Iraqi Journal of Agricultural Sciences –2026:57(5):1489-1497
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/8gyy7117

INFLORESCENCE TRAITS OF *STEVIA REBAUDIANA* BERTONI AFFECTED BY PLANTING DATE AND FOLIAR BORON APPLICATION

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ABSTRACT

A field experiment was conducted at the agricultural research station, to study the effect of planting dates (three spring dates: February 15, March 1, and March 15), and spray with boron at three concentrations (0, 20, and 30 mg L^{-1}, and 10) on the inflorescence traits of the sweet leaf plant (Stevia). The results showed some floral vital parameters superiority, especially the anthesis date, number of inflorescences on the plant, inflorescence diameter, pollen viability percentage (in *vitro*), pollen germination percentage of (in *vitro*), and boron concentration in the flower ovary (221.00 days, 3963 inflorescences $plant^{-1}$, 7.922 mm, 91.67%, 47.33%, and 51.10 mg L^{-1}, respectively). It could be conclude from this study the significant impact of changing planting dates and boron concentrations on anthesis dates, pollen vitality, fertility percentages, and seeds produced, as inflorescence parameters increased with delaying planting dates to mid-March and increasing boron concentrations.

Keywords: pollen vitality, fertility percentage, temperatures, nutrients.

Received: 28/8/2023, Accepted: 31/12/2023, Published: 30/5/2026

INTRODUCTION

The importance of the sweet leaf plant (Stevia rebaudiana Bertoni) is due to its dried leaves containing many essential amino acids. It is considered a source of folic acid, ascorbic acid, vitamin B, vitamin C, and vitamin B2, in addition to the elements such as potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), iron (Fe), and zinc (Zn) (17). Due to its medical and economic importance, producing this promising raw material for zero-calorie sugar has expanded in countries where its cultivation is successful .The flowering system in the stevia plant is one of the most sensitive systems to environmental conditions and planting dates, which is ultimately a result of the number of daylight hours. Anthesis occurs within 46 days at 11 hours of daylight, while the flowering period was prolonged to 96 days when the daylight was 12 and a half hours. (Abdul-Qader and Rabie, 2019) found that the sweet leaf plant is an obligate short-day plant. (Benelli et al, 2017) referred to the phenomenon of protandry (pollen grains maturation and release before the stigma maturity) in the florets of the sweet leaf plant, indicating that cross-pollination is predominant in the plant above and is often mediated by insects (Entomophile), which affects the flowers, fertility rates and the number of formed seeds. (Raina et al, 2013) noticed that the behavior of chromosomes in the stevia plant was abnormal when pollen vitality decreased, as there was a defect in the non-pairing of some chromosomes in the fourth and fifth stages (diplotene and diakinesis) of meiosis I, which causes sterility in the stevia plant pollen grains (Farco and Dematteis, 2014). The flowering date and duration are among the primary factors for normalizing and completing the plant's life cycle in a specific environment; hence, changing the planting date has a direct effect on the number of hours of heat accumulation,

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which is reflected in the vegetative growth and anthesis of a plant (Hussein, 2011). Studies have shown that boron plays a crucial role in the growth and germination of pollen grains. When boron is absent from the growth medium, pollen grains tend to shrink and dry up. This element contributes to the nutrition of pollen grains, and its presence stabilizes the pollen tube, and improvement number of fruits plant and total yield thereby stimulating the proliferative process (Abdullateef et al, 2011). Accordingly, boron has a positive relationship with the number of flowers and the percentage of non-aborted flowers. Boron deficiency directly affects pollen production and its performance in seed formation, vitality, and germination (Islam et al, 2018). Boron deficiency can cause flowers to drop before they even open and decrease the setting rate in higher plants because the setting process is sensitive to boron deficiency. On the other hand, having enough boron can help increase flower production and improve pollen germination and pollen tube growth, so the flowering stage is clearly affected by this element. The high pH Iraqi soil reduces the availability of diboron, exposing it to stabilization and sedimentation processes. Hence, its absorption decreases, and the plant does not benefit from it sufficiently (Rose et al, 2023). Most of the treatments adopted by previous studies to increase the vitality of seeds and their field establishment are based on treating the seeds immediately before planting, and this indicates that the problem exists in the seeds themselves before or during harvest since plants in the flowering and seed formation stages are exposed to many stresses (Al-Khafaji, 2021) Therefore, adding foliar reduces this element's losses. Crops generally respond to different concentrations of boron and the appropriate stage for its spray. Based on the information provided, the objective of this study was to investigate the significance of planting dates and foliar nourishment with boron on the flowers, the vitality of pollen grains, and its impact on the fertility of the seeds produced by the stevia plant.

