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Carbonic anhydrase, net photosynthetic rate and yield of black cumin (*Nigella sativa*) plants sprayed with kinetin

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Foliar sprays of water, 10^{-6}, 10^{-5} or 10^{-4} M aqueous solutions of kinetin were applied to 40-day-old plants of black cumin (*Nigella sativa* L.) to study the effect on net photosynthetic rate, nitrogen metabolism, and the seed yield. A 10^{-5} M solution of kinetin maximally increased the activities of nitrate reductase, and carbonic anhydrase, chlorophyll and total protein contents and net photosynthetic rate in the leaves, along with capsule number and seed yield per plant at harvest.

Key words: *Nigella sativa*, kinetin, leaf area, total dry matter, capsule number, carbonic anhydrase, chlorophyll, black cumin, net photosynthetic rate, nitrate reductase, stomatal conductance, seed yield, total protein content.

Abbreviations; CA – carbonic anhydrase, NR – nitrate reductase, RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase

Introduction

Phytohormones play an essential role in regulating plant growth and development. Cytokinins have been implicated in many developmental processes and environmental responses of plants, such as regulation of cell division, apical dominance, chloroplast development, anthocyanin production and maintenance of source-sink relationship (HUTCHINSON and KIEBER 2002). In fact, cytokinins are considered the most important senescence-retarding hormones (FAISS et al. 1997), and their exogenous application has been demonstrated to prevent the degradation of chlorophyll and photosynthetic proteins (WINGLER et al. 1998), to cause induction of flower/ pod set (MA et al. 1998), to reverse leaf and fruit abscission, to release seed dormancy (POSPÍŠILOVÁ et al. 2000) and to modify substantially plant responses to a variety of environmental stresses (ALDESUQUY and GABER 1992, EL-SHIHABY et al. 2002).

However, in spite of these pleiotropic effects, few studies have critically analysed the physiological effects of kinetin application and their overall impact on plant productivity. Therefore, this study was designed to study the physiological and yield responses of *Nigel-*

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la sativa to foliar application of various concentrations of kinetin, in an attempt to develop methods for enhanced commercial cultivation of this greatly appreciated medicinal herb.

Materials and methods

Seeds of *Nigella sativa* L. were obtained from the Regional Research Institute of Unani Medicine, Aligarh (UP), India. They were surface sterilized with mercuric chloride solution (0.01 %), followed by repeated washings with double-distilled water. The seeds were then sown in earthen pots filled with sandy loam soil and farmyard manure, mixed in a ratio of 9:1. A uniform basal dose (45, 300 and 78 mg) of N, P and K, in the form of urea, single superphosphate and potassium chloride, was applied at the time of sowing to each pot.

Kinetin was obtained from *Sigma Chemicals* Co., St. Louis, USA. Forty-day-old plants were sprayed with 10^{-6}, 10^{-5} or 10^{-4} M kinetin at a rate of 5 cm^3 $plant^{-1}$. Control plants were sprayed with double-distilled water only. Each treatment was set simultaneously in triplicate. The pots were lined up in the department's net house, according to simple randomized block design. The various parameters were studied at 50 and 70 days after spraying. Leaf area was measured using a portable leaf area meter (LI-COR-3100 Lincoln, NE, USA). Total dry mass (TDM) was determined by drying the plant material at 80 °C for 24 h.

Carbonic anhydrase (CA) (E.C. 4.2.1.1) activity was assayed by the procedure adopted by Dwivedi and Randhawa (1974). 200 mg fresh leaf was cut into small pieces in 0.2 M cystein, at 4 °C. These pieces were transferred to a test tube containing 4 mL of 0.2 M sodium phosphate buffer (pH 6.8) to which 4 mL 0.2 M sodium bicarbonate and 0.2 mL bromothymol blue were added. CO_2 liberated during catalytic action of the enzyme on $NaHCO_3$ was estimated by titrating the reaction mixture against 0.01 M HCl, using methyl red as an indicator. Nitrate reductase (NR) (E.C. 1.6.6.1) activity was determined in fresh leaves of the plants by the method of JAWORSKI (1971). Total chlorophyll (Chl) content was estimated following the method of MACKINNEY (1941). Net photosynthetic rate (P_N) and stomatal conductance at each stage of sampling were measured in fully expanded leaves by an infra red gas analyzer *(*LI-COR 6200*,* Lincoln, NE, USA). Protein content in the leaves was estimated by the method of LOWRY et al. (1951). Treatment means were compared by analysis of variance using the statistical package *SPSS* (*SPSS 7.5.1 for Windows,* standard version 1996). Least significant differences were estimated at the 0.05 level of probability.

