 and 100 mg kg⁻¹, as well as with the farm treatment (TC), where the leaves of these treatments showed higher chlorophyll content of 59.00 SPAD units (Figure 6A). At 67 DAT, statistical differences were observed in relative chlorophyll content ($P < 0.05$) in leaves treated with the farm treatment (TC) (Figure 6A). Finally, at 75 DAT, statistical differences ($P < 0.05$) were obtained in leaves treated with 6-BAP at 33.3, 66.6, and 100 mg kg⁻¹, with 69.58 SPAD units being a lower value for this variable than that recorded in leaves without CK addition (Figure 5B).

Leaf abscission in 'Shrek' and 'Bomber Green' chrysanthemum plants

In 'Shrek' chrysanthemum plants, both with and without CK application, leaf loss associated with chlorosis and senescence symptoms began at 59 DAT, with a loss of 5 to 6 leaves, increasing to a total loss of 10 to 11 leaves by the end of the evaluation at 75 DAT in both CK-added and control plants (Tab. 3). In contrast, in 'Bomber Green' plants, leaf loss due to senescence began with 2 leaves across all treatments (Tab. 3). At 67 DAT, plants treated with 33.3 mg kg⁻¹ of 6-BAP and the farm treatment (TC) lost 3 leaves, while with the other treatments only 2 leaves were lost (Tab. 3). By the end of the trial at 75

DAT, the plants treated with 33.3 mg kg⁻¹ of 6-BAP lost 4 leaves, while the other treatments resulted in a loss of 6 leaves (Tab 3).

Behavior of vase life in 'Shrek' chrysanthemum stems in response to 6-BAP application at different developmental stages

In 'Shrek' chrysanthemum plants, the vase life of cut stems treated with foliar application of 100 mg kg⁻¹ of 6-BAP was 8.7 d, longer than the 6.3 d obtained for plants subjected to the farm treatment (TF), which was the lowest value in this study. Meanwhile, stems from plants that did not receive CK application had a vase life of 7.4 d (Tab. 4). The leaves of the stems from both CK-treated and untreated plants exhibited a severity of yellowing at grade 1 when exposed to vase conditions, with slight yellowing of the leaf blade and no progression of symptoms. However, the commercial value of the stems was lost due to the degradation of the green color of the foliage (Figure 2).

Table 3. Quantification of foliage abscission (number of leaves) in 'Shrek' and 'Bomber Green' chrysanthemum plants with CK application.

Assessment / Treatment	0 mg kg ⁻¹	33.3 mg kg ⁻¹	66.6 mg kg ⁻¹	100 mg kg ⁻¹	Farm (TC)
'Shrek'					
35 DAT	-	-	-	-	-
43 DAT	-	-	-	-	-
51 DAT	-	-	-	-	-
59 DAT	6	6	5	6	5
67 DAT	8	8	7	7	8
75 DAT	11	10	10	11	10
'Bomber Green'					
35 DAT	-	-	-	-	-
43 DAT	-	-	-	-	-
51 DAT	-	-	-	-	-
59 DAT	2	2	2	2	2
67 DAT	2	3	2	2	3
75 DAT	6	4	5	5	6

Regarding the yellowing of the foliage, this began after 5 d of exposure to vase conditions, with signs of leaf blade discoloration in plants treated with 100 mg kg⁻¹ of 6-

BAP and the farm treatment (TC). In contrast, yellowing in the leaves of stems that received 33.3 mg kg⁻¹ of 6-BAP started after 7 d. However, in the leaves of control plants without CK application, yellowing began 6 d after being placed in the vase (Tab. 4).

Table 4. Days to the onset of yellowing, percentage of affection in stems, total duration of vase life, and severity grade in cut stems in response to the application of different concentrations of CK in 'Shrek' and 'Bomber Green' chrysanthemum plants.

Treatm ent	Concentration of 6-BAP (mg kg ⁻¹)	Yellowing onset (d)	Severity	Affected stems (%)	Vase life (d)
'Shrek'					
0	0	6	1	100	7
1	33.3	7	1	100	7
2	66.6	6	1	100	9
3	100	5	1	100	9
4	Farm (TC)	5	1	100	6
'Bomber Green'					
Treatm ent	Concentration of 6-BAP (mg kg ⁻¹)	Yellowing onset (d)	Severity	Affected stems (%)	Vase life (d)
0	0	12	1	25	14
1	33.3	15	0	0	15
2	66.6	15	0	0	15
3	100	15	0	0	15
4	Farm (TC)	12	2	20	14

Behavior of vase life in cut stems of 'Bomber Green' chrysanthemum in response to 6-BAP application at different developmental stages

The vase life duration of cut stems from this cultivar supplied with 6-BAP was 15 d, which is a longer duration of adequate commercial appearance, without loss of the characteristic green coloration of the cultivar, than that of control plants without CK source application, which lasted 14.3 d (Tab. 4). With the application of 6-BAP concentrations of 33.3, 66.6, and 100 mg kg⁻¹, the foliage ended its vase life without any yellowing

affectation; that is, with a severity of 0. Additionally, the foliage of the stems belonging to the farm treatment exhibited yellowing with a severity grade of 2 (Figure 3). In control stems without CK source application, as well as those treated with the farm treatment, the onset of symptoms associated with leaf yellowing began after 12 d of exposure to vase conditions. In plants without CK sources, yellowing was observed in 25% of the evaluated stems, while this was observed in 20% of plants with the farm treatment (Tab. 4).

