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EFFECT OF PLANTING DATE, SPARYING WITH KINETIN ON VITALITY AND VIGOUR OF SEEDS OF *STEVIA REBAUDIANA* BERTONI

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ABSTRACT

A laboratory experiment was carried out in the Seed Technology Laboratory of the Field Crops Department - College of Agricultural Engineering Sciences - University of Baghdad- Al-Jadriyah, aiming to study the effect of planting dates and spraying with kinetin and the interaction between them on the vitality and vigor parameters of the resulting (*stevia rebaudina)* plant seeds. The experiment was implemented according to the completely randomized design (CRD) with three replications comprising two factors. The first factor represented the dates of planting seedlings, as it included three spring dates: February 15, March 1, and March 15,. The second factor was spraying with three concentrations of kinetin (25, 50, and 75mg L^{-1}), in addition to the control treatment of spraying with distilled water only, referred to as K_0. The results showed that the late planting date was superior in the first count and the second count in the standard laboratory germination test, radical length, plumule length, seedling dry weight, cold test, and seedling vigor index (21.50%, 55.56%, 15.936 mm, 8.719 mm, 3.494 mg, 24.23%, 1374.0 mg, respectively). The highest concentration of kinetin in the first count and the second count was superior in the standard laboratory germination test, radical length, plumule length, seedling dry weight, cold test, and the seedling vigor index (20.00%, 49.58%, 14.998 mm, 7.745 mm, 2.758 mg, 20%, 1170.5 mg, respectively). Based on the findings of this study, altering the planting date and using kinetin spray significantly impacts the viability of the resulting seeds. Delaying the planting date until mid-March and using the highest concentration of kinetin (75 mg L^{-1}) has been observed to increase the seed viability.

Keywords: *Stevia rebaudian* Bertoni, plant growth regulators, temperatures, seed germination.

تأثير موعد الزراعة والرش بالكاينتين في حيوية وقوة بذور الستيفيا *Stevia rebaudiana* **Bertoni**

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الخلاصة

نفذت تجربة مختبرية في مختبر تكنولوجيا البذور التابع لقسم المحاصيل الحقلية ـ كلية علوم الهندسة الزراعية ـ جامعة بغداد- الجادرية، بهدف دراسة تأثير مواعيد الزراعة والرش بالكاينتين في حيوية البذور والتداخل فيما بينهم في مؤشرات حيوية البذور لنبات ورق السكر. اجريت التجربة وفقأ لتصميم تام التعشية (CRD) بواقع ثلاثة تكرارات، مثل العامل الأول مواعيد زراعة الشتلات، إذ ضم ثلاثة مواعيد ربيعية هما: 15 شباط و1أذار و15 اذار والعامل الثاني الرش بثلاثة تراكيز من الكاينتين وهي (25 و50 و75 ملغم لتر$^{-1}$) فضلاً عن معاملة المقارنة الرش بالماء المقطر فقط بينت النتائج تفوق موعد الزراعة المتأخر في العد الاول والعد الثاني في فحص الانبات المختبري القياسي وطول الجذير والرويشة والجاف للبادرة والفحص البارد ودليل قوة البادرة 21.50%، 55.56%، 15.936ملم، 8.719 ملم، 3.494 ملغم ، 24.23% ،1374.0ملغم على التتابع . وتفوقت التركيز الاعلى من الكاينتين في العد الاول والعد الثاني في فحص الانبات المختبري القياسي وطول الجذير والرويشة والوزن الجاف للبادرة والفحص البارد ودليل قوة البادرة 20.00%،

49.58 %، 14.998ملم، 7.745ملم، 2.758 ملغم، 20%، 1170.5 ملغم على التتابع. يستنتج من نتائج هذه الدراسة الدور البارز لتغير موعد الزراعة والكاينتين في حيوية البذور الناتجة والتي ارتفعت مع تأخر موعد الزراعة الى منتصف اذار والرش بالتركيز الاعلى من الكاينتين (75 ملغم $لتر^{-1}$).
الكلمات المفتاحية: ستيفيا، منظمات النمو النباتية، درجات الحرارة ، انبات البذور.

