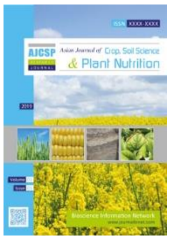
Published with Open Access at Journal BiNET
Vol. 07, Issue 01: 272-280

Asian Journal of Crop, Soil Science and Plant Nutrition

Journal Home: https://www.journalbinet.com/ajcsp-journal.html



Differential responses of Boron, Calcium and Zinc on growth and flowering of Lisianthus

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Article received: 17.06.2022; Revised: 24.07.2022; First published online: 30 August, 2022.

ABSTRACT

An experiment was conducted to assess the differential responses of Boron, Calcium and Zinc_on the growth and flowering of Lisianthus during the period from January 2021 to May 2021 at Sher-e-Bangla Agricultural University, Bangladesh. The experiment was conducted with a Complete Randomized Design (CRD) with ten replications. Treatments comprising Control, Boron (B), Calcium (Ca), Zinc (Zn), B+Ca, B+Zn, Ca+Zn, B+Ca+Zn were used in the study. Collected data on growth and flower yield attribute parameters showed significant variations. According to the results, the maximum plant height (79.1 cm), stem length (42.7 cm), Peduncle height (12 cm), Stem dia. (6.2 mm), SPAD value (60.9) and 100% rosette free plants were found from the Ca treated plants. The application of Zn also enhanced stem no. (8.9), bud no./plant (28.7), flower dia. (73.5 mm), and weight/stick (11.9 g) of the Lisianthus and decreased the rosetting rate (1.6%) compared to control. From the study, we found that the combination of B+Ca+Zn showed the minimum result on plant height (56.2 cm), stem no (4.8), bud no (16.9), peduncle height (8.0 cm), stem dia (3.6 mm), and SPAD value (52.0). So, in a nutshell, we observed that the sole application of calcium and zinc is best for enhancing lisianthus growth and flower-related attributes.

Key Words: *Macro and micro nutrients, Eustoma grandiflorum, Nandini and Cut flower.*

Cite Article: Sultana, M. N., Maliha, M., Husna, M. A., Raisa, I. and Uddin, A. F. M. J. (2022). Differential Responses of Boron, Calcium and Zinc on Growth and Flowering of Lisianthus. Asian Journal of Crop, Soil Science and Plant Nutrition, 07(01), 272-280.
Crossref: https://doi.org/10.18801/ajcsp.070122.33

I. Introduction

Lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.] is a neophyte in the domain of cultivated ornamentals (Halevy and Kofranek, 1984). In the early 1980s, Japanese producers developed and disseminated F1 hybrids, which increased consumer interest in this species (Halevy and Kofranek, 1984; Harbaugh, 2007). Lisianthus was introduced to Mexico in the late 1990s and quickly became a popular cut flower option for producers. As a result, consumers are becoming more interested in this species (Halevy and Kofranek, 1984). It is a cold-tolerant annual or biennial ornamental plant used for the cut flower and pot flower owing to its large rose-like blooms with varying size and form, long stems, and extended duration in vases (up to 3 weeks) (Roh and Lawson, 1988; Uddin et al., 2004; Shimizu and Ichimura, 2005). This series' sturdy stem and broad petals offer it a high market value in handling and transportability. Its versatility in arrangements, as well as its excellent consumer appeal, attracts florists. Plant nutrition is critical for the optimal development of flower crops, whether it is in open fields or controlled conditions. Plant growth in flower crops is severely influenced by the availability of micronutrients as well as the presence of macronutrients in adequate amounts and proportions. Soil naturally provides these trace elements, but because of intensive farming, an increase in salinity and soil pH, these nutrients are not always accessible to plants (Ahmad et al., 2010).

Boron is a critical micronutrient for plant growth (Bolanos et al., 2004). Without boron, calcium cannot be used properly, even with a sufficient supply of calcium at the roots. The B element aided the plant in absorbing more calcium in a given time or increasing the efficiency with which it is metabolized when taken (Ganmore et al., 1993). Flower production, pollen germination, fertilization, seed production and fruit abscission are all affected by it. Misra, H. P. (2001) reported that soil application of borax increased plant height, days to flower bud opening, flower count, flower weight, and chrysanthemum yield, but other parameters were not affected. So, the current study looked into the effects of B on Lisianthus.

