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Effect of cytokinin on the number of capsules per leaf node in sesame under field conditions

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ABSTRACT

Sesame has valuable oil which is beneficial for human health. This plant has one capsule in each leaf node (the place where the leaf joins the stem), and by manipulating plant hormones, the number of capsules in each leaf node can increase, which leads to an increase in yield. To increase the number of capsules per leaf node, a factorial experiment was carried out in a randomized complete block design. The first factor was two types of seeds [seeds from one capsule per leaf node (CAP1) and seeds from triple capsule per leaf node (CAP2)], and the second factor was three concentrations of cytokinin (CK) (zero, 50, 100 ppm). The results showed that the maximum plant length (83.5 cm) was obtained from the 50 ppm CK treatment. The application of 50 ppm CK in CAP1 was increased the number of single capsule nodes about 48%. Compared with the control and 100 ppm treatments, the 50 ppm CK treatment had the greatest effect on the number of nodes with triple capsules (7.71), with increases of 38.6 and 72.8%, respectively. The greatest number of triple capsule nodes per plant (10.3) was obtained in the CAP2 treatment by using 50 ppm CK. The greatest amount of fresh and dry weight (84.5 and 30.7 g per plant, respectively) was obtained from the 50 ppm CK treatment group. The maximum number of capsules per plant and number of seeds per capsule were obtained from the 50 ppm CK×CAP2 treatment. Other results showed that the application of 50 ppm CK led to the highest number of seeds produced per plant (6.75 grams). In addition, the exogenous application of cytokinin to plants has the greatest effect on the areas to which it moves or the areas where cytokinin is synthesized, but to improve the effectiveness of this hormone and obtain an economic crop, it is necessary to use an appropriate ratio of other plant growth regulators.

Highlights

- Cytokinin increased sesame yield by boosting capsule production.
- 50 ppm cytokinin was optimal for growth and yield.
- Combining cytokinin with superior seeds maximized yield.
- Cytokinin application can enhance sesame yield.
- Balancing cytokinin with other hormones is crucial for optimal results.

1. Introduction

Sesame (*Sesamum indicum* L.) is an important oil crop because its seeds contain valuable substances, such as oil (50-60%), protein (20%), and carbohydrates (14-20%), and because they contain endogenous antioxidants, such as sesamol, sesaminol and tocopherol, which have remarkable stability (Ball et al., 2000). Compared to other crops, this plant has a low yield capacity due to its low harvest index (HI), capsule shattering, susceptibility to disease, and indeterminate habitat. One of the main components of yield is the number of capsules per leaf node (node: is place that leaf join to stem or branch). In natural ecotypes, there is one capsule in each leaf node. Studies have shown that some ecotypes have three or more capsules per leaf node. This trait is controlled by a single gene, and the recessive allele produces triple capsules. In a few ecotypes, three capsules

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are produced in all leaf nodes of the plant (three capsules in each node). Most three-capsule lines have single capsules at the bottom and top of the plant (Langham and Wiemers, 2002). Yield enhancement, especially for oil crops, is the main issue in our agricultural agenda. Yield is a complex trait, and yield components such as the number of seeds per capsule, the number of capsules per plant, and the weight of 1000 seeds are significantly positively correlated with the number of capsules per plant (Fazeli Kakhki et al., 2014). Based on these findings, it seems that cytokinin regulates the growth of reproductive meristems that can produce more than one capsule per leaf node and is effective in determining sesame yield.