INTRODUCTION

The medicinal importance of the sweet leaf plant is due to the high content of a very sweet group of calorie-free compounds in its leaves (**Al Amrani *et al.*, 2018**), which is called steviol glycosides, extracted and purified from the leaves of this plant (Al-Hamdani,2015). Among the most important compounds of steviol glycosides are Stevioside and Rebaudioside. Despite the importance of this plant medically and nutritionally, as well as its high economic feasibility (**Javed & Yücesan, 2022., Azzam *et al.*,2021**), There are not enough previous studies dealing with growing this plant and its seed production in the field of Iraq because of the problem this plant suffers from which is the rapid loss of its seeds' vitality, and leading to a decrease in its germination percentage and field emergence (**Yadavannavar *et al*.,2021**) resulting in reducing the crop growth and productivity, at the same time, the reliance on tissue culture to propagate the sweet leaf plant greatly increases the cost of its production. All studies and treatments mentioned formerly relied on treating the seeds immediately before planting (**Gawa & Cheyed, 2017**) indicating that the problem exists in the seeds before or during harvest (**Aziz & Al-Taweel, 2019**) and dates of their inflorescence and seed formation may coincide with dramatic temperature rises as the seeds at this stage are exposed to a lot of from stress(**Abdul-Qader & Rabie, 2019**). The effect of heat stress can be overcome or reduced by determining the appropriate planting date. The difficulty of its reproductive life characterizes the sweet leaf plant due to its many complexities and contradictions. Although its flowers are hermaphrodite, the plant requires cross-pollination. It also exhibits excessive sensitivity to various environmental factors, including photoperiod length (**AL-Taweel *et al.*,2021**). some growth regulators interaction (Kinetin) and cadian concentration have a significant effect on some characteristics under study (**Hashim&. Ahmed, 2017**). leaf area, plant dry weight, adicle length, stalk length, seedling strength index (**Al-Karawi1& Al-Jumaily , 2023., Hussain & AL-Rawi, 2023**), Plant growth regulators and nutrients increase the photosynthesis efficiency and thus affect inflorescence, seed formation, the number weight, and size of seeds formed, and their impact on seed vigor (**Ali& Abraheem, 2023**). Kinetin is a types of cytokinin that plays an important role in influencing the morphological, physiological and biochemical characteristics (**Al-Obaidi & Nazim, 2017**). It affects the translocation of nutrients towards the regions treated with as for they are of high metabolism, in addition to its effects on the development of flowers and fruits and encouraging cell division (**Ali& Abraheem, 2023**), as well as the photosynthesis properties of stevia and the germination of the resulting seeds (Mohammed & Ahmed, 2016). Therefore, this study aimed to determine the optimal date for planting stevia seedlings, evaluate the impact of spraying them with the growth regulator kinetin, and assess the quality of the resultant seeds and their performance in laboratory and field conditions.

MATERIALS AND METHODS

A laboratory experiment was carried out in the Seed Technology Laboratory of the Field Crops Department- College of Agricultural Engineering Sciences- University of Baghdad - Al-Jadriyah, aiming to study the effect of planting dates and spraying with kinetin and the interaction between them on the vitality and vigor parameters of the resulting Stevia plant seeds. The experiment was implemented according to the CRD with three replications comprising two factors. The first factor represented the dates of planting seedlings, as it included three spring dates: February 15, March 1, and March 15, symbolized as D_1, D_2, and D_3, respectively. The second factor was spraying with three concentrations of kinetin 25, 50, and 75mg L^{-1}, symbolized as K_{25}, K_{50}, and K_{75}, respectively, in addition to the control treatment of spraying with distilled water only, referred to as K_0. Tissue seedlings were obtained from the Jannat Al Nakheel Company for Tissue culture. The tissue seedlings were grown in dishes, and the plants were acclimatized inside a lath house prepared for this purpose until the seedlings became six weeks old. Then, they were transferred and planted in soil on the dates referred to above. Kinetin solution was prepared by dissolving 1 gm of kinetin powder in distilled water with 50% ethanol as a dissolution catalyst and 3-5 drops of HCl at a temperature of 50 °C. The volume was then completed to 1 liter to reach a concentration of 1000 mg L^{-1}. After that, the required concentrations were prepared. The studied traits included the following:

Standard laboratory germination test

From all the sprayed treatments in the field experiment, 200 seeds were taken at a rate of 50 seeds in each replicate. These seeds were distributed on germination papers, which were wrapped and inserted into clean nylon bags to maintain their humidity; then, they were placed in a germinator at a temperature of 25±2°C for twelve days, which is the period for experimenting.