Calcium (Ca) is another necessary nutrient for plant growth (Hepler, 2005). Calcium expedites plant growth while also strengthening the floral stem's structure. Furthermore, it improves plant resilience to microbial infections (Ustun et al. 2007). The addition of calcium boosted the diameter and plant height of Lisianthus, as well as the number of flower buds and diameter (Hernández-Pe'rez et al., 2016). So we decided to study the effect of Ca on Lisianthus in Bangladesh.

Although Zn is only minimally necessary, it is crucial for agricultural plants' physiological processes. Photosynthesis and the normal operation of enzymes are both impacted by Zn shortage. To raise the caliber of Lilium blooms, the effects of Zn were investigated. According to the findings, Zn had the largest impact on flower characteristics such as flower size, quantity of flowers, stem dia, and length (Shaheen et al., 2015). On Iris (Iridaceae) plants, foliar sprays of zinc sulfate (ZnSO4) produced similar outcomes (Khalifa et al., 2011). Plant height, branch number per plant, blooming time, and floral production were all significantly raised by soil treatment of zinc sulfate at 2.5g m-2 in chrysanthemum (Misra, 2001). Applying zinc to a tuberose cultivar's soil increased flowering time and bulb production for the cultivar Double (Yadav et al., 2002).

Lisianthus needs balanced nutrient administration in order to grow and flourish. Uneven growth and a decrease in the number of flowers produced are known physiological effects of micro- and macronutrient deficiencies. In order to investigate the varied effects of boron, calcium, and zinc on the development and flowering of Lisianthus in Bangladesh, this experiment was carried out.

II. Materials and Methods

The effects of Boron, Calcium, Zinc and their combination on the growth and flowering of Lisianthus were investigated during the period from January/2021 to May/2021 at Horticulture Farm, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The experiment was conducted in CRD Design with 10 replications. This was a single-factor experiment with eight treatments. The experiment was established in a truncated cone pot. Before filling the soil in the pot, vermicompost, sand and chemical fertilizer (Cowdung-7 ton/ha, Urea-225 kg/ha, TSP-200 kg/ha, MoP-150 kg/ha) were added to the soil. Then, the initial treatment was put on the soil before transplanting the plants. After that, the 2nd application was done at 30 DAT and the further application was done at 60 DAT in a corresponding manner. The sources of B, Ca and Zn were boric acid (17% Boron), Gypsum ($CaSO_4$) and zinc oxide (36% Zn), respectively. According to the treatment layout, 6.13 mg boric acid, 337.5 mg Gypsum and 2.3 mg ZnO were applied each time in one truncated pot containing 4.5 kg soil.

Lisianthus seeds were collected by Takii Seed, Japan. A Super Magic type Blue variety was used in this experiment. Seeds were sown in October, 2020 in plug trays (128-hole) filled with vermicompost and placed in the Nandini plant factory, which is used as the growth chamber for germination and subsequent growth of the seedlings. Critical care for proper development of seedlings was taken, and 80 days old seedlings were taken for transplanting in the field. All routine cultural practices, like watering, weeding and staking during crop growth and development, were kept the same and consistent.

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Data on different parameters of growth and flowering of treated Lisianthus like plant height (cm), stem length (cm) and peduncle length (cm) were measured using a measuring scale (Plate 01). Flower head diameter (mm) (Plate 01. C) and stem diameter (mm) (Plate 01. b) were measured using a Digital Caliper-515 (DC-515) in millimeters (mm) and the mean was calculated. SPAD value was measured using SPAD-502 chlorophyll meter (Plate 01. e). Colorimetric measurement of the experiment of the lisianthus flower was done using CIELAB Colorimeter colorimeters (Shenzhen Wave) following L* (Lightness), a* and b* (two Cartesian coordinates) including C* and *hab* (chroma & hue angle) based on the CIELab scale with standard observer 10" and standard illumination D65.