Cytokinins (CKs) was discovered in 1955. Since kinetin was isolated from autoclaved products of herring sperm DNA as a cell division-promoting factor (Amasino, 2005; Sakakibara, 2006), several compounds with CK activity have been identified, including zeatin (Z), a natural CK substance (Sakakibara, 2006). Other CK synthetic compounds include kinitin, benzyladenine (BA), tetrahydropyranylbenzyladenin (PBA) and ethoxyethyl adenine, as well as several natural CKs with aromatic side chains (Vylíčilová et al., 2020). Our knowledge of CKs is incomplete, but based on the available data, CKs occur not only as free bases and ribonucleosides and ribonucleotids but also as constituents of particular tRNA species (Marquez-lopez et al., 2019). CK was synthesized in the roots. It moves acropetally through the transpiration system in the xylem and promotes bud growth and plant growth by inducing cell division. Cytokinin is involved in lateral branching and has indirect effects, unlike auxin (Nordstrom et al., 2004; Nagarathna et al., 2010). The results have shown that hormones are synthesized and act at various sites in the plant body, although the physiological differentiation and mechanisms of the dual signaling system have not been fully elucidated (Sakakibara, 2006). Researchers have shown that the effect of CK in the physiological stage is dissimilar due to the differences between the concentrations used and the plant growth stage. The results of study showed that reduced cytokinin content was decreases the size and the activity of the shoot apical meristem (SAM), demonstrating that cytokinin is a positive regulator of the SAM, in contrast, enhancing the cytokinin content was increases SAM activity that led to formation of more flowers by reproductive meristems (Schwarz et al., 2020). One of the functions of CK is to increase the activity of the meristematic zone. In this regard, Giulini et al. (2004) reported an increase in the size of the meristem in a maize mutant. Studies have shown that simultaneous mutation of two CKX genes in Arabidopsis delays the differentiation of cells in reproductive meristems; as a result, the flowers are formed more and larger, and subsequently, more seeds are produced. A study by Bartrina et al. (2011) showed that an increase in cytokinin content caused an approximately 50% increase in seed yield; they suggested that cytokinin has a major role in reproductive development. Cytokinin plays a central role in the induction and regulation of buds and nodes in plants (Bartrina et al., 2011; Li and Tang, 2002). Based on the research conducted, the aim of this research was to investigate different concentrations of cytokinin in the production of more than one capsule at the node of each leaf in sesame plant.

2. Materials and methods

The field trial was conducted in the spring of 2023 at Khorasan Razavi Agricultural and Natural Resources Research and Education Center, Mashhad, Iran. Geographical experimental site: Longitude 59.6 degrees east and latitude 36.2 degrees north and an altitude of 985 meters. The average rainfall of the region is 255 mm and the maximum and minimum annual absolute temperature is 42 and -27.7 degrees Celsius, respectively. The climate of the region is determined based on the Amberozheh method, cold and dry (IRIMO, 2016). The experiment was performed in a factorial arrangement based on a randomized complete block design with three replications. The first factor was two types of seeds obtained from three consecutive years. First, 13 sesame seed accessions were prepared from the oilseed section of the center in 2009 and then tested for morphological components and yield at the research farm of the Faculty of Agriculture of Ferdowsi University of Mashhad (Nezami et al., 2014). In this year, seeds were collected from plants that produced three capsules per leaf node, and to obtain pure seeds of three capsules per leaf node, these seeds were planted in pots for three consecutive years. For the plants that produced three capsules per leaf node, seeds with three capsules per leaf node (CAP2) and one capsule per leaf node (CAP1) were prepared separately and considered as the first factor for the two types of seeds. The second factor was three concentrations of cytokinin (CK) (zero, 50, and 100 ppm) applied during the life cycle of sesame plants (seed priming and foliar spraying at 55 and 80 days after planting: DAP), so the total treatments were zero, 50, and 100 ppm for T1, T2, and T3, respectively.

Table 1. Physical and chemical characteristics of the experimental (0 – 30 cm depth)

Sand	Silt	Clay	EC	pH	OC	TNV	SP	NT	P_{ave}	K_{ave}
%	%	%	$dS.m^{-1}$	-	%	%	%	%	ppm	ppm
31	49	20	1.08	7.6	0.61	15.5	36.4	0.076	227	12.8

TNV: (Total Neutralising Value), this is a quality index system used to express the effectiveness of the material to reduce or neutralise soil acidity
SP: (The Saturation Percentage), equals the weight of water required to saturate the pore space divided by the weight of the dry soil.
NT: (Total Nitrogen Content)

To prepare for hormonal treatments, 0.1 g of cytokinin powder was mixed with 10 ml of a 1 N solution of NaOH, and after stirring for 5 minutes on a shaker, 10 ml of 1 N hydrochloric acid (HCl) was added to the mixture. The resulting solution was stirred well until the NaOH was completely neutralized in the solution. Finally, 980 ml of

distilled water was added. This solution was 100 ppm of cytokinin stock solution. The same procedure was used to prepare 50 ppm cytokinin solution. The seeds were disinfected with fungicides and planted on 20 May. Land preparation and planting operations were carried out according to the custom of the region. In order to ensure uniformity of nutrition, before planting, soil samples were prepared from five points of land and sent to the laboratory (Table 1). After the plants were established and reached the four-leaf stage, they were thinned until the distance between two plants in a row reached seven centimeters.

Irrigation was done every week. After elimination of the marginal effects in each plot, and the plants ripened, 10 plants were selected, and several morphological traits (such as plant length, number of branches per plant, branch length, number of nodes with one capsule, number of nodes with three capsules, number of capsules per plant, number of seeds per capsule, 1000 seed weight, and fresh and dry weight) and yield components were measured in the laboratory.

The data were analyzed with Mstat-C software, and the means were compared with the least significant difference (LSD) test at five percent probability.

3. Results

3.1. Morphological traits

Analysis of variance revealed that cytokinin (CK) significantly affected the length and number of plant branches (Table 2). The maximum plant length (83.5 cm) was obtained from the 50 ppm CK treatment, which increased by 4 and 31% compared to those in the zero and 100 ppm CK treatments, respectively (Table 4). The greatest plant lengths in CAP2 and CAP1 under 50 ppm CK were 97.2 and 68.7 cm, respectively (Table 5). The number of branches per plant was obtained after the application of 100 ppm CK in the CAP1 treatment (6.66), and the other interaction treatments did not significantly affect this trait. The lowest number of branches per plant was recorded in the T3×CAP2 treatment (1.00), which was a decrease of 84.9% compared to the greatest reduction (Table 5). There was no significant difference in branch length between the zero and 50 ppm CK treatments (42.8 and 45.8 cm, respectively), but as the CK concentration increased to 100 ppm, the branch length decreased (Table 4). The length of the branches in the T2×CAP1 and T2×CAP2 treatments were 34.4 and 46.0 cm, respectively. The maximum length of this trait was obtained in the 50 ppm CK treatment (T2) in CAP2 treatment (57.2 cm), which was 42% greater than effect it in the CAP1 treatment (Table 5).

3.2. Comparison of CAP1 and CAP2 treatments

Table 2 shows that plants grown from the seeds of one-capsule plants (CAP1) differed significantly from those grown from the seeds of three-capsule plants (CAP2) in terms of traits such as the number of one-capsule nodes and the number of three-capsule nodes. In the CAP1 and CAP2 treatments, the number of one-capsule nodes per plant was 25.1 and 15.5, respectively. In CAP2, the number of three capsule nodes per plant was greater than that in CAP1 (Table 3). Compared with the use of 100 ppm CK, the use of 50 ppm CK in CAP1 increased the number of one-capsule nodes by 48% (Table 5). With the application of 50 ppm CK, the maximum number of three-capsule nodes was approximately 7.71, which was 38.6 and 72.8% greater than that in the control and 100 ppm CK treatments, respectively (Table 4). The interaction results of treatments showed that the maximum and minimum number of one-capsule nodes were in T2×CAP1 (32.2) and T3×CAP2 (9.33), respectively. The highest and lowest number of three-capsule nodes in CAP2 were 10.3 and 2.02, respectively, which were obtained from the application of 50 and 100 ppm CK, respectively (Table 5). The number of one-capsule nodes in CAP2 was much lower than that in CAP1. The lowest number of three-capsule nodes was obtained for T1×CAP1 (1.01) treatment (Table 5).

Table 2. Results of variance analysis of morphological and yield components traits of sesame plants affected by different concentrations of cytokines under field conditions.

S.O.V	df	Plant length	No. of branches per plant	Branch length	No. of one-capsule nodes per plant	No. of three-capsule nodes per plant	No. of capsules per plant	No. of seeds per capsule	1000 seed weight	seed weight per plant	Plant fresh weight	Plant dry weight
Block	2	499*	18.4	16.8	47.1	5.52	1394	42.5	96.4	12.2	3109	231
Capsule (CAP)	1	640**	290**	2.51ns	420**	0.907*	354*	530**	9.46ns	0.27ns	351ns	2.56ns
cytokinin	2	1010**	282**	1001**	290**	46.7**	1540*	269*	171ns	39.3	1940ns	181*
Capsule×cytokinin	2	448*	124**	733**	35.3*	96.1**	488*	8.37*	30.1ns	0.45*	3234*	134ns
Error	10	6025	7.41	34.2	18.6	2.03	503	40.4	70.5	12.1	1002	61.5

ns, * and ** are nonsignificant and significant at the 5% and 1% probability levels, respectively.

Table 3. Mean comparison of morphological and yield components traits of in two sesame plant. sesame plant.

Treatment	Plant length (cm)	No. of branches per plant	Branch length (cm)	No. of one-capsule nodes per plant	No. of three-capsule nodes per plant	No. of capsules per plant	No. of seeds per capsule	1000 seed weight (g)	seed weight per plant (g)	Plant fresh weight (g)	Plant dry weight (g)
CAP1	65.8 b	11.8 a	35.6 a	25.1 a	4.6 b	49.8 b	30.64 b	3.84 a	3.59 a	72.8 a	17.2a
CAP2	78.2 a	3.77 b	36.4 a	15.5 b	5.05 a	55.9 a	41.4 a	3.59 a	4.12 a	83.9 a	19.4a

CAP1: plants grown from one-capsule plant seeds; CAP2: plants grown from three-capsule plant seeds.

Table 4. Mean comparison different concentrations of cytokinin on morphological and yield components traits of sesame plant.

Treatment	Plant length (cm)	No. of branches per plant	Branch length (cm)	No. of one-capsule nodes per plant	No. of three-capsule nodes per plant	No. of capsules per plant	No. of seeds per capsule	1000 seed weight (g)	seed weight per plant (g)	Plant fresh weight (g)	Plant dry weight (g)
T1	79.5 a	14.7 a	42.8 a	23.1 a	4.73 b	49.3 b	28.7 c	1.78 a	3.16 b	79.7 a	20.4 b
T2	83.5 a	4.16 b	45.8 a	24.1 a	7.71 a	56.7 a	47.1 a	2.45 a	6.75 a	84.5 a	30.7 a
T3	57.4 b	3.83 b	21.1 b	12.4 b	2.09 c	28.0 c	33.7 b	2.30 a	1.48 c	49.9 b	11.1 c

Means with common letters are not significantly different from each other based on the least significant difference (LSD) test at the 5% probability level.
* T1: zero of cytokinin (CK), T2: 50 ppm prime with CK, T3: 100 ppm prime with CK.

Table 5. Mean comparison of the cytokinin×capsule interaction effect on morphological and yield components traits of sesame plant.

Interaction effect of treatments	Plant length (cm)	No. of branches per plant	Branch length (cm)	No. of one-capsule nodes per plant	No. of three-capsule nodes per plant	No. of capsules per plant	No. of seeds per capsule	1000 seed weight (g)	seed weight per plant (g)	Plant fresh weight (g)	Plant dry weight (g)
T1 × CAP1	68.3 b	4.01 ab	39.6 bc	28.9 a	1.01 c	48.8 b	26.0 c	1.81 a	3.28 c	57.4 d	15.3 cd
T2 × CAP1	68.7 b	4.00 ab	34.4 c	32.2 a	8.91 a	59.9 a	44.4 ab	2.31 a	6.91 b	93.6 a	25.5 b
T3 × CAP1	56.7 b	6.66 a	33.0 c	15.4 b	2.15 c	33.8 c	28.7 c	2.10 a	1.33 d	67.3 cd	13.3 cd
T1 × CAP2	89.3 a	4.66 ab	46.0 b	17.0 b	5.11 b	49.3 b	31.4 c	1.76 a	1.26 d	75.2 cd	19.3 bc
T2 × CAP2	97.2 a	4.33 ab	57.2 a	13.8 b	10.3 a	64.5 a	49.8 a	2.59 a	13.2 a	102 a	42.2 a
T3 × CAP2	58.0 b	1.00 b	9.33 d	9.33 c	2.02 c	22.1 d	39.4 b	2.50 a	3.08 c	31.2 e	8.69 d

Means with common letters are not significantly different from each other based on the least significant difference (LSD) test at the 5% probability level.
T1: zero of cytokinin (CK), T2: 50 ppm prime with CK, T3: 100 ppm prime with CK.
CAP1: plants grown from one-capsule plant seeds; CAP2: plants grown from three-capsule plant seeds

3.3. Yield components

The results of the analysis of variance showed that type of seed (CAP1 and CAP2), CK concentrations (T2, T3 and T4) and the interaction between them had significant effects on the number of capsules per plant and the number of seeds per capsule (Table 2). CAP2 had the greatest number of capsules per plant (Table 3), and with the application of 50 ppm CK, the greatest number of capsules per plant (56.7) and the greatest number of seeds per capsule (47.1) were obtained (Table 4). The minimum number of capsules per plant and the lowest number of seeds per capsule were recorded in the 100 ppm CK (T3) and control treatments, respectively (Table 4). The results of the interaction between the treatments showed that the maximum number of capsules per plant (64.5) and the maximum number of seeds per capsule (49.8) were recorded for T2×CAP2 (Table 5). The concentrations of CK, CAP treatment and their interaction had no significant effect on the 1000-seed weight (Tables 2, 3, 4 and 5). The results in Table 3 show that with the application of 50 ppm CK, the seed weight per plant was 6.75 g, which was 53 and 78% greater than that in application the zero and 100 ppm CK treatments, respectively. The highest (13.2 g) and lowest (1.26 g) seed weights per plant were obtained by application of 50 ppm CK and the control treatments in CAP2, respectively (Table 5). In CAP1, when 50 ppm CK was used, the maximum seed weight per plant (6.91 g) was recorded (Table 4). In compare of 50 ppm CK treatment (T2), the seed weight per plant was decreased by approximately 80% in the 100 ppm CK treatment (T3) (Table 5). There was a significant difference in the effect of CK and CAP on the fresh and dry weights of the plants (Table 2). The highest fresh and dry weights of the plants (84.5 and 30.7 g, respectively) were obtained from plants treated with 50 ppm Ck (Table 4). Similar results were recorded for the interaction between 50 ppm CK (T2) and CAP1 and CAP2, and the highest fresh weights of the plants were 93.6 and 102 g, respectively (Table 5). In Cap1, the lowest plant fresh weight was recorded for the zero Ck treatment, while in CAP2, the lowest plant fresh weight of 31.2 g was recorded in the 100 ppm CK (T3) treatment (Table 5). In the T2×CAP2 treatment, the greatest amount of plant dry weight (42.2 g) was recorded, and the lowest amount of this trait (8.69 g) was obtained in the T3×CAP2 treatment (Table 5). The lowest plant dry weight (13.3 g) was recorded in the T3×CAP1 treatment, (Table 5).

4. Discussion

Plant development and growth are affected by the activity of meristematic zones. Low-molecular-weight transcription factors, including plant hormones, regulate this region (Bartrina et al., 2011). In recent years, cytokinin metabolism and signal transduction have been elucidated (Werner and Schmulling, 2009), and it has been demonstrated that cytokinin positively regulation of seed germination, shoot elongation and proliferation, induction of flowering, fruiting, seed set, and senescence (Jameson and Song, 2015). The studies of Bartrina et al. (2011) also showed that the application of cytokinin in arabidobsis mutants caused the formation of a larger inflorescence meristem compared to that in wild type plants. In the present study, we observed an increase in the number of capsules per leaf node that originated from axillary buds in leaf nodes, and these results are consistent with the results of Bartrina et al. (2011). It seems that the division and differentiation of axillary bud cells in the presence of cytokinin increase the number of flower buds, and the synthesis of more photosynthates meets the needs of the growing auxiliary buds, and glucose is provided for their growth. In a research Akter et al. (2014) reported that

application of CK at 150 mg.l^{-1} was excellent resulting in a 106% yield advantage compared to drought stress and 79.9% increase relative to well-watered controls. Some researchers (Koprna et al., 2021; Yeh et al., 2015) reported that depending on the crop species, the exogenous application of cytokinin was increased reproductive tillers in winter wheat, and in rice plant, was increase in the number of tillers (34.7%), number of panicles (38.5%), and paddy yield (21.6%). The results of our study on these morphological traits are consistent with the results of these studies. In contrast, Leite et al. (2003) showed that the application of 30 mg. L^{-1} cytokinin during vegetative growth had no effect on the evaluated plant growth variables. Studies have shown that the efficacy of cytokinin depends on the amount of auxin; at lower levels of auxin, more cytokinin initiates bud break. Leite et al. (2003) reported that the application of GA3 and cytokinin increased plant height by 34% in compare of the control. In our study, plant height increased in response to 50 ppm CK. Importantly, in our study, the number of three capsule nodes increased with the use of 50 ppm CK. Increasing the concentration of CK was decreased the auxin/cytokinin ratio, and as a result, the effect of auxin was decreased in apical dominance, and the number of lateral buds increased. In this regard, Bartrina et al. (2011) showed that the levels of biologically active trans-zeatin and trans-zeatin riboside were approximately fourfold greater in the inflorescences of Arabidopsis mutants than in those of the wild type. In another study, increased chlorophyll contents were observed when plants were applied with BAP in 5 µM concentration under drought stress due to increased cell division, cell elongations and increased stay green foliage (Pandey et al., 2003). It seems that the appropriate concentration of CK causes a delay in leaf senescence, and as a result, by increasing photosynthesis, enough glucose is produced for the growth of other parts of the plant. In this regard, Hamdani et al. (2024) suggested that with foliar application cytokinin during the leaf development stage in potato plant led to accelerate growth in the early vegetative phase, resulting provided energy for formation and development of tubers. Thus, cytokinin present in the leaves stimulates the division and enlargement of young leaf cells to their normal size, which in turn increases the leaf area that leads to the increase of photosynthetic materials and the allocation of these materials to growth sites (Taiz and Zeiger, 2006). Therefore, cytokinin creates new source–sink relationships and preserves the synthesis of nucleic acid and protein in the leaf, thereby delaying leaf senescence.

5. Conclusion

The research findings of this research showed that the appropriate concentration of CK stimulates the division and enlargement of the meristic region, which in turn increases the morphological index (increasing the bud that produces leaves, stems, and flowers). In this regard, the use of 50 ppm external cytokinin in the CAP2 treatment has caused a significant increase in the number of multi-capsule nodes, the number of capsules per plant, the number of seeds per capsule and the weight of seeds per plant. However, for better efficiency, this hormone should be used with the appropriate growth ratio of other plant growth regulators to obtain an economic product.

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