First count for the standard laboratory germination test (%)

The number of germinated seedlings was calculated in each replicate on the seventh day after planting, and the results were converted into percentages (**Rossi *et al*., 2018**), according to the following law:

Germination percentage at the first count= (number of germinated seeds after seven days/number of total seeds) x 100

Final count for the standard laboratory germination test (%)

Final count= (number of germinated seeds after twelve days/number of total seeds) x 100 (**Rossi *et al*., 2018**)

Radical length

Ten natural seedlings were taken after the end of the examination period (ten days), then the radical was separated from its point of contact with the seed, and the plumule was separated from its point of connection with the epicotyl, then the length of the root and the shoot were measured separately with a ruler (**ISTA, 2023**).

Seedling vigor index

It was calculated based on the germination percentage in the final count and the length of plumule and radical, which were measured previously, according to the following equation:(**Murti *et al*., 2004**)

Seedling vigor index = germination percentage in the final count (%) x [radical length (cm) + plumule length (cm)].

Cold test

An even amount of sand and clean field soil free of waste, in which the experiment was carried out, was mixed in a ratio of 1:1 to cover the seeds after planting (**ISTA, 2023**). The resulting soil was moistened with cold water at 10°C. Then, 200 seeds were planted in four replicates, 50 seeds in each, using germination paper moistened with cold water at 10°C. After that, they were placed in the germinator at 10±0.5°C for seven days. After the cold treatment period ended, the temperature was changed to 25±1°C for four days, i.e., temperature and humidity of the standard germination test. After the end of the period, 11 days, Appendix (11-B), the number of normal seedlings was later counted. The percentage of germination under the conditions of this test was calculated according to the equation:

% of normal seedlings in cold test = (number of normal seedlings / total number of seeds) x 100.

Statistical analysis

The data were statistically analyzed using the GenStat Release 10.3DE software, and the value of the least significant difference (LSD) was calculated to compare the arithmetic means of the treatments at the 5% level (**Al-Obaidi &Nazim, 2017**).

RESULTS AND DISCUSSION

First count for the standard laboratory germination test (%)

The planting late date (D3) gave the highest germination percentage in the first count, amounting to 21.52%, differing significantly from the other dates. In contrast, the early date plants (D1) gave the lowest average for the trait, amounting to 7.64% (Table 1). The superiority of the third planting date in the standard laboratory germination percentage in the first count for stevia plant seeds and its development may be due to the optimal environmental conditions it provides during the plant's growth period, which is reflected positively in plant possession of potential energy and the seedlings capability (**Al-naqeeb *et al*.,2018**) who found that the planting date of the plant has a positive role in improving seed germination in the early growth stages. Results in the table showed that spraying with kinetin at a concentration of 75 mg L^{-1} gave the highest germination percentage in the first count, reaching 19.96%, with a significant difference from the other kinetin treatments as well as the control treatment, which gave the lowest percentage of 12.17%. The superiority of spraying treatment with kinetin in increasing the germination percentage in the first stage may be due to its role in transferring micronutrients among plant cells and tissues and increasing seeds' ability to germinate. Alternatively, perhaps the noticeable increase in the physiological parameters of germination with treatment with a high kinetin concentration may be due to its positive effect on the plasma membrane and several metabolic processes in the cell, as kinetin regulates the transport mechanism in the plasma membrane and activates plant hormones involved in increasing cell division and size. This result is consistent with what was mentioned by. (**Lucho *et al*.,2021**). Results in Table 1 were characterized by the absence of significant differences between the treatments of the binary interaction, i.e., between planting dates and spraying with kinetin (D×K) in the field emergence percentage.