Plate 01. Photographs showing, a. Measurement of plant height using measuring scale after transplanting. b. Measurement of stem diameter using Digital Caliper-515, c. Measuring flower head diameter using Digital Caliper-515, d. Measurement of plant height using a measuring scale after 30 days of transplanting, e. SPAD value measurement using SPAD-502 chlorophyll meter, f. Harvesting

Statistical analysis: The data collected for each parameter was statistically analyzed using Statistix-10 scientific analysis software to determine the significance of variation among treatments, and the treatment means were compared using the Least Significant Difference (LSD) test at the 5% level of probability

III. Results and Discussion

Plant height

Plant height showed significant variation in the performance of B, Ca, Zn, and their interactions at 30 to 100 days after transplanting. The maximum plant height was found in Ca-treated plants at 30 DAT (11.4 cm), 40 DAT (15.9 cm), 50 DAT (18.3 cm), 60 DAT (25.4 cm), 70 DAT (37.3 cm), 80 DAT (47.8) and 90 DAT (64.3 cm) (Table 01). After 100 days of transplanting, the mean plant height ranged from 55.2 to 79.1 cm and the tallest plant was found in Ca (79.1 cm), while the shortest one was recorded in B+Zn (55.2 cm), which is statistically similar to B+Ca+Zn (56.2 cm). These results are closely related to Bolanos et al. (2003), who said that adding Ca to the nutrition solution made the flowering stem longer and wider. These results also agree with Ehret et al. (2005) they stated that adding B and Ca to the soil made the rose stems grow longer and wider. In 1993, Ganmore-Neumann and Davidov discovered that increasing the Ca level only causes roses to grow faster if the B level is also changed.

Stem length

Stem height of Lisianthus varied under study, exposing significant variations (Table 02). The longest stem was recorded in the Ca application (42.7 cm) and the shortest stem was found in the B+Zn (32.8 cm), Control (33.0 cm) and B+Ca+Zn (33.4 cm) applications (Plate 2. b). Shams et al. (2012) observed the same results in the case of Rose. They claimed that the B and Ca concentrations substantially impacted flowering stem length, but their combination had no effect. Our results were also in line with

EISSN 2706-5510

those of Volpin and Elad (1991), who concluded that the greenhouse rose's Ca treatment raised the concentration of stem, leaf and flower.

Number of stems/plants

The number of stems per plant varied significantly depending on the application of B, Ca and Zn (Table 02). The maximum number of stems (8.9) per plant was recorded in Zn treated plant, while least number (4.8) was observed in B+Ca+Zn, which is statistically similar to control (4.9) and the combination of B+Ca (5.1). According to Shams et al. (2012), Ca treatment had a large impact on the quantity of blooming stems of Rose, despite the B concentration.

Table 01. Effects of Boron, Calcium and Zinc and their interactions on plant height of Lisianthus on different days after planting

Treatments	30 DAT	40 DAT	50 DAT	60 DAT	70 DAT	80 DAT	90 DAT	100 DAT
Control	7.1de	12.9b	14.2bc	17.0cd	24.1cd	33.7e	48.2e	61.9d
B	9.5b	11.5b	14.3bc	21.5b	31.9ab	40.8c	59.9b	75.5ab
Ca	11.4a	15.9a	18.3a	25.4a	37.3a	47.8a	64.3a	79.1a
Zn	10.1b	15.1a	16.6ab	23.5ab	32.6ab	42.9b	59.8b	72.8bc
B+Ca	7.4cd	9.5c	12.0cd	17.5c	28.3bc	40.9c	55.7c	70.5c
B+Zn	6.1ef	8.8c	11.5cd	14.5de	20.5d	28.5g	41.6g	55.2e
Ca+ Zn	8.1c	11.8b	13.0cd	15.5cde	25.6cd	38.8d	52.7d	70.7c
B+Ca+Zn	5.4f	8.1c	11.0d	13.4e	21.8d	30.6f	44.6f	56.2e
LSD	0.95	1.86	3.03	2.68	6.01	1.74	2.35	3.96
CV %	4.13	5.6	7.72	5.11	7.64	1.62	1.55	2.06

Here, B: Boron, Ca: Calcium, Zn: Zinc; Means with similar letters in a column are statistically identical, while means with distinct letters differ significantly at 0.05 probability.

Stem diameter

Stem diameter was significantly affected by the B, Ca and Zn applications. The thickest flower stem was recorded in Ca (6.2 mm), which is statistically similar to Zn (5.9 mm) and B+Zn (5.9 mm), where the minimum was observed in control (3.7 mm) and B+Ca+Zn (3.6 mm) (Table 02). Stem diameter is a crucial factor in determining the sturdiness of the stem that will support the flowers. When collected for the vase, a larger diameter ensures a greater surface area for water absorption, extending the vase's life. Anuradha and Narayanagowda (2002) stated that the diameter of the stalk directly affected the production of cut flowers. In the case of Rose, Shams et al. (2012) found the same results. They said the flowering stems of the plants treated with calcium were the thickest.