DISCUSSION

The lower relative chlorophyll content in 'Shrek' and 'Bomber Green' in the lower leaves, along with the variation of this in the middle and upper thirds, may be related to the leaves' competition for light due to planting density, the erectophile growth habit of the stems, and low light penetration in the lower canopy, leading to mechanisms that produce lower chlorophyll content (Kamble *et al.*, 2015). However, the variation and heterogeneity in chlorophyll content, resulting from the structural organization of molecules in chloroplasts and the adaptation between and within leaves, maximize the photosynthetic rate in the canopy (Zhao *et al.*, 2019), which is a common response.

In some species, chlorophyll content is higher in the lower leaves during the vegetative phase, and this pattern is reversed at the beginning of the reproductive phase (Zhang *et al.*, 2022). This contrasts with the findings of this study, as the relative chlorophyll content in the lower stratum leaves was lower from the time of transplanting. This implies that the greening and chlorophyll concentration are specific and depend on the age of the leaf blade (Kamble *et al.*, 2015), the amount of intercepted light, and stored sugars, which are reflected in the response of chloroplasts, particularly in the early stages of aging (Zubo *et al.*, 2008). This also suggests that the developmental state of the leaf and its age are determining factors related to the effect of 6-BAP on the onset of yellowing.

The modification of chrysanthemum architecture associated with agronomic management may have increased the interception of photosynthetic active radiation in the middle and upper leaves, favoring photosynthesis (De Villa *et al.*, 2022). However, the variability in relative chlorophyll content could be related to higher light intensity

activating the defense mechanism of degrading photosynthetic pigments (Hallik *et al.*, 2009). This confirms that both leaves and chloroplasts are adapted to specific light conditions, and these changes can alter the functioning of the photosynthetic apparatus, reducing photosynthetic efficiency (Swoczyna *et al.*, 2022). Chrysanthemums exhibit specificity in their morphological response to light conditions and floral capacity (Ochiai *et al.*, 2015); thus, the variation in chlorophyll content could be linked to the cultivar and its interactions with climatic conditions during growth, determining the increase or decrease in agronomic yield, commercial value, and quality parameters of this cut flower.

Applications of CK to induce the delay of leaf aging were not effective in the lower third of the plant (Tab. 4). Similarly to findings in rose, carnation, petunia, and lily, the anti-senescence response of CK was variable and depended on the type, concentration, and developmental stage at which it was used (Chang *et al.*, 2003; Trivellini, 2014). This contrasts with findings in cilantro leaves (Aminifard *et al.*, 2020), carnation (*Dianthus caryophyllus*) (Ramtin *et al.*, 2016), and bird of paradise (*Strelitzia* spp) (Rayya *et al.*, 2015), where foliar spraying of CK (benzyladenine (BA)) increased chlorophyll and carotenoid content, as well as the number and size of chloroplasts and photosynthetic reactions.

In the evaluated cultivars, the onset of yellowing appears to be related to the duration of the leaf area of the plant and the developmental stage at which CK application was made. At around 51 DAT, the relative chlorophyll content in basal leaves began to decrease, coinciding with floral bud differentiation and the formation of secondary branches, events associated with high translocation of photoassimilates from mature leaves to newly forming and developing organs (Zhao *et al.*, 2019) or for storage (Zwack and Rashotte, 2013), determined by the source-sink relationship. Since leaves are assimilation and storage organs, the onset of senescence could be considered a transition from carbon to nitrogen source. If this demand is not met through the roots, it is drawn from more mature tissues (Davies and Gan, 2012). CKs maintain the green color of their mature leaves by inducing changes in nitrogen mobilization from leaves (Glanz-Idan *et al.*, 2022), translocating photoassimilates to the new developing structures (Guiboileau *et al.*, 2010). This suggests

that, in the evaluated chrysanthemum cultivars, in addition to CK applications, it may be necessary to review nutritional demands at each phenological stage, as optimal nitrogen content would help meet the demands of sinks, potentially delaying the senescence of the lower third leaves of the plants.