Table (1): Effect of planting date and spraying with kinetin on the first count for the standard laboratory germination test (%).

Average	kinetin concentrations. ($mg^{-1}L$)				Planting dates
	K75	K50	K25	K0	
7.64	12.17	8.14	6.13	4.13	D1
18.20	22.05	18.33	18.17	14.23	D2
21.52	25.66	22.08	20.20	18.15	D3
					*(P≤0.05)
1.143	19.96	16.18	14.84	12.17	Average
	1.32				*(P≤0.05)

Statistically significant differences (P<0.05)

Second count for the standard laboratory germination test (%)

Planting on the late spring date (D3) gave the highest average germination percentage in the second count, amounting to 56.07%, compared to the other dates, especially the early date (D1), which gave the lowest value for the trait, amounting to 21.78%. Perhaps the reason for the superiority of the D3 planting date in the standard laboratory germination percentage in the second count of stevia seeds is because of the optimal environmental conditions this date provides during the plant's growth period, which reflects positively on the seeds in obtaining the highest germination percentage and improves the process of seed germination in the early stages of growth, which was clearly shown in the germination percentage in the first count (Table 1). As for the early planting dates, the low temperatures may have negatively affected plant growth, directly affecting the seed formation and germination (**Hussein, 2011**)

The results also showed a superiority of the $75mg.L^{-1}$ spray treatment concentration, recording 49.85%, compared to the other treatments and the control treatment (36.52%). The noticeable increase in the physiological parameters of germination may be due to the positive effect of kinetin on the plasma membrane and several metabolic processes in the cell, leading to encouraging the transfer of nutrients, especially when sprayed in batches during the stages of seed formation on the plant, which improved seed formation, dry matter accumulation, and the enzymatic system of seeds, reflecting positively on the seed during germination (**Simlat *et al.*, 2019**).

The interaction treatment between the late planting date and spraying with kinetin at the highest concentration (D3K3) recorded the highest germination percentage in the second count (60.57%), compared to the treatment of the first planting date without spraying kinetin D1K0, which recorded the lowest value in the trait amounting to 12.07%.

Table (2): Effect of planting date and spraying with kinetin on the second count for the standard laboratory germination test (%).

Average	kinetin concentrations. (mg^{-1}L)				Planting dates
	K75	K50	K25	K0	
21.78	36.30	22.10	16.64	12.07	D1
47.83	52.66	48.19	46.26	44.21	D2
56.07	60.57	56.21	54.21	53.29	D3
	4.14				*(P≤0.05)
2.073	49.85	42.17	39.04	36.52	Average
	2.39				*(P≤0.05)

Statistically significant differences (P<0.05)

Radical length

Results in Table 3 demonstrate that the seeds produced by planting seedlings at the late date (D3) had the highest average radical length of 15.936 mm, which differed from the other dates. In contrast, the early date (D1) gave the lowest average for the trait, amounting to 10.665 mm. The reason for late planting date superiority may be because the seed produced then was with high vitality and rapid growth (Tables 1 and 2), reflected in the increasing growth of the embryo's main parts (the root and the petiole). It was also found that the optimal planting date is vital in increasing the velocity of metabolic activities related to root cell division, which increases its length (**Al-Amairi, 2021**).

The results also refer to the superiority of the kinetin spray treatment at 75 mg L^{-1}, giving the highest radical length, averaging 14.998 mm, compared to the kinetin spray treatments and the control treatment, which gave the lowest trait average, amounting to 11.970 mm. The superiority of the kinetin spray treatment on the plant can be explained by its positive role in improving seed growth and formation due to transferring nutrients to the effective regions (**Akram & Aftab, 2015**) this may also be because this treatment was superior in the germination percentage in the first and second counts (Tables 1 and 2), taking the shortest time to complete germination and was the fastest in germination, which allowed for a more extended time for the radical growth and development until the end of the testing period. As for the binary interaction between planting dates and kinetin concentration, the results showed that the late planting date and spraying kinetin at the concentration of 75 mg L^{-1} (D3K3) gave the highest average radical length (90.73 mm). Compared to the early planting date treatment and the control treatment (spraying with water only) (D1K0), which recorded the lowest value for the trait (69.62 mm).