Leaf width

Lisianthus leaf width varied significantly depending on the application of B, Ca, Zn and their combinations (Table 02). The maximum width of leaf (4.9 cm) per plant was observed in Zn-treated plants, while Ca + Zn (4.6 cm) and B + Zn (4.4 cm) also showed wider leaves than others. Moreover, the lowest width (3.9 cm) was recorded in control, which is statistically similar to the B (3.9 cm) treated plant.

Table 02. Effects of Boron, Calcium, Zinc and their interactions on growth related parameters

Treatments	Stem length (cm)	Stem no/plant	Stem diameter (mm)	Leaf length (cm)	Leaf width (cm)	Rosetting (%)
Control	33.0d	4.9e	3.7d	7.0e	3.9e	58.7a
B	38.9bc	6.9cd	4.3c	8.0cd	3.9e	30.3c
Ca	42.7a	8.1ab	6.2a	8.6ab	4.1cde	0.0e
Zn	37.0c	8.9a	5.9a	8.2bc	4.9a	1.7e
B+Ca	30.3e	5.1e	4.7c	9.0a	4.0de	9.3d
Ca+ Zn	40.6ab	7.4bc	5.4c	7.9cd	4.7ab	39.3b
B+Zn	32.8d	6.4d	5.9a	7.1e	4.4bc	6.0de
B+Ca+Zn	33.4d	4.8e	3.6d	7.5de	4.4bcd	30.3c
LSD	2.14	0.82	0.45	0.57	0.4	6.25
CV %	2.1	4.44	3.2	2.58	3.3	10.06

Here, B: Boron, Ca: Calcium, Zn: Zinc; Means with similar letters in a column are statistically identical, while means with distinct letters differ significantly at 0.05 probability.

Leaf length

Application of B, Ca, Zn and their combinations demonstrated statistically significant variation in the leaf length of Lisianthus (Table 02). The highest leaf length was recorded at 9.0 cm in the B+Ca application, which is very close to Ca (8.6 cm) and Zn (8.2 cm). On the other hand, the lowest leaf length (7.0 cm) was recorded in control, which was statistically similar to B+Zn (7.1 cm). So, in terms of leaf length, the combination of B+Ca and single application of Ca and Zn resulted in the maximum leaf length of Lisianthus, whereas control and the combination of B+Zn showed the minimum leaf length (Plate 2. a).

(a)

(b)

Plate 02. Photographs showing, (a) Variation of Leaf length and width under different treatments, (b) Variation of stem length under different treatments (Here, C_0: Control, B: Boron, Ca: Calcium, Zn: Zinc).

Rosette percentage

Variation in Rosette % was observed during the study of different Treatments**.** The minimum rosetting percentage was found in Ca (0.0), which is statistically similar to Zn (1.7) application and the highest percentage (58.7) was observed in control. We know that stress conditions induce rosetting. So, our findings are correlated with the research of Plieth (2005), who stated that calcium is required in various metabolic pathways as a secondary messenger for the defensive response to abiotic stresses.

SPAD value

Due to the application of B, Ca, Zn and their combinations, a significant variation in SPAD value were noted (Figure 01). Ca (60.9) showed the highest SPAD percentage, whereas B + Ca + Zn was the lowest (52.0). According to Jiang and Huang (2002), the effects of zinc on the amount of highest SPAD boosted wheat yield and its components. As the amount of chlorophyll in the environment increases, so does the amount of photosynthesis. Paul and his colleagues had a similar experience in 2016. The presence of chlorophyll in leaves increases photosynthetic activity, resulting in the production of carbohydrates that provide energy for expanding buds, flower opening and floral lifetime. These combined variables produce robust and long flower stalks, huge buds and high-quality blooms (Tarannum and Naik, 2014).