MATERIALS AND METHODS

A field experiment was conducted at the agricultural research station affiliated with Al-Khasib Agricultural Equipment Company located at Yusufiyah, at latitude 33 and longitude 44 south of Baghdad, aiming to study the effect of planting dates and spraying with boron on the flowering traits of the sweet leaf plant (Stevia) *Stevia rebaudian* Bertoni. The experiment was conducted according to a randomized complete block design (RCBD) within a split plot arrangement, with three replicates and involved two factors. The main factor (Main plot) was the planting date of seedlings, as it included three spring dates: February 15, March 1, and March 15, which were distributed randomly among the complete plots and coded as D1, D2, and D3, respectively. The secondary factor (Sub plot) was boron, which included three levels (0, 10, 20, and 30 mg L^{-1}) symbolized by B0, B10, B20, and B30, respectively. Thus, there were experimental units (4×3×3) 72 total experimental units, each containing 32 plants. The six-week-old tissue seedlings were obtained from Jannat Al Nakheel Company for plant tissue culture. Fertilizers were added as recommended by Verma et.al (2020) with a fertilizer combination that included adding urea in an amount of 160 kg ha^{-1} as a source of N in three batches during the plant growth stages, and P_2O_5 fertilizer was added as a total amount of 180 kg ha^{-1} at the planting, as well as K_2O fertilizer at 300 kg ha^{-1}. The soil was moistened using a drip irrigation system before planting the seedlings, and then immediately after planting, the plants were irrigated. The seedlings were prepared beforehand and planted in four lines per experimental unit. There was a distance of 25 cm between plants and 50 cm between lines, resulting in 32 plants per treatment. Boron solutions of boric acid H_3BO_3 were prepared by calculating the amount of boric acid containing boron with a weight of 04.0 grams, relying on the molecular weight of boric acid, which is 62 grams, and the molecular weight of boron is 11 grams. Then, boron concentrations were prepared by diluting the above concentration.

Floral life parameters (Inflorescence parameters): Five plants were selected from the midline of each experimental unit for each replicate randomly, and the following traits were calculated:

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Days from planting to 50% anthesis: The number of days was calculated from planting until plant flowering by 50%.

Number of inflorescences.plant^{-1}: The number of inflorescences of all plants was calculated and averaged per plant.

Inflorescence diameter (mm): The diameter of the inflorescence was measured from the widest area with a vernier after blooming for five of each experimental unit. Percentage of pollen viability using acetocarmine dye in-*vitro*: Five flowers at the anthesis stage were selected from a group of randomly selected flowers from all experimental units and for each replicate, and the anther contents were emptied onto a glass slide with 1-2 drops of acetocarmine dye on it and examined under a compound microscope. The number of pollen grains stained with the dye, as well as the total number of pollen grains in the slide, was counted by five readings of the glass slide; the average was calculated, as well as the percentage of pollen vitality according to the following equation:

Pollen vitality= Number of pollens dyed with acetocarmine/ number of pollens per reading x 100…….. (Hussein, 2011)

Percentage of pollen germination in-*vitro*:

Germination medium preparation: To prepare the media, 20g of sucrose ($C_{12}H_{22}O_{11}$) and 50 mg of boric acid (H_3BO_3) were weighed and dissolved in distilled water, then the solution volume was completed to 100 ml. five flowers were selected from a group of randomly selected flowers from each experimental unit and for each replicate during the blooming stage. The contents of the anthers were emptied of pollen; then they were placed on a glass slide on which 1-2 drops of germination medium were placed, and then they were placed in Petri dishes containing moistened towel paper. Next, they were kept at room temperature for 24 hours, after which 1-2 drops of acetocarmine dye were added to stop the reaction and stain the germinated pollen grains and tubes; finally, the pollen germination percentage was calculated according to the following equation:

Pollen germination percentage= Number of germinated pollen grains/number of total pollen grains in the slide × 100 (Matlob and William, 1974).

Estimation of boron in ovaries: Boron concentrations in the ovary florets were estimated using the wet digestion method and measured with a spectrophotometer.

Statistical analysis: The data were statistically analyzed using the Genstat software (version 20.1). The means were compared according to the least significant difference (L.S.D) test at the probability level of P<0.05. Correlation analysis was also conducted between the studied traits based on the computer, and the correlation coefficient was found using the Genstat software (Kadem and Abed, 2018).

RESULTS AND DISCUSSION

Days from planting to 50% anthesis (days): The third planting date (D3) was characterized as taking the most significant number of days (239.25 days) to flower by 50% compared to the second and first planting dates (D2 and D1), which recorded less number of days 224.50 and 209.58 days, respectively for flowering by 50% (Table 1). The third planting date had fewer days because of late planting, decreased solar brightness, and lower temperatures in the fall season. These factors forced the plant to enter the flowering stage earlier. On the other hand, the first and second planting dates had a more extended period of growth, resulting in the highest number of days recorded, possibly due to planting the seedlings early (Yadav et.al, 2014). Results in the Table (1) also showed superiority in the treatment of boron spray B30, giving the least number of days to anthesis on plant by 50%, taking 221.00 days compared to the other treatments and the control treatment, as the treatments of control and the boron at the concentration of 10mg.L^{-1} had the highest number of days to reach anthesis by 50%, taking 226.00 and 226.78 days, respectively, without any significant difference between them. This effect of boron on the anthesis date is due to its inducing and stimulating role for growth and development and in synthesizing protein, as well as its influential role in cell division through activating some enzymes and regulating the effectiveness of plant hormones. Boron is responsible for shortening the vegetative period by promoting early plant

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growth, strong root growth, and increased root nodules. It also regulates subsequent growth stages of vegetative growth and is involved in synthesizing proteins, amino acids, and some vitamins. Ultimately, these physiological roles affect the process of stimulating flowering and transforming vegetative buds into floral ones (Jayaprakash et.al, 2018). The interaction between planting dates and boron spray treatments, had insignificant effect on the number of days to 50% anthesis

Table 1. Effect of planting date and foliar spray with boron on the number of days to 50% anthesis

Planting date	Boron concentration (mg L^{-1}) B0	B10	B20	B30	Mean
D1	241.00	240.00	239.00	237.00	239.25
D2	226.00	227.00	224.00	221.00	224.50
D3	211.00	213.33	209.00	205.00	209.58
L.S.D 0.05		N.S			
Mean	226.78	226.00	224.00	221.00	1.34
L.S.D 0.05		1.55			

Number of inflorescences per plant (inflorescences.plant^{-1}): Data in Table 2 shows that the late planting date (D3) had the largest number of inflorescences (4257 inflorescences plant^{-1}) compared to the early dates, especially D1, which produced 3320 inflorescences plant^{-1}. The increases in the inflorescences number per plant is due to the vegetative growth parameters, especially the number of main and secondary branches and the suitability of weather conditions prevalent during this date, precisely temperature, leading to an increase in the efficiency of the photosynthesis process and the transfer its products to the various plant parts, leading ultimately to an increase in the number of inflorescences per plant. This result is consistent with what was mentioned by Hussein (2011), as the number of inflorescences produced by the plant varies according to planting dates. The results in the Table also showed the superiority of the boron spray treatment B3, giving the highest number of inflorescences per plant, which had 3963 inflorescences $^{-1}$, compared to the other boron treatments as well as the control treatment B0, which produced with the lowest number of inflorescences, amounting to 3556 inflorescences $^{-1}$. The effect of boron could be due to its role in increasing vegetative growth parameters, especially the number of main and secondary branches, the number of leaves, and leaf area, which led to an increase in the efficiency of photosynthesis and the transfer of products to the various plant parts, resulting ultimately in an increase in the number of inflorescences per plant and number of inflorescences in the plots. In addition, the peduncles receive the appropriate nutrition due to the transfer of the assimilated materials to sinks, which contributed to their increases, formation, and proper growth (Islam et.al, 2018). The results also showed that the differences between the interaction treatments were insignificant in the number of inflorescences per plant.

Table 2. Effect the planting date and spray with boron on the number of inflorescences

Planting date	Boron concentration (mg L^{-1}) B0	B10	B20	B30	Mean
D1	3166	3213	3446	3455	3320
D2	3548	3669	3769	3872	3714
D3	3955	4090	4422	4562	4257
L.S.D 0.05		N.S			
Mean	3556	3657	3879	3963	74
L.S.D 0.05		85			

Inflorescence diameter (mm)

Results Table (3) shows that the late planting date (D3) was superior, producing the highest inflorescences diameter average, reaching 7.208mm, differing significantly from the other two dates, as the early date (D1) recorded the smallest inflorescence diameter, averaging 5.192mm (Table 3). The superiority

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of the late planting date in inflorescence diameter is due to the suitable environmental conditions, especially the optimal temperatures that were available during this date, which were reflected positively in vegetative growth parameters and increased the efficiency of photosynthesis and the transfer of products to the active regions, hence increasing cell division and expansion, which led to an increase in diameter; or perhaps the size of the inflorescence is due to the interaction of the environmental factor with the genetic factor related to the genotype (Al-Khafaji, 2021). Results in the Table (3) demonstrate the superiority of the boron spray treatment B3 in producing the largest inflorescence diameter, averaging 7.922mm, compared to the other boron treatments and the control treatment, which recorded the lowest diameter reaching 4.156 mm. It became clear from field observations that the best umbel architecture of the stevia plant is represented by its large diameter and giving many complete flowers inside its inflorescences, as this type of inflorescences is characterized by the fact that they are not compact and are permeated by air, which facilitates the movement of pollinators within them and thus increases the percentage of set as well as inflorescence strength due to the strength and thickness of its peduncle makes it not susceptible to breakage or dispersal. This distinguished umbel architecture was obtained at the highest boron concentration (Hussein, 2011). The interaction between the late planting date and spraying with boron at the highest concentration (D3B30) recorded the highest inflorescence diameter of 10,067 mm, compared to the lowest inflorescence diameter recorded in the first planting date treatment with no boron-added D1B0, which amounted to 3,900 mm.

Table 3. Effect of planting date and boron spray on the inflorescence diameter average

Planting date	Boron concentration (mg L^{-1}) B0	B10	B20	B30	Mean
D1	3.900	4.400	6.067	6.400	5.192
D2	4.100	4.900	6.200	7.300	5.625
D3	4.467	5.600	8.700	10.067	7.208
L.S.D $_{0.05}$		0.517			
Mean	4.156	4.967	6.989	7.922	0.258
L.S.D $_{0.05}$		0.298			

Pollen viability percentage in *vitro*

Late planting date (D3) was characterized by producing the highest percentage of pollen viability, reaching 93.08%, compared to the early date plants (D1), which had the lowest percentage, reaching 86.75% (Table 4). The late date superiority could be due to suitable the weather conditions and the positive effect of temperature and humidity during plant flowering during the late planting, which positively affected the stamens' function in producing pollen with high vitality and without exposing plants to environmental stress, in particular temperature (Rane et.al, 2022), Beltrán et.al (2019) reported that it is the most crucial main factor in its influence in this aspect. The results also showed superiority in the boron spray treatment B30 by giving it the highest percentage of pollen vitality, to (91.67%) compared to the other boron treatments and the control treatment B0, which recorded the lowest percentage, amounting to 89.11%. Increasing the vitality of pollen grains in *vitro* is due to the role of nutrients, especially boron, which is essential in stimulating vital reproductive processes, including increasing the vitality of pollen grains and helping the plant withstand various harsh conditions during the flowering stage. (Amanullah et.al, 2010). The results showed did not significant differences in the interaction between the two factors of planting dates and boron spraying in the percentage of pollen vitality.

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Table 4. Effect of planting date and spraying with boron on the pollen viability percentage in *vitro*

Planting date	Boron concentration (mg L^{-1}) B0	B10	B20	B30	Mean
D1	85.67	86.00	87.33	88.00	86.75
D2	90.00	90.00	91.00	92.00	90.75
D3	91.67	91.67	94.00	95.00	93.08
L.S.D $_{0.05}$		N.S			
Mean	89.11	89.22	90.78	91.67	1.05
L.S.D $_{0.05}$		1.22			

Percentage of pollen germination in-*vitro*

Results in Table 5 demonstrate the superiority of the late planting date (D3), recording the highest pollen germination percentage, reaching 48.42%, compared to the other two date, where the early date (D1) gave the lowest percentage, recording 18.50%. The differences in the in-*vitro* pollen germination percentage could be due to the genetic nature of the variety and the extent to which it is affected by temperature; such an explanation is consistent with what was stated by. Rose et al (2023), who suggested that the percentage of pollen tube germination and growth is affected by temperature depending on the variety, as some varieties that are affected and others are not affected by temperature change (Souza et al,2022). The data from the Table indicates that the boron treatment B30 was superior, with the highest percentage of pollen germination at 47.33%, compared to the other boron treatments and the control treatment, which showed the lowest percentages reaching 14.22%. This impact of boron could be due to its role in pollen tube growth (pollen germination), as boron facilitates the germination of pollen grains on the stigmas, initiates pollen tube formation (Budeguer et al,2018), and directs its growth (directional cue) through what is called chemotropism or the chemotactic, meaning that the growth of the pollen tube is attracted towards the highest concentrations of boron, starting from the stigma, where the concentration is lowest, and down to the ovary, where the concentration is highest (calcium gradient), in addition to boron exceptional importance in nourishing the pollen grains, as its deficiency leads to their shrinkage and failure pollen tube to grow (Abdullateef et al, 2012). Results also exhibit significant differences in the variation of boron concentrations in pollen tube growth in *vitro*; however, the pollen tube constitution is still followed by difficulties in seed growth, despite the high percentage of their viability percentage, which may reach 90%, and this difference between their viability and pollen tube growth, denoting the presence of abnormalities in pollen grains. The interaction treatment between the late planting date and spraying with boron of the third concentration (D3B30) recorded the highest pollen germination percentage of 65.00%, compared to the lowest percentage resulting from the combination of the early planting date treated with distilled water (D1B0), which had to 8.00%.

Table 5. Effect of planting date and spraying with boron on pollen germination percentage in-*vitro*

Planting date	Boron concentration (mg L^{-1}) B0	B10	B20	B30	Mean
D1	8.00	12.00	25.00	29.00	18.50
D2	14.00	20.00	42.00	48.00	31.00
D3	20.67	49.00	59.00	65.00	48.42
L.S.D $_{0.05}$		2.04			
Mean	14.22	27.00	42.00	47.33	1.02
L.S.D $_{0.05}$		1.17			

Boron concentration in the stevia plant ovary (mg kg^{-1}): Plants of the late date (D3) had the highest average of boron concentration in the ovary, reaching 45.57 mg kg^{-1}, which differed significantly from the other dates in which the early date (D1) gave the lowest concentration, recording 37.02 mg kg^{-1} (Table 6). This differences could be due to the effect