Table (3): Effect of planting date and spraying with kinetin on radical length Plumule length (mm).

Average	kinetin concentrations. ($mg^{-1}L$)				Planting dates
	K75	K50	K25	K0	
10.665	11.912	11.402	10.250	9.095	D1
13.752	15.727	13.967	13.110	12.205	D2
15.936	17.355	16.265	15.515	14.610	D3
	0.334				*(P≤0.05)
0.167	14.998	13.878	12.958	11.970	Average
	0.193				*(P≤0.05)

* Statistically significant differences (P<0.05)

From the data, we noticed that the seeds resulting from planting at the late date (D3) gave the highest plumule length average, reaching 8.719 mm, which differed significantly from the other dates, in particular, the early date (D1), which gave the lowest length of plume, averaging 6.436 mm (Table 4), which may be due to that late planting date produced seeds with the highest biological germination velocity (Tables 1 and 2), which was reflected in increasing radical and plumule growth (Table 3). The results also refer to the superiority of spraying kinetin at 75 mg L^{-1} by giving the highest average length of the plumule, reaching 7.955 mm, compared to the other kinetin spray treatments and the control treatment, which resulted in 7.056 mm. This difference may be due to the prominent role of kinetin in stimulating the embryo, enhancing seed vitality and vigor, and accelerating the initiation of germination, which contributes to increasing the growth and development of embryonic axes and achieving the highest average plume length (**Al-Amiri,2022**). Moreover, this may also be because this treatment was superior in the germination percentage in the first and final counts (Tables 1 and 2), which allowed it a longer time for radicals to grow and initiate the root and shoot system until the end of the test period (Table 3). Regarding the binary interaction, no significant differences appeared between the two study factors in the plumule length.

Table (4): Effect of planting date and spraying with kinetin on plumule length.

Average	kinetin concentrations. ($mg^{-1}L$)				Planting dates
	K75	**K50**	**K25**	**K0**	
6.436	6.900	6.630	6.267	5.947	D1
7.476	7.745	7.663	7.518	6.980	D2
8.719	9.220	8.867	8.550	8.240	D3
6.267	5.947				*(P≤0.05)
7.476	7.745	7.663	7.518	6.980	Average
	0.139				*(P≤0.05)

* Statistically significant differences (P<0.05)

Seedling dry weight (mg)

Results in (Table 5) show that the seeds resulting from planting seedlings at the late date (D3) gave the highest average seedling dry weight of 3.494 mg, which differed significantly from the other dates. In comparison, the early date (D1) gave the lowest average for the trait, amounting to 1.013 mg. The reason for this superiority is that the late date may have been optimal for planting, which was reflected in the growth, development, and vitality of the resulting seeds (Tables 1 and 2), as well as the speed of metabolic activities related to cells and its division, represented by the length of the radical and the plumule (Tables 3 and 4), resulting in increasing the dry weight of seedlings. The results demonstrate that the kinetin spray treatment at a concentration of 75 mg L^{-1} was superior, giving the highest average dry weight of 2.758 mg, differing significantly from the other treatments and the control treatment, which gave the lowest dry weight of seedlings averaging 1.792 mg. The superiority of the highest kinetin spraying treatment (K75) regarding seedling dry weight is due to its superiority in root and shoot lengths (Tables 3 and 4). The results showed insignificant differences between the binary interactions for the study factors on the same trait.