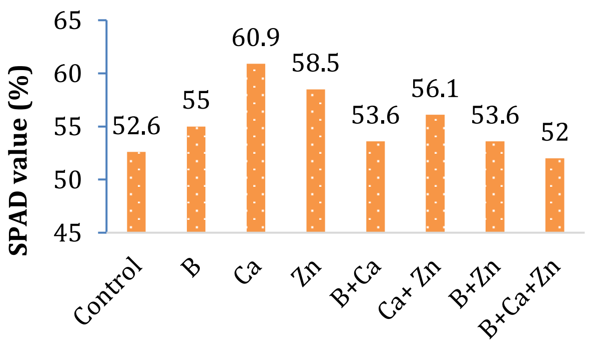


Figure 01. Performance of Boron (B), Calcium (Ca), Zinc (Zn) and their combinations on SPAD value % of lisianthus leaves

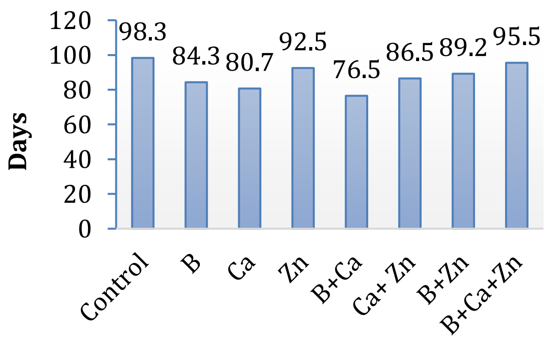


Figure 02. Effect of Boron (B), Calcium (Ca), Zinc (Zn) and their combinations on days to flower bud initiation

Days to Flower Bud Initiation

The application of B, Ca, Zn and their combinations resulted in a considerable variation in the number of days needed for floral bud initiation (Figure 02). B+ Ca had the lowest number of days (76.5) needed to initiate flower buds, while the maximum days (98.3) needed in control. This result partially correlates with Mohammadi et al. (2019). They claimed that while foliar spraying nano ZnO delayed flowering time, nano $CaCO_3$ reached the flowering stage and bloomed roughly 15 days sooner than the control plants. They claimed that blooming was delayed by zinc oxide, whether it was nano or not.

Bud number/Plant

The number of flower buds/plant of Lisianthus varied significantly among the application of B, Ca, Zn and their combinations under study (Table 03). The highest number of flower buds/plant was observed in Zn (28.7), where Ca application plants show the 2nd highest no of buds (25.2) and the Lowest no was recorded in B +Ca +Zn (16.9). These findings are similar to Misra et al. (2001), who claimed that soil-applied zinc sulfate dramatically increased chrysanthemum flower yield. According to research by Yadav et al. (2002), applying zinc to the soil caused tuberose cultivars to blossom more frequently and produce more bulbs. Frett et al. (1988) also reported that calcium application in Lisianthus resulted in a high bud count.

Table 03. Effects of Boron, Calcium and Zinc and their interactions on different yield attributing parameters

Treatments	Bud/Plant	Flower dia (mm)	Peduncle length (cm)	Weight/stick (g)
Control	20.5cd	57.2d	8.7ab	5.7e
B	22.1c	63.2bc	11.3ab	7.1d
Ca	25.2b	71.9a	12.0a	10.7b
Zn	28.7a	73.5a	9.0ab	11.9a
B+Ca	17.9de	64.8bc	8.7ab	6.8d
Ca+ Zn	19.5cde	66.1b	12.0a	10.1b
B+Zn	18.2de	70.4a	11.2ab	8.5c
B+Ca+Zn	16.9e	62.1c	8.0b	6.9d
LSD	2.97	3.73	3.44	1.13
CV %	4.98	2.0	12.03	4.74

Here, B: Boron, Ca: Calcium, Zn: Zinc; Means with similar letters in a column are statistically identical, while means with distinct letters differ significantly at 0.05 probability.

Number of flowers/plants

Number of flowers/plants of Lisianthus also varied because of the application of B, Ca, Zn and their combinations. (Figure 03). The Ca treatments showed the highest number of flowers (15.8), while the 2nd highest was found in Zn (12.7) and the lowest was observed in Ca + Zn (6.4). The findings of this study are consistent with a study by Shaheen et al. (2015), who examined the effect of Zn fertilizer on the quantity of lilium flowers, claiming that these components boosted the number of flowers.

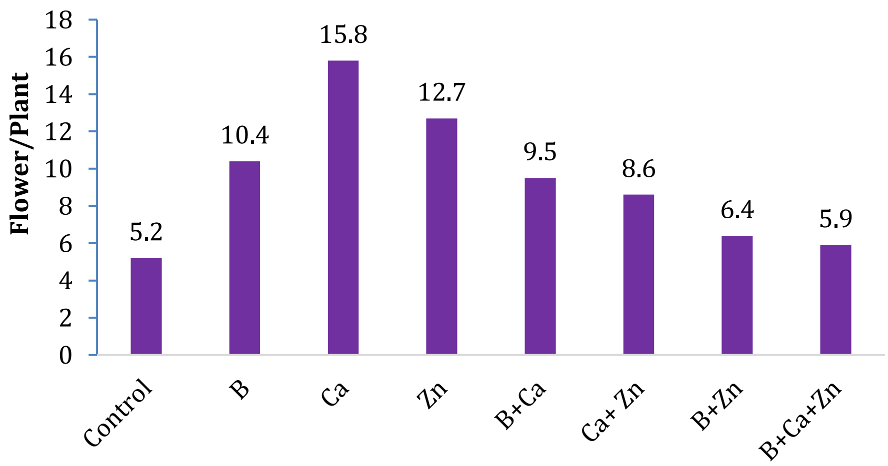
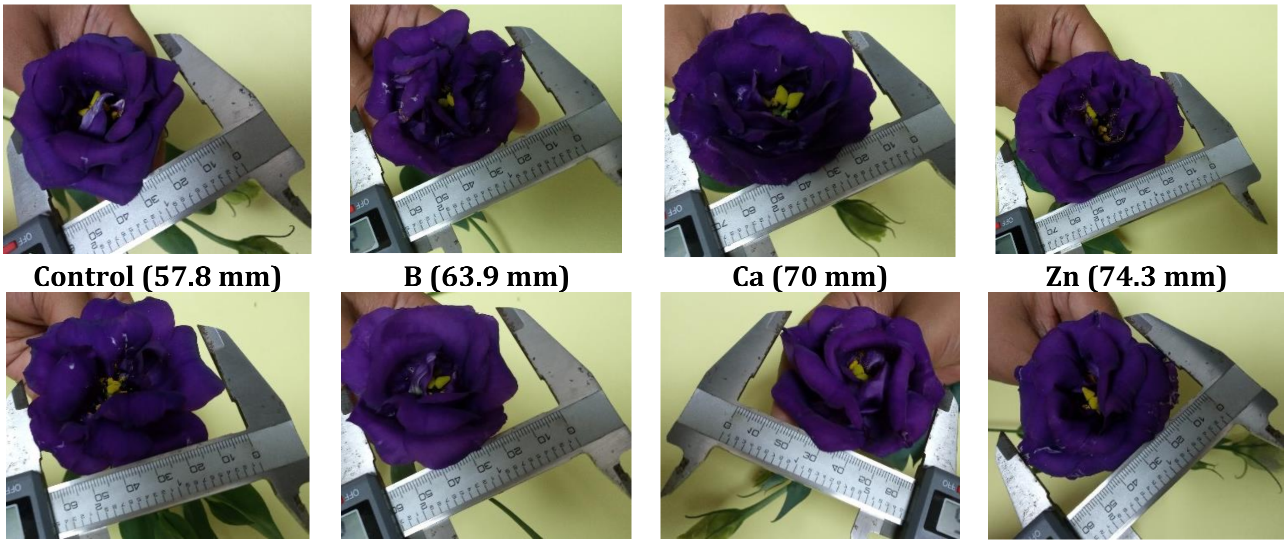


Figure 03. Effect of Boron (B), Calcium (Ca), Zinc (Zn) and their combinations on flower no/plant

Flower head diameter

Flower head diameter varied significantly among the application of B, Ca, Zn and their combinations under study. When examining several treatments after full bloom, variations in flower head diameter were seen (Plate 3). Zn exhibited the largest flower head diameter (73.5 mm), which is statistically similar to Ca (71.9) and B+ Zn (70.4), whereas control showed the smallest (57.2 mm) (Table 03).



Control (57.8 mm) | B (63.9 mm) | Ca (70 mm) | Zn (74.3 mm)

B+Ca (64.2 mm) | B+Zn (69.5mm | Ca +Zn (66mm) | B+Ca+Zn (62.1mm)

Plate 03. Photographs showing differential responses of Boron (B), Calcium (Ca) and Zinc (Zn) on the flower head diameter

Peduncle length

In terms of peduncle height, there was significant variation in the performance of B, Ca, Zn and their combinations (Table 03). The maximum peduncle height (12 cm) was observed in Ca and Ca+Zn treated plants. While B + Ca + Zn had the smallest Peduncle length (8 cm).