Results

In the present study, foliage treatment of *Nigella sativa* with kinetin was found to produce significant results in all the considered parameters (Tab. 1-2). Among various applied concentrations, 10^{-5} M proved to be optimum by causing maximum stimulation. However, a higher concentration (10^{-4} M) failed to bring about any significant effect, possibly by inducing an increase in endogenous auxin to supra- optimal levels, thereby initiating an inhibitory regulation of its own action (KAMÍNEK et al. 1992). Nevertheless, optimum kinetin supplementation (10^{-5} M) produced a 22 and 32% increase in the P_N over the control, as monitored at the 50th and the 70th day after sowing (DAS). Correspondingly, an enhancement of 24% and 47% was recorded in the Chl and stomatal conductance of the test plants at similar samplings. Moreover, the activity of CA was found to be elevated by 30 and 38%

over the control, whereas that of NR increased by 15 and 24% at the respective stages. Protein content in leaves was enhanced by 29 and 43%. Finally, seed yield was improved by 41% at harvest (130 DAS) relative to the untreated control.

Discussion

Following the kinetin treatment a substantial increase was observed in the amount of total dry mass of the test plants as compared to the control (Tab.2). Moreover, this effect was found to arise as a direct consequence of a similar stimulation in P_N (Tab.1), indicating that the capacity for CO_2 assimilation and photosynthate production was highly optimized. Although kinetin is known to have a direct influence on photosynthesis (SYNKOVÁ et al. 1997), in the present study, the hormone was found to exert pleiotropic effects at multiple levels to achieve an enhancement in P_N. One such level is the light harvesting efficiency, which was potentiated by the increase in leaf area and levels of chlorophyll (Chl) (Tab.1-2) in the leaves of the test plants. Enhancement in LA following kinetin application is known to be caused through enhanced cell division and enlargement induced by the hormone (JABLONSKI and SKOOG 1954). Moreover, supplementation of kinetin can possibly increase the number of chloroplasts in the leaf, by increasing the intensity of cell growth, ultrastractural morphogenesis of chloroplasts and stimulating the activity of ribosomes, and consequently enhancing chlorophyll synthesis (POSPÍŠILOVÁ et al. 2000, EL-SHIHABY et al. 2002). In addition, kinetin is also known to prevent the degradation of Chl thereby facilitating both the retention and the accumulation of this important photosynthetic pigment.

Another aspect of photosynthesis acted upon by the hormone treatment was the CO_2 availability, which was optimized by the enhancement of stomatal conductance (Tab.1). Kinetin is known to facilitate stomatal opening by causing an increase in guard cell K^+ content (LECHOWSKI 1997). A lesser stomatal resistance implies a greater stomatal conductance and as such, results in a freer exchange of gases (ARTECA and DONG, 1981). The diffusion of CO_2 into the stomata is followed by its transport across several membranes into the chloroplast, where it is duly reduced by ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO). It is this supply of CO_2 to RuBPCO that requires the activity of CA, which catalyses the reversible dehydration of HCO_3^- to CO_2 in close proximity to the CO_2 fixing enzyme. The functioning of this entire mechanism is likely to improve by enhancement of the activity of both CA (Tab.1), as well as RuBPCO (CHERNYAD'EV and MONAKHOVA 1998) brought about by kinetin application. Cytokinins have been postulated to affect the levels of a number of plant enzymes by enhancing translation/transcription of the respective genes and stabilization of the transcripts (SUGIHARTO and SUGIYAMA 1992, PREMABATIDEVI 1998). Thus, we observed a simultaneous increase in the activity of NR (Tab.1). This enzyme is responsible for the initiation of N metabolism and, consequently, determines the degree of protein synthesis. Enhancement in the activity of NR could, therefore, have boosted N assimilation, hence causing the increase in protein content of the test plants (Tab.2). Increase in protein content might, in turn, have further optimized the availability of photosynthetic components, such as light harvesting chlorophyll protein-complexes (LHCP) and various CO_2 assimilating enzymes, to influence the rate of photosynthesis.

Lastly, an equally potent effect on the photosynthetic capacity of the test plants could have arisen from the enhancement of LA and extension of the effective life of leaves (LA

Tab. 1. Net photosynthetic rate (P_N) [nmol (CO_2) m^{-2} s^{-1}], stomatal conductance (g_s) [mol m^{-2} s^{-1}], carbonic anhydrase (CA) activity [mol (CO_2) kg^{-1} s^{-1}], nitrate reductase (NR) activity [nmol (NO_2) g^{-1} min^{-1}], protein content [%(d.m.)] and chlorophyll (Chl) content [g kg^{-1} (f.m.)] in *Nigella sativa* leaves, sprayed with water (control), kinetin (KIN: 10^{-6}, 10^{-5} or 10^{-4}) at 40 d, after sowing and sampled at 50 and 70 days after sowing (DAS). LSD for $P = 0.05$, mean±SD.

	P_N		g_s		CA		NR		Chl	
	50 DAS	70 DAS	50 DAS	70 DAS	50 DAS	70 DAS	50 DAS	70 DAS	50 DAS	70 DAS
Control	15.16±1.5	16.58±1.4	0.245±0.005	0.285±0.007	2.11±0.20	2.65±0.21	7.11±0.70	7.69±0.80	1.21±0.13	1.27±0.12
10^{-6}	16.51±1.6	19.62±1.7	0.285±0.002	0.320±0.012	2.45±0.22	3.11±0.24	7.35±0.71	8.51±0.61	1.33±0.10	1.45±0.11
10^{-5}	18.45±1.8	21.91±1.8	0.360±0.008	0.415±0.016	2.74±0.19	3.65±0.26	8.10±0.59	9.51±0.69	1.48±0.12	1.61±0.15
10^{-4}	17.01±1.5	21.41±1.4	0.321±0.010	0.375±0.010	2.65±0.25	3.42±0.18	7.95±0.71	9.45±0.75	1.53±0.11	1.58±0.14
LSD	0.82	0.56	0.016	0.015	0.065	0.045	0.35	0.69	0.10	0.12

Tab. 2.. Dry mass (DM) [g], leaf area (LA) [cm^2], protein content [% DM], (sampled at 50 and 70 d, after sowing), and number of capsules and seed yield $plant^{-1}$ [sampled at 130 DAS) in *Nigella sativa* plants, sprayed with water (control), or kinetin (KIN: 10^{-6}, 10^{-5} or 10^{-4}M) at 40 days after sowing (DAS). LSD for $P = 0.05$, mean±SD.

	DM		LA		Protein			
	50 DAS	70 DAS	50 DAS	70 DAS	50 DAS	70 DAS	Capsule number	Seed yield
Control	1.33±0.11	2.10±1.21	136.2±10.2	285.1±22.1	11.45±1.1	12.41±1.1	16.34±1.4	1.16±0.15
10^{-6}	1.79±0.15	2.88±0.24	210.6±18.4	365.2±27.1	13.15±1.2	14.65±1.3	18.45±1.7	1.30±0.20
10^{-5}	1.97±0.14	3.47±0.26	301.5±21.6	430.8±31.1	14.71±1.4	17.70±1.5	21.75±1.9	1.64±0.19
10^{-4}	2.11±1.17	3.30±0.21	290.8±24.6	415.6±32.4	14.35±1.5	17.40±1.4	21.25±1.6	1.56±0.16
LSD	0.079	0.031	31.4	21.16	0.92	1.53	1.51	0.17

duration) brought about by minimization and delay of senescence resulting from the kinetin treatment (POSPÍŠILOVÁ et al. 2000)

At harvest, the seed yield of the treated *Nigella* plants was also found to be significantly enhanced over the control (Tab.2). In lupines, kinetin causes enhanced induction of pod set as well as prevention of abscission of the developing pods (MA et al. 1998), ultimately resulting in an increased number of capsules (Tab.2) reaching maturity. This causes a substantial increase in the size of the reproductive sink to attract more photoassimilate. Also, cytokinins induce the formation of more secondary vascular tissue (MA et al. 1998), presumably resulting in a greater potential for assimilate translocation from vegetative structures to the pods. Given the sufficient availability of nutrients, this increased sink strength and vascular capacity results in enhanced seed filling and thus increases the seed yield (Tab.2). Similar beneficial effects of kinetin on seed yield have been reported by FARRIDUDIN et al. (2004) and SHAH (2007).

In accord with the results of the present study, the use of foliar kinetin application, especially at a concentration of 10^{-5} M, can be strongly suggested as a prospective method for the augmentation of *Nigella sativa* cultivation at a commercial level.

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