During leaf senescence, CK reduces sugar accumulation, increases chlorophyll synthesis, and prolongs leaf activity (Wu *et al.*, 2021). Previous results indicate that the distribution of carbon skeletons in these chrysanthemums primarily favors the stem; leaves may remobilize assimilates to the stem and secondary branches, promoting the senescence of mature leaves. In contrast, in the stems of chrysanthemum 'Shrek', after cutting, the demand for substrates used in respiration may have exceeded the total amount of assimilates accumulated in their storage organs, leading to rapid yellowing of the foliage and a shorter vase life compared to the 'Bomber' cultivar. As observed in cut *lisianthus* sp. and marigold plants, a high carbohydrate content in the stem positively affected flower longevity (Ahmad *et al.*, 2014). Additionally, during the post-harvest period of the cut stem, sugars are translocated from the leaves to the flower petals, enhancing the quality of the flower buds; however, when floral stems are exposed to vase conditions, yellowing of the foliage may occur, even though it has been observed that flower buds maintain their quality for a longer time. Nonetheless, more evidence is needed regarding sugar metabolism and its translocation during the post-harvest process of cut flowers (Horibe, 2020).

The delay in aging due to CK supply does not appear to correlate with the total amount accumulated in the leaf, and the molecular mechanism of CK-mediated control in leaves is still not entirely clear (Zwack and Rashotte, 2013; Glanz-Idan *et al.*, 2022). Similarly, the environmental and physiological factors related to chrysanthemum growth are not yet fully understood, although this knowledge is essential for regulating their growth and yield to promote uniformly high-quality plants for commercial production (Nakano *et al.*, 2019).

Yellowing of the foliage during the post-harvest stage is characterized by the change in color of the leaf blade, which is the most perceptible phenotypic sign (Horibe, 2020; Wu *et al.*, 2021). This leads to the activation of senescence and a reduction in the vase life of

plants (Cavalcante da Costa *et al.*, 2021). Counteracting or delaying these normal processes of reserve degradation and subsequent senescence to ensure optimal quality of the stems is a focus of interest in cut flower production. It was confirmed that the response to the use of CK in increasing vase life depends on the species and cultivar as well as the CK concentration used (Cavalcante da Costa *et al.*, 2021). The synthesis of pigments as an effect of CK application may be associated with biomass formation and the leaf's assimilation surface, correlated with the formation of the photosynthetic apparatus at the cellular and chloroplastic levels. Furthermore, the rate of leaf aging depends on species characteristics, position in the plant, and environmental conditions (Hönig *et al.*, 2018). Applications of 6-BAP may not increase carbohydrate content in the leaves, as previously found (Al-Dalwi and Al-Bakkar, 2023).

The foliage of 'Shrek' chrysanthemum plants treated with foliar sprays of 66.6 and 100 mg kg⁻¹ of 6-BAP had the longest vase life, of 9 d. These results are consistent with those found in lily plants, calla lily (Kapri *et al.*, 2018), some varieties of the Asteraceae family (El-Kinany, 2019), and saffron (Al-Saad, 2021), where the application of BA at a concentration of 100 mg kg⁻¹ extended vase life; reduced and delayed leaf abscission, lipid peroxidation in cells, and ion loss; and led to differences in vegetative growth and increased longevity of inflorescences, influenced by the timing of BAP application.

In "Punjab Shyamli" chrysanthemum plants, the application of BA did not result in yellowing of the leaves of cut stems; however, as in this study, the longest vase life was found in control plants without CK application. On the other hand, 'Bomber Green' chrysanthemum stems treated with different concentrations of 6-BAP had a longer vase life; after 13 d, the foliage showed no yellowing, fully meeting the post-harvest quality conditions required by producers. It has been found that in species such as heliconia, the application of 300 mg kg⁻¹ of BA favors vase life (Malakar *et al.*, 2023), which is different to what was found in the chrysanthemum plants used in this study.

CONCLUSIONS

Throughout the development of 'Shrek' and 'Bomber Green' chrysanthemum plants, the relative chlorophyll content increased in the leaves of the middle and upper thirds; however, the magnitude of the chlorophyll content varied by cultivar. The greatest variation in relative chlorophyll content was observed in the leaves of the lower third of the 'Shrek' cultivar after day 51 DAT, reaffirming that the onset of this physiological disorders is linked to the appearance of the floral bud and new branching. The application of cytokinins (CK) did not delay the onset of leaf yellowing in the different developmental stages of the basal leaves; however, the vase life of cut stems of 'Shrek' chrysanthemum was longer with the use of 6-BAP at concentrations of 66.6 and 100 mg kg⁻¹. Therefore, this physiological disorders is related to the plant's interaction with the environmental conditions in which it is cultivated.

Conflict of interest: The manuscript was prepared and reviewed with the participation of the authors, who declared that there exists no conflict of interest that puts at risk the validity of the presented results.

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