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and the environmental conditions prevailing during the late planting date were optimal in giving the highest boron content (Majkowska et al, 2014). The results show that increasing sprayed boron at treatment B30 was superior, giving it the highest concentration of boron in the flower ovary, amounting to 51.10 mg kg^{-1}, compared to the other boron treatments, in particular the control treatment B0, which had the lowest concentration, amounting to 29.28 mg kg^{-1}. The increased boron concentration in the flower ovary is due to spraying with a higher concentration of boron, which increases the accumulation of the element in the vegetative parts and its transfer to the florets. The combination of the late planting date and spraying with boron at the highest concentration (D3B30) recorded the highest concentration of boron in the ovary, amounting to 57.10 mg kg^{-1}, compared to the lowest concentration found in the first planting date treatment with no boron added (D1B0), which amounted to 25.60 mg kg^{-1}.

Table 6. Effect of planting date and spraying with boron on boron concentration in the stevia plant ovary

Planting date	Boron concentration (mg L^{-1})				Mean
	B0	B10	B20	B30	
D1	25.60	36.50	40.57	45.40	37.02
D2	28.50	39.40	44.50	50.80	40.80
D3	33.73	42.70	48.73	57.10	45.57
L.S.D $_{0.05}$		0.97			
Mean	29.28	39.53	44.60	51.10	0.48
L.S.D $_{0.05}$		0.56			

CONCLUSIONS

From the results of this study, we conclude the significant impact of changing planting dates and boron concentrations on anthesis dates, pollen vitality, and the fertility percentage of the produced seeds, as flowering parameters increased with delaying planting dates to mid-March. Boron, in all concentrations, especially the highest concentrations, also increased these parameters. These findings lead us to recommend delaying the planting of this crop within the environmental conditions of the central Iraq regions and testing the dates that follow in the future, in addition to adopting fertilization with boron at a concentration of 30 mg L^{-1} to increase seed production.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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Iraqi Journal of Agricultural Sciences –2026:57(5):1489-1497
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI:

صفات التزهير لنبات ستيفيا *rebaudian Bertoni* المتأثرة بموعد الزراعة والرش الورقي بالبورون

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المستخلص

نفذت تجربة حقلية في المحطة البحثية الزراعية في اليوسفية التابعة الى شركة الخصيب للتجهيزات الزراعية التي تقع جنوب بغداد بهدف دراسة تأثير مواعيد الزراعة (ثلاثة مواعيد ربيعية هما: 15 شباط و1 أذار و15 اذار)، والرش بالبورون (وشمل على ثلاثة تراكيز 0 و10 و20 و30 ملغم لتر$^{-1}$)، في الصفات الزهرية لنبات ورق السكر (الستيفيا). أظهرت النتائج بعض المؤشرات الحياتية الزهرية لاسيما موعد التزهير وعدد النورات في النبات وقطر النورة والنسبة المئوية لحيوية حبوب اللقاح (in *vitro*) والنسبة المئوية للإنبات حبوب اللقاح (in *vitro*) وتركيز البورون في المبيض الزهرة (221.00 يوم ،3963 نورة نبات$^{-1}$، 7.922 ملم، 91.67%، 47.33 %، 51.10 ملغم لتر$^{-1}$) على التتابع. نستنتج من هذه الدراسة التأثير الكبير لتغيير مواعيد الزراعة وتراكيز عنصر البورون في مواعيد التزهير وحيوية حبوب اللقاح ونسب الخصب والبذور المنتجة، اذ ارتفعت مؤشرات التزهير مع تأخير مواعيد الزراعة الى منتصف شهر اذار وزيادة تراكيز البورون.

الكلمات المفتاحية : **Stevia rebaudian Bertoni، حيوية حبوب اللقاح، نسبة الخصب، درجات الحرارة، المغذيات.**