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BRIEF COMMUNICATION

Responses of *Nigella sativa* to foliar application of gibberellic acid and kinetin

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Abstract

Foliar sprays of water or 1, 10 and 100 μM aqueous solutions of gibberellic acid (GA_3) or kinetin (KIN) were applied to 40-d-old plants of *Nigella sativa* (L.) to study their effects on net photosynthetic rate, nitrogen metabolism, and the seed yield. 10 μM solutions of both the hormones, especially GA_3, appreciably 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 plant^{-1}, at harvest.

Additional key words: black cumin, capsule number, carbonic anhydrase, leaf protein, net photosynthetic rate, nitrate reductase, seed yield.

Gibberellins (GAs) are a class of endogenous plant growth substances actively involved in the control of a number of key developmental processes including endosperm mobilization and stem elongation, as well as flower and fruit development (Huttly and Phillips 1995). Plants subjected to exogenous application of GAs have been found to exhibit increased activities of carbonic anhydrase (CA), nitrate reductase (NR) (Khan 1996, Hayat *et al.* 2001, Afroz *et al.* 2005), CO_2 fixation, stomatal conductance (Bishnoi *et al.* 1992), and ribulose-1,5-biphosphate carboxylase/oxygenase (RuBPCO) (Arteca and Dong 1981, Yuan and Xu 2001). Besides, GAs are also known to alter membrane permeability to ions (Manuel *et al.* 1980, Gilroy and Jones 1992.), induce fruit set (Arteca 1996) and greatly enhance the translocation potential of the sink (Peretó and Beltrán 1987).

Despite the known importance of cytokinins (CKs) in plant growth and development, few studies have critically investigated the physiological impact of their exogenous application *e.g.*, increase in activities of nitrate reductase (Roa *et al.* 1984, Saxena and Saxena 2002), glutamate synthetase (Ghisi and Passera 1987), and enhancement of chlorophyll and protein contents (Towne and Owensby 1983, Synková *et al.* 1997). CKs may also be employed to improve the photosynthetic rate and the activity of RuBPCO (Chernyad'ev 1994, Chernyad'ev and Mankhova 1998, Singh *et al.* 2001). Owing to the beneficence of mentioned physiological effects and the interwoven nature of actions of these hormones, we were tempted to study their impact on *Nigella sativa* L. in context of photosynthesis, nitrogen metabolism and yield.

Nigella sativa L. (black cumin) is a miraculously remedial herb, used intensely in medicinal as well as food formulations (Babayan *et al.* 1978, Takruri and Dameh 1998). 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 muriate of potash, was applied at the time of sowing to each pot.

Gibberellic acid (GA_3) and kinetin (KIN) were obtained from *Sigma Chemicals Co.*, St. Louis, USA. The plants of 40 d age were sprayed with 1, 10 or 100 μM GA_3 or KIN at the rate of 5 cm^3 plant^{-1}. Control plants were sprayed with double distilled water only.

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Abbreviations: CA - carbonic anhydrase; Chl - chlorophyll; CK - cytokinin; DAS - days after sowing; GAs - gibberellins; GA_3 - gibberellic acid; KIN - kinetin; NR - nitrate reductase; P_N - net photosynthetic rate.

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Each treatment was replicated three times. The pots were lined in the Department's net house, according to simple randomized block design. The various parameters were studied at 50, 70 and 90 d after spray.

Carbonic anhydrase (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 test tube with phosphate buffer and 0.2 M sodium bicarbonate and bromothymol blue were added. CO_2 liberated during catalytic action of enzyme on $NaHCO_3$ was estimated by titrating the reaction mixture against 0.01 M HCl, using methyl red as an indicator. Nitrate reductase (E.C. 1.6.6.1) activity was determined in fresh leaves of the plants by the method of Jaworski (1971). Total chlorophyll content was estimated following the method of Mackinney (1941). Net photosynthetic rate (P_N) at each stage of sampling was measured in fully expanded leaves by a portable photosynthetic system (*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 statistical package *SPSS* (*SPSS 7.5.1 for Windows*, standard version 1996). Least significant difference (LSD) was estimated at 0.05 level of probability.

Although, spray of either GA_3 or KIN was found to promote activity of CA, GA_3 was more efficient with 45 % increase in CA activity (relative to control) in 70-d old plants when sprayed with 10 μM GA_3 at 40-d-stage (Table 1). Enzyme activity perceivably increased with the increase in hormone concentration from 1 to 10 μM, however, a higher concentration, *i.e.*, 100 μM of either KIN or GA_3, failed to bring about any appreciable stimulation. Maybe KIN induced an increase in endogenous auxin content to supraoptimal level, thereby initiating an inhibitory regulation of its own biosynthesis and action (Kamínek *et al.* 1997). Similarly, GAs are known to check their own effects through feedback control under excess concentrations (Martin *et al.* 1996). The observed increase in CA activity induced by 1 to 10 μM GA3 can be attributed to its effect on the *de novo* synthesis of CA involving the translational and transcriptional mechanisms (Okabe *et al.* 1980, Khan *et al.* 2004). Similarly, the mode of action of KIN can be traced to the influence of cytokinins at the level of transcription and/or stabilization of the transcripts as they increased the content of CA-mRNA (Sugiharto *et al.* 1992).

In context to the NR activity, GA_3 again outperformed KIN as better inducer of such enzyme activity (Table 1). The positive effect of GAs on NR activity is well known (Khan 1996, Premabatidevi 1998). A marked rise in the protein content (Table 1) of the treated leaves may well be a consequence of this GA_3 intensified NR activity (Saroop *et al.* 1998). This postulation is further complemented by the positive correlation between the content of proteins and that of the enzyme (r = 0.905**). Here also, the influences of KIN and GA_3 on the translation/transcription mechanisms, as mentioned above, may be stated in explanation for the increase in NR activity and protein content.

Leaves of the plants receiving KIN treatment, had higher chlorophyll content compared to control (Table 1),

Table 1. Carbonic anhydrase (CA) activity [mol(CO_2) kg^{-1} s^{-1}], nitrate reductase (NR) activity [nmol(NO_2) g^{-1} min^{-1}], chlorophyll (Chl) [g kg^{-1}(f.m.)] and protein content [%(d.m.)], and net photosynthetic rate (P_N) [μmol(CO_2) m^{-2} s^{-1}] in *Nigella sativa* leaves, sprayed with water (control), kinetin (KIN), or gibberellic acid (GA_3) at 40 d after sowing and sampled at 50, 70 and 90 DAS. LSD for $P = 0.05$, mean ± SE.

Parameter	Treatment	[μM]	50 DAS	70 DAS	90 DAS
CA	control		2.07±0.18	2.54±0.19	1.35±0.12
	KIN	1	2.41±0.21	3.02±0.22	1.65±0.14
		10	2.68±0.19	3.43±0.26	1.95±0.20
		100	2.56±0.23	3.34±0.23	1.85±0.15
	GA_3	1	2.51±0.22	3.15±0.25	1.75±0.14
		10	2.87±0.29	3.68±0.27	2.15±0.21
		100	2.65±0.19	3.25±0.24	1.87±0.16
LSD			0.071	0.051	0.041
NR	control		7.04±0.72	7.66±0.84	4.88±0.91
	KIN	1	7.42±0.59	8.52±0.57	5.33±0.76
		10	7.87±0.63	9.52±0.71	5.86±0.73
		100	8.03±0.89	9.50±0.82	5.68±0.63
	GA_3	1	7.64±0.61	8.65±0.72	5.50±0.69
		10	8.67±0.67	9.92±0.76	6.01±0.71
		100	8.09±0.74	9.42±0.87	5.41±0.62
LSD			0.41	0.75	0.31
Chl	control		1.24±0.11	1.26±0.11	0.97±0.09
	KIN	1	1.36±0.12	1.38±0.12	1.05±0.10
		10	1.48±0.11	1.57±0.15	1.15±0.11
		100	1.50±0.12	1.54±0.14	1.14±0.10
	GA_3	1	1.42±0.13	1.46±0.11	1.06±0.10
		10	1.62±0.15	1.68±0.17	1.17±0.12
		100	1.52±0.14	1.53±0.14	1.15±0.11
LSD			0.07	0.08	0.05
Protein	control		11.40±1.2	12.35±1.2	10.40±1.1
	KIN	1	13.01±1.1	14.74±1.3	12.01±1.2
		10	14.65±1.3	17.60±1.4	13.51±1.1
		100	14.80±1.5	16.80±1.3	12.62±1.2
	GA_3	1	12.95±1.1	15.06±1.4	12.42±1.1
		10	15.41±1.4	18.70±1.7	14.24±1.2
		100	14.21±1.2	17.71±1.6	13.21±1.3
LSD			0.71	0.52	0.65
P_N	control		15.10±1.4	16.10±1.5	13.25±1.3
	KIN	1	16.21±1.5	18.41±1.8	14.32±1.4
		10	17.29±1.6	19.75±1.7	15.42±1.5
		100	16.85±1.4	20.01±1.4	15.01±1.4
	GA_3	1	16.35±1.3	19.25±1.5	14.75±1.4
		10	19.01±1.7	22.15±1.9	16.80±1.6
		100	17.15±1.6	20.45±1.4	15.55±1.5
LSD			0.65	0.46	0.75

which is in agreement with Ghosh and Biswas (1991), Saroop *et al.* (1994) and Saxena and Saxena (2002). However, such an effect of GA_3 was more pronounced than that of KIN (Table 1), which may be attributed to the GA_3-generated enhancement of ultrastructural morphogenesis of plastids, coupled with the retention of Chl and delay of senescence caused by the hormone treatment (Arteca 1996, Ouzounidou and Illias 2005), and the increase in protein content. Similarly, CKs can also be said to have promoted Chl content through conversion of etioplasts into chloroplasts, and by governing the composition and ultrastructure of plastids (Pospíšilová *et al*. 2000). A subsequent expression of the cumulative effect of increased activities of NR and CA, and protein and Chl contents was the high P_N rate (Table 1) of the hormone (GA_3 or KIN) treated plants. Various other factors such as, ample availability of CO_2 due to increased CAactivity, along with GA_3 induced promotion of the rates of cyclic and non-cyclic phosphorylation (Saroop *et al.* 1994, Naidu and Swamy 1995) and the activity of RuBPCO, may also have supplemented the above stance. Indeed, the activity of CA was positively correlated with P_N ($r = 0.831$**).

Vegetative growth of a test plant is highly responsible for desired seed yield, as the number of photosynthesizing sites is detrimental in the production of photoassimilates. The rise in photosynthetic rate implies more vegetative growth due to sufficient availability of nutrients, which in turn, increases the number and size of the reproductive sink, *i.e.*, number of flowers and capsules (Table 2). This, when coupled with the GA_3 induced mobilization of metabolites to the developing pods (Hoad *et al*. 1977, Naidu and Swamy 1995, Ouzounidou and Illias 2005), may have stimulated their growth and hence, caused an increase in seed yield, as observed herein (Table 2). Similary, KIN was also seen to have some effect on yield, though relatively subdued, which may be ascribed to the increased opportunity for formation of new buds (Bruinsma 1977), and successful reversal of leaf and fruit (pod) abscission . Such favourable effect of KIN or GA_3 in enhancing economic yield of *Nigella sativa* and various other crops has also been reported by Chrungoo and Farooq (1984), Khan (1996), Hayat *et al.* (2001), Mousa *et al.* (2001), and Azam (2002). Summarily, we can say, that the observed increase in seed yield is therefore, an expression of the cumulative effect of the hormones on the leaf metabolism, re-directed mobili-zation of metabolites, and delayed senescence of plant organs.

Table 2. Number of capsules and seed yield plant^{-1} in *Nigella sativa* L. plants, sprayed with water (control), gibberellic acid (GA_3) or kinetin (KIN) at 40 d after sowing and sampled, at harvest (130 DAS), LSD for $P = 0.05$, mean ± SE.

Treatment	[μM]	Number of capsules [plant^{-1}]	Seed yield [g plant^{-1}]
Control		16.20±1.5	1.12±0.16
KIN	1	18.35±1.8	1.25±0.19
	10	21.50±1.9	1.50±0.22
	100	20.91±1.4	1.41±0.24
GA_3	1	19.70±1.5	1.32±0.18
	10	23.75±2.1	1.72±0.19
	100	21.52±1.9	1.52±0.20
LSD		1.02	0.08

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