Weight/stick

Application of B, Ca, Zn and their combinations had a substantial impact on weight/stick (Table 03). The highest weight/stick (11.9 g) was found during Zn application, and the lowest weight/1 stick (5.7 g) was discovered during control conditions. Ca (10.7 g) and Ca + Zn (10.1 g) combinations also produced better results.

Colorimetric measurement of Lisianthus under study using CIELab

The colorimetric measurement of the lisianthus varieties with the application of B, Ca, Zn, and their combinations were conducted using a precision colorimeter IWAVE WF32 (Shenzhen Wave) and L* (lightness), a* and b* (two Cartesian coordinates) including C* and *h*ab (Chroma & Hue angle) based on CIELab scale with standard observer 100 and standard illumination D65 (CIE, 1986; McGuire, 1992). The maximum for L* is 100, representing a perfect reflecting diffuser. The respective data for each of the treatments are presented in Table 04. In presence of B and Ca the petal lightness (b*) decreases, it means the flower petal color becomes darker. Furthermore, Table 04 showed that the petal color became more vivid (a*) with the presence of B+Ca+ Zn.

Table 04. Effects of Calcium, Boron and Zinc on variations in petal color attributes in lisianthus flower.

Treatments	L*	a*	b*	C*	*hab*
Control	44.0	33.2	-16.6	37.1	333.5
B	38.2	32.9	-18.2	37.6	331.0
Ca	51.8	32.0	-12.4	34.3	338.8
Zn	37.9	36.8	-14.6	39.6	338.4
B+Ca	39.7	34.7	- 8.5	35.7	346.2
B+Zn	46.9	33.8	-12.3	36.6	337.6
Ca+ Zn	47.4	31.0	-13.9	33.9	335.8
B+Ca+Zn	31.0	36.8	- 20.1	41.9	331.3

Here, B: Boron, Ca: Calcium, Zn: Zinc; L*, Lightness; a* and b**, chromatic components; and C*, chromas (brightness); *hab,* hue angle(degree)= tang (b*/a*)

IV. Conclusion

Results revealed that applying calcium and zinc suits Lisianthus plant growth and flowering. In comparison to other treatments in the study, the application of calcium increased plant height, stem length, peduncle length, stem diameter, flower diameter and SPAD value and Zn increased stem no., bud no., flower dia., and weight/stick. We found rosette free plants from Ca treatment also. These findings may provide a source of significant information that may be used in developing sustainable techniques for producing lisianthus flowers in Bangladesh. More studies are required to investigate the level and role of Macro and Micronutrients in physiological processes of Lisianthus during the growth period.

Acknowledgement

We would like to thank 'Ministry of Science and Technology', Bangladesh and the 'Krishi Gobeshona Foundation (KGF)' for their financial support of this research.

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HOW TO CITE THIS ARTICLE?

MLA

Sultana, M. N. et al. "Differential Responses of Boron, Calcium and Zinc on Growth and Flowering of Lisianthus". Asian Journal of Crop, Soil Science and Plant Nutrition, 07(01), (2022): 272-280.

APA

Sultana, M. N., Maliha, M., Husna, M. A., Raisa, I. and Uddin, A. F. M. J. (2022). Differential Responses of Boron, Calcium and Zinc on Growth and Flowering of Lisianthus. *Asian Journal of Crop, Soil Science and Plant Nutrition*, 07(01), 272-280.

Chicago

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Harvard

Sultana, M. N., Maliha, M., Husna, M. A., Raisa, I. and Uddin, A. F. M. J. 2022. Differential Responses of Boron, Calcium and Zinc on Growth and Flowering of Lisianthus. Asian Journal of Crop, Soil Science and Plant Nutrition, 07(01), pp. 272-280.

Vancouver

Sultana, MN, Maliha, M, Husna, MA, Raisa, I and Uddin, AFMJ. Differential Responses of Boron, Calcium and Zinc on Growth and Flowering of Lisianthus. Asian Journal of Crop, Soil Science and Plant Nutrition, 2022 August 07(01), 272-280.