Table (5): Effect of planting date and spraying with kinetin on seedling dry weight (mg)

Average	kinetin concentrations. ($mg^{-1}L$)				Planting dates
	K75	K50	K25	K0	
1.013	1.500	1.200	0.725	0.625	D1
2.144	2.725	2.350	1.875	1.625	D2
3.494	4.050	3.500	3.300	3.125	D3
	N.S				*(P≤0.05)
0.220	2.758	2.350	1.967	1.792	Average
	0.254				*(P≤0.05)

*Statistically significant differences (P<0.05)

Seedling vigor index

The seedlings of late planting date D3 were distinguished by giving the highest seedling vigor index, averaging 1374.0 mg, which differed significantly from the other two dates, as the early date (D1) showed the lowest mean for the trait, amounting to 379.7 mg (Table 6). The superiority of the late date treatment is due to the superiority of the same treatment in the germination percentage and length of the radical and plumule (Tables 2, 3, and 4), which are directly proportional to the seedling vigor index. The spraying Kinetin(K75) treatment was superior, giving the highest average for the trait, reaching 1170.5 mg, while the control treatment gave the lowest average (737.4 mg). The reason for the superiority of the high concentration treatment is due to its superiority in the germination percentage and the length of the radical and plumule (Tables 2, 3, and 4), indicating the vigor and vitality of the seed, as seeds that grow rapidly in the early stages of plant formation produce large and robust seedlings. The interaction between the two study factors showed no significant differences in the seedling vigor index.

Table (6): Effect of planting date and spraying with Kinetin on seedling vigor index (mg).

Average	kinetin concentrations. ($mg^{-1}L$)				Planting dates
	K75	K50	K25	K0	
379.7	677.2	396.8	264.3	180.4	D1
1016.0	1233.0	1038.3	948.8	843.9	D2
1374.0	1601.2	1407.4	1299.4	1188.0	D3
	N.S				*(P≤0.05)
45.73	1170.5	947.5	837.5	737.4	Average
	52.80				*(P≤0.05)

*Statistically significant differences (P<0.05)

Cold test

Results in Table 7 show that the seedlings resulting from planting on the third date (D3) gave the highest average percentage of normal seedlings in the cold germination test, amounting to 24.23%, differing significantly from the other dates where the early date (D1) recorded the lowest average for the trait, amounting to 8.70%. The superiority of seeds of late planting date plants in this test may be due to the optimal environmental conditions provided during the plant's growth period, which is reflected positively in activating the metabolic processes that protect the seeds from various stresses through strengthening and protecting the cell walls, Thus resulted in an increase in the seeds' resistance to pathogens and reducing rotting under wet and cold soil conditions, and the harmful effect of low temperatures, and eventually increasing the germination percentage (**Ashraf *et al*., 2015**). According to the results, the treatment of kinetin spray at 75 mg L^{-1} was the most effective. It produced the highest average germination percentage in the cold test, reaching 21.26%, significantly higher than the other treatments, including the control treatment, which had the lowest average germination percentage at 11.85%. The superiority of this treatment is due to the vital role of kinetin in stimulating the efficiency of cell membranes by activating the enzymes' action and creating positive changes, improving the transfer of micronutrients between plant cells and tissues, which enhanced seeds ability to withstand low temperatures and thus increasing the germination percentage under the conditions of this test (**Al-Jaf, Hamza, 2022**). Concerning the interaction between the two study factors, the seeds resulted from the late planting date treated with the highest concentration of kinetin (D3K3) showed the highest value of the normal-seedling percentage in the cold germination test (29.43%), compared to the interaction of the early planting date sprayed with water only (D1K0), which recorded the lowest value for the trait, reaching 5.25%.

Table (7): Effect of planting date and spraying with kinetin on germination percentage in cold test (%).

Average	kinetin concentrations. ($mg^{-1}L$)				Planting dates
	K75	K50	K25	K0	
8.70	13.19	8.68	7.70	5.25	D1
15.67	21.16	17.12	13.28	11.14	D2
24.23	29.43	25.18	23.15	19.15	D3
	2.40				*(P≤0.05)
2.44	21.26	16.99	14.71	11.85	Average
	2.89				*(P≤0.05)

* Statistically significant differences (P<0.05)

CONCLUSIONS

It is noticed from the study results that changing planting dates affects the vitality and activity of the resulting seedlings, as delaying the planting date from mid-February to mid-March led to an increase in the vitality and vigor of the seeds. The growth regulator, kinetin, played a positive role in the activity of the resulting seedlings, especially at the highest concentration (75 mg L^{-1}), which gives good possibilities for introducing it into stevia seed production programs.

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