DOI: 10.1007/s11099-011-0011-5 PHOTOSYNTHETICA 49 (1): 154-160, 2011

BRIEF COMMUNICATION

Growth and photosynthetic characteristics of *Nigella sativa* L. as affected by presowing seed treatment with kinetin

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Abstract

Black cumin (*Nigella sativa* L.) seeds were surface-sterilized and soaked for 5, 10, or 15 h in 1, 10, or 100 μM aqueous solution of kinetin (KIN). The potted plants were then analyzed at 30, 50, and 70 days after emergence (DAE) for dry mass (DM), leaf area (LA), chlorophyll (Chl) content, stomatal conductance (g_s), carbonic anhydrase (CA), and nitrate reductase (NR) activity, total protein content, and net photosynthetic rate (P_N). Capsule number and seed yield were determined at harvest (90 DAE). Treatment with the growth regulator was found to appreciably enhance all the determinants, with most prominent results being obtained following 10-h soaking with 10 μM KIN, in which case the values for DM, LA, P_N, CA and NR activity, and seed yield were elevated by 55, 63, 43, 38, 29, and 23% respectively over the control at the 50-day stage.

Additional key words: carbonic anhydrase; chlorophyll; kinetin; *Nigella sativa*; nitrate reductase; photosynthetic rate; protein; seed treatment; stomatal conductance.

Cytokinins (CYTs) play an important regulatory role in plant growth, and, acting both in synergy and antagonism with other plant hormones, influence a wide range of developmental processes. These have been particularly implicated in apical dominance, chloroplast development, anthocyanin production, and regulation of cell division as well as source-sink relationships (Hutchison and Kieber 2002). Moreover, CYTs are regarded as the most important senescence-retarding hormones (Faiss *et al*. 1997) and their exogenous application has been demonstrated to inhibit the degradation of Chl and photosynthetic proteins (Wingler *et al*. 1998), besides elevating photosynthetic rate and levels of key photosynthetic enzymes (Synková *et al*. 1997, Chernyad'ev and Monakhova 1998, Shah 2007, Shah *et al.* 2007), and substantially modifying plant responses to varied environmental stresses (Aldesuquy and Gaber 1992, El-Shibaby *et al.* 2002, Shah 2011).

In quest for the potential beneficence of exogenous hormone supplementation on plant performance and productivity, innumerable studies have explored the effects of foliar treatments on various aspects of plant metabolism. With regard to presowing seed treatment, CYT have been demonstrated to promote germination, yield, and fiber properties of cotton (Sawan *et al*. 2000), besides enhancing nitrogen metabolism and productivity of chickpea (Fatima *et al*. 2008). Moreover, seed priming with kinetin has also been shown to counteract the adverse effects of salinity on germination of lettuce (Khan and Huang 1988), wheat (Afzal *et al*. 2005), and sorghum (Ismail 2003), as well as growth, photosynthetic activity, ion contents and yield of wheat (Iqbal and Ashraf 2005). In this milieu, it is foreseeable that further elucidation of the effects of KIN pretreatment *per se*, especially on key enzyme activities, concurrent to photosynthetic and yield attributes, would provide valuable comparative information for tolerance-enhancing strategies focusing on presowing hormone supplementation. The speculation of the effects of KIN pretreatment on later developmental processes arises from the opportunity of a plant to assimilate more nutrients following an early germination. Moreover, CYTs are known to function *via* the basic processes of translation and transcription, particularly by enhancing gene-specific transcription and/or increasing mRNA stability (Sugiharto and Sugiyama 1992). As such, optimization of the levels

Received 19 August 2010, *accepted* 4 February 2011.
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Abbreviations: CA – carbonic anhydrase; Chl – chlorophyll; CYT – cytokinin; DAE – days after emergence; DM – dry matter; g_s – stomatal conductance; LA – leaf area; KIN – kinetin; NR – nitrate reductase; P_N – net photosynthetic rate.

of structural and functional proteins possibly occurs, which might sustain the potency of CYT effects for an appreciable period of time. In this context, the present experiment was designed to study the growth, enzyme activities, and photosynthetic and yield responses to KIN seed treatment using *Nigella sativa* L., a Middle Eastern herb greatly demanded in the domestic and international markets for its immense remedial and culinary properties and high aromatic value (Shah 2004).

Pure strains of *Nigella sativa* L. were obtained from the Regional Research Institute of Unani Medicine, Aligarh, Uttar Pradesh, India. The plant is an annual herb, about 60 cm high, about 20–40 pinnatisect leaves, divided into linear segment. The flowers are usually pale blue and white, with 5–10 petals. The fruit is a large inflated capsule composed of 3–7 united follicles, each containing numerous seeds (Shah 2004).

Homogenous seeds were surface-sterilized by soaking in 1 mol m^{-3} $HgCl_2$ solution for 3 min, washed thoroughly with distilled water, and divided into twelve sets which were soaked in distilled water (control) or distilled water containing 1, 10, or 100 μM KIN respectively, for 5, 10, or 15 h. These were subsequently sown (10 per pot) in earthen pots (25 cm diameter) filled with sandy loam soil and farmyard manure, mixed in a ratio of 9:1. A uniform basal dose (45, 300, and 78 mg, respectively) of N, P, and K, in the form of urea, single superphosphate and muriate of potash respectively, was applied at the time of sowing to each pot. The pots were irrigated with tap water as required. After germination only 5 uniform seedlings were left in each pot. The experiment was carried out on a completely randomized block design, under natural day/night conditions: average 12-h photoperiod, photosynthetically active radiation (PAR) >1,100 μmol(photon) m^{-2} s^{-1}; temperature 21 ± 3ºC and RH 64–52%. Each treatment was replicated five times. Samplings were carried out during the preflowering, flowering and fruiting stages of the plant, at 30, 50, and 70 DAE, respectively. Five plants from each replicate were randomly selected for the measurement of LA, DM, Chl content, CA and NR activity, protein content, P_N, and g_s. Capsule number and seed yield $plant^{-1}$ were recorded at harvest (90 DAE). CA activity was assayed by the procedure adopted by Dwivedi and Randhawa (1974). 200 mg of leaf fresh mass was cut into small pieces in 0.2 M cystein, at 4°C. These pieces were transferred to a test tube with phosphate buffer (0.2 M, pH 6.8), 0.2 M sodium bicarbonate, and bromothymol blue. 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. NR activity was determined in fresh leaves of the plants by the method of Jaworski (1971). Protein content in the leaves was estimated by the method of Lowry *et al.* (1951). P_N and g_s were recorded using a portable infrared gas analyzer (*LICOR-6200*, Lincoln, NE, USA) on the fully expanded, uppermost leaf of the main plant axis, at saturating irradiance between 11:00 and 12:00 h under atmospheric conditions: PAR – 1,012–1,084 μmol m^{-2} s^{-1}, atmospheric CO_2 – 350 μmol mol^{-1}, RH 62 ± 2%, and temperature 23 ± 2°C. LA per plant was measured using a leaf area meter (*LI-3000*, *LI-COR Inc.,* Lincoln, NE, USA). DM [g $plant^{-1}$] [of all the above ground plant tissue] was determined on oven-dried plant material (80°C for 48 h). Total Chl content was estimated following the method of MacKinney (1941). The data were subjected to an analysis of variance (*ANOVA*) and means were compared using the least significant difference (*LSD*) (P=0.05) calculated according to Gomez and Gomez 1984).

Nigella plants grown from the KIN-treated seeds exhibited an appreciable enhancement of all the studied parameters, and presented with a superior state of metabolism as compared to the control. Among the various concentrations, 10 μM KIN proved to be most stimulatory, irrespective of the soaking duration, eliciting maximum response following 10-h soaking; all other treatments were either mild, or supraoptimal (Table 1). Conceptually, exogenous application of phytohormones is intended to achieve only a limited desired deviation in the finely regulated set pattern of hormonal ratio. As such, supraphysiological dosages might result in saturation of the signaling systems, besides induction of feedback controls (Weyers and Paterson 2001), and hence prove relatively ineffective.

At 30 and 50 DAE, leaves of the test plants photosynthesized at a rate faster by 36 and 43%, respectively, as compared to the control – an apparent consequence of their enhanced g_s, activities of CA and NR, and increased Chl and protein contents (Table 1). A lesser stomatal resistance implies a greater stomatal conductance and as such, results in a freer exchange of gases (Arteca and Dong 1981). At 50 DAE, g_s of the test plants was elevated by 41%, which can be ascribed to the influence of KIN on guard cell K^+ content that results in stomatal opening (Lechowski 1997). Diffusion of CO_2 through the stomata is followed by its transport into the chloroplast, wherein it is duly reduced. Here, the enzyme CA regulates the reversible hydration of CO_2 and as such, provides for an effective reservoir for photosynthetic carboxylation. Enhancement in activity of CA by KIN, as observed by 38% at 50 DAE, may have thus upgraded the C-fixing assembly, and favorably affected photosynthesis – a conjecture supported by the positive correlation obtained between CA activity and P_N of the test plants (r = 0.906**). With reference to the enzyme activity, KIN has been demonstrated to induce *de novo* synthesis, by stimulating gene-specific transcription and increasing the stability of the mRNA transcripts (Sugiharto and Sugiyama 1992). Evidently, similar enhancements of 29 and 41% observed in NR activity and protein content respectively, at 50 DAE, were also an expression of this effect of the growth regulator on translation/transcription, as also observed by Lu *et al.*

Table 1. Leaf carbonic anhydrase (CA) activity [mol(CO_2) kg^{-1}(FM) s^{-1}], nitrate reductase (NR) activity [nmol(NO_2) g^{-1}(FM) min^{-1}], protein content [% (DM)], chlorophyll (Chl) content [g kg^{-1}(FM)], stomatal conductance (g_s) [mol m^{-2} s^{-1}], and net photosynthetic rate (P_N) [μ mol (CO_2) m^{-2} s^{-1}], in *Nigella sativa*, raised from seeds soaked in water (control), 1, 10, or 100 μM KIN for 5, 10, or 15 h and sampled at 30, 50, and 70 (DAE). Least significant difference (LSD) for p=0.05, mean ± SE, (n = 5), S – soaking; NS – nonsignificant; KIN – kinetin.

		30 DAE			50 DAE			70 DAE		
	Treatment [h]	5	10	15	5	10	15	5	10	15
CA	Control	1.80 ± 0.14	1.85 ± 0.15	1.84 ± 0.16	2.53 ± 0.21	2.55 ± 0.23	2.56 ± 0.18	1.33 ± 0.12	1.35 ± 0.11	1.36 ± 0.14
	1 μM	2.17 ± 0.18	2.25 ± 0.21	2.24 ± 0.19	2.79 ± 0.22	3.11 ± 0.25	2.84 ± 0.27	1.45 ± 0.14	1.44 ± 0.14	1.55 ± 0.13
	10 μM	2.39 ± 0.21	2.49 ± 0.23	2.51 ± 0.18	3.31 ± 0.23	3.52 ± 0.27	3.45 ± 0.28	1.48 ± 0.16	1.69 ± 0.19	1.62 ± 0.16
	100 μM	2.23 ± 0.22	2.33 ± 0.21	2.45 ± 0.23	3.23 ± 0.25	3.35 ± 0.26	3.31 ± 0.24	1.42 ± 0.12	1.57 ± 0.15	1.58 ± 0.13
LSD		S = 0.09			S = 0.11			S = 0.08		
		KIN = 0.12			KIN = 0.18			KIN = 0.11		
NR	Control	7.09 ± 0.73	7.05 ± 0.70	7.08 ± 0.76	7.49 ± 0.59	7.54 ± 0.62	7.51 ± 0.80	4.74 ± 0.91	4.80 ± 0.85	4.82 ± 0.82
	1 μM	7.55 ± 0.88	7.58 ± 0.86	7.45 ± 0.61	8.30 ± 0.89	8.52 ± 0.63	8.42 ± 0.72	5.25 ± 0.69	5.47 ± 0.71	5.33 ± 0.75
	10 μM	8.30 ± 0.91	8.74 ± 0.72	8.59 ± 0.55	9.25 ± 0.85	9.74 ± 0.77	9.62 ± 0.81	5.52 ± 0.66	6.11 ± 0.68	5.89 ± 0.74
	100 μM	7.95 ± 0.79	8.28 ± 0.79	8.21 ± 0.73	9.11 ± 0.84	9.36 ± 0.81	9.42 ± 0.79	5.16 ± 0.92	5.50 ± 0.89	5.35 ± 0.90
LSD		S = 0.14			S = 0.13			S = 0.15		
		KIN = 0.19			KIN = 0.18			KIN = 0.22		
Protein	Control	11.31 ± 1.2	11.32 ± 1.4	11.40 ± 1.5	12.34 ± 1.2	12.36 ± 1.1	12.42 ± 1.3	10.55 ± 1.1	10.51 ± 1.3	10.45 ± 1.2
	1 μM	12.16 ± 1.3	12.90 ± 1.4	12.45 ± 1.3	14.65 ± 1.2	15.16 ± 1.4	14.85 ± 1.3	11.76 ± 1.5	12.43 ± 1.2	12.13 ± 1.3
	10 μM	14.38 ± 1.4	14.95 ± 1.5	14.73 ± 1.4	16.91 ± 1.6	17.39 ± 1.7	17.83 ± 1.6	13.59 ± 1.2	14.61 ± 1.3	13.88 ± 1.1
	100 μM	13.72 ± 1.1	14.06 ± 1.4	14.22 ± 1.3	16.35 ± 1.5	17.12 ± 1.6	17.25 ± 1.5	12.76 ± 1.4	13.32 ± 1.2	13.12 ± 1.4
LSD		S = 0.15			S = 0.30			S = 0.26		
		KIN = 0.31			KIN = 0.69			KIN = 0.50		
Chl	Control	1.03 ± 0.09	1.06 ± 0.10	1.08 ± 0.11	1.21 ± 0.11	1.25 ± 0.12	1.22 ± 0.09	0.84 ± 0.08	0.83 ± 0.06	0.78 ± 0.07
	1 μM	1.09 ± 0.08	1.23 ± 0.07	1.18 ± 0.10	1.38 ± 0.10	1.47 ± 0.12	1.46 ± 0.13	1.01 ± 0.06	1.15 ± 0.08	1.03 ± 0.09
	10μM	1.21 ± 0.12	1.41 ± 0.14	1.37 ± 0.12	1.55 ± 0.14	1.76 ± 0.17	1.71 ± 0.16	1.11 ± 0.11	1.32 ± 0.12	1.26 ± 0.10
	100 μM	1.14 ± 0.10	1.37 ± 0.13	1.23 ± 0.12	1.42 ± 0.15	1.51 ± 0.16	1.52 ± 0.15	1.07 ± 0.07	1.15 ± 0.09	1.10 ± 0.10
LSD		S = 0.10			S = 0.09			S = 0.08		
		KIN = 0.16			KIN = 0.13			KIN = 0.07		

Table 1 continues on the next page.

Table 1 (continued)

	Treatment [h]	30 DAE 5	10	15	50 DAE 5	10	15	70 DAE 5	10	15
g_s	Control	0.303 ± 0.01	0.295 ± 0.01	0.290 ± 0.02	0.351 ± 0.03	0.340 ± 0.02	0.353 ± 0.02	0.258 ± 0.01	0.251 ± 0.02	0.253 ± 0.01
	1 µM	0.326 ± 0.03	0.332 ± 0.02	0.322 ± 0.02	0.392 ± 0.01	0.415 ± 0.04	0.405 ± 0.03	0.259 ± 0.01	0.274 ± 0.03	0.273 ± 0.02
	10 µM	0.375 ± 0.04	0.401 ± 0.04	0.391 ± 0.03	0.442 ± 0.05	0.479 ± 0.03	0.462 ± 0.02	0.314 ± 0.03	0.361 ± 0.02	0.334 ± 0.02
	100 µM	0.366 ± 0.03	0.382 ± 0.04	0.385 ± 0.02	0.433 ± 0.04	0.443 ± 0.05	0.441 ± 0.03	0.312 ± 0.02	0.324 ± 0.03	0.304 ± 0.01
LSD		S = 0.010 KIN = 0.020			S = NS KIN = 0.032			S = NS KIN=0.014		
P_N	Control	10.98 ± 1.1	11.07 ± 1.2	11.12 ± 1.1	12.49 ± 1.4	12.58 ± 1.3	12.4281.2	10.12 ± 1.1	10.28 ± 1.3	10.25 ± 1.2
	1 µM	12.11 ± 1.4	13.99 ± 1.5	12.56 ± 1.4	14.72 ± 1.6	15.87 ± 1.5	15.36 ± 1.3	10.66 ± 1.4	11.73 ± 1.4	11.55 ± 1.1
	10 µM	14.04 ± 1.5	15.11 ± 1.6	14.86 ± 1.6	16.45 ± 1.8	18.01 ± 1.8	17.68 ± 1.6	12.45 ± 1.5	13.55 ± 1.6	13.23 ± 1.4
	100 µM	13.87 ± 1.3	14.61 ± 1.4	14.25 ± 1.3	15.86 ± 1.6	17.31 ± 1.7	17.21 ± 1.5	12.11 ± 1.3	12.25 ± 1.2	13.02 ± 1.5
LSD		S = 0.88 KIN = 1.11			S = 0.84 KIN = 1.20			S = 0.71 KIN = 0.93		

Table 2. Dry mass (DM) [g plant^{-1}], leaf area (LA) [cm^2 plant^{-1}], (sampled at 30, 50, and 70 DAE), and number of capsules and seed yield [g plant^{-1}], (sampled at 90 DAE), of *Nigella sativa* plants, raised from seeds soaked in water (control), 1, 10, or 100 µM KIN for 5, 10, or 15 h. Least significant difference (LSD) for p=0.05, mean ± SE, (n = 5), DAE – days after emergence; S – soaking; KIN – kinetin.

		30 DAE			50 DAE			70 DAE / 90 DAE		
	Treatment [h]	5	10	15	5	10	15	5	10	15
DM	Control	1.35 ± 0.21	1.41 ± 0.11	1.38 ± 0.19	2.08 ± 0.25	2.10 ± 1.21	2.12 ± 0.24	3.48 ± 0.12	3.52±0.12	3.50 ± 0.14
	1 µM	1.55 ± 0.11	1.79 ± 0.15	1.62 ± 0.16	2.35 ± 0.27	2.60 ± 0.19	2.45 ± 0.31	3.98 ± 0.14	4.60±0.16	4.45 ± 0.13
	10 µM	1.75 ± 0.14	1.99 ± 0.14	1.89 ± 0.22	2.93 ± 0.19	3.26 ± 0.26	3.08±0.27	5.16 ± 0.16	5.87±0.15	5.64 ± 0.16
	100 µM	1.69 ± 0.12	1.82 ± 0.18	1.78 ± 0.25	2.43 ± 0.22	3.01 ± 0.21	2.90±0.30	5.11 ± 0.12	5.53±	5.42 ± 0.13
LSD		S = 0.10 KIN = 0.12			S = 0.10 KIN = 0.20			S = 0.09 KIN = 0.15		
LA	Control	130.1 ± 11.2	136.2 ± 10.2	140.2 ± 18.6	255.4 ± 18.7	265.1 ± 22.1	262.5 ± 25.1	243.5 ± 21.4	245.3 ± 19.7	251.1 ± 22.3
	1 µM	149.5 ± 14.3	170.6 ± 18.4	162.4 ± 13.6	301.4 ± 22.3	355.2 ± 27.1	332.3 ± 20.1	276.5 ± 23,5	316,3 ± 25.3	306.4 ± 23.2
	10 µM	171.4 ± 18.4	197.5 ± 21.6	190.2 ± 16.2	365.2 ± 20.3	430.8 ± 31.1	410.6 ± 24.2	323.6 ± 20.5	390.6 ± 23.1	372.6 ± 26.1
	100 µM	159.2 ± 16.7	180.8 ± 24.6	175.3 ± 14,5	323.7 ± 17.4	415.6 ± 32.4	401.4 ± 24.2	311.7 ± 23.3	380.3 ± 24.5	355.1 ± 21.8
LSD		S = 9 KIN = 15			S = 10 KIN = 16			S = 15 KIN = 15		
Capsule number	Control							16.22 ± 1.7	16.32 ± 1.4	16.38 ± 1.5
	1 µM							17.25 ± 1.6	18.80 ± 1.8	18.34 ± 1.7
	10 µM							18.97 ± 1.9	20.69 ± 2.1	20.62 ± 1.9
	100 µM							17.65 ± 1.7	20.46 ± 1.8	20.41 ± 1.5
LSD								S = 0.42 KIN= 0.72		
Seed yield	Control							1.22 ± 0.17	1.31 ± 0.12	1.24 ± 0.15
	1 µM							1.18 ± 0.15	1.45 ± 0.14	1.36 ± 0.13
	10 µM							1.40 ± 0.14	1.61 ± 0.17	1.51 ± 0.15
	100 µM							1.32 ± 0.16	1.52 ± 0.14	1.44 ± 0.13
LSD								S = 0.13 KIN = 0.24		

(1990), and Premabatidevi (1998). NR is responsible for the initiation of nitrate metabolism and consequently for protein synthesis at various levels in the plant body. The increase in NR activity by CYT is expressed at the level of NR mRNA, which is increased by this hormone but suppressed by ABA (Lu *et al*. 1992). In addition to NR activity, binding of CYKs to ribosomes conceivably could have also had profound consequences for the regulation of protein synthesis (Moore 1989). With an opulent protein status, the availability of structural and functional photosynthetic components might have been optimized, reflecting in turn as an enhanced P_N. The correlation observed herein between NRA and Chl content ($r = 0.915^{**}$) and NRA and P_N ($r = 0.925^{**}$), further supports this stance.

At 50 DAE, Chl content of the leaves of the test plants was enhanced by 41% as compared to the control. In addition to the aforementioned influence of KIN, the observed increase in Chl content might also have resulted from the stimulation of the intensity of cell growth and activity of ribosomes, which could have also possibly increased the number of chloroplasts in the leaf (Aldesuquy and Baka 1998), and consequently enhanced Chl content (Pospíšilová *et al*. 2000, El-Shihaby *et al*. 2002). Moreover, kinetin is also known to prevent the degradation of Chl (Pospíšilová *et al*. 2000), thereby facilitating both the retention and the accumulation of this important photosynthetic pigment.

Besides the increase in Chl content, the capacity of the leaves of the test plants to garner light was also potentiated by the 63% increase of their LA (Table 2). Moreover, in addition to promoting meristematic activity and cell enlargement (Jablonski and Skoog 1954), KIN also functions to enhance the transport of nutrients through the seedling stems (Seth and Wareing 1964), and regulate the partitioning of assimilate among different plant parts (Hutchison and Kieber 2002). Therefore, the supplementation of KIN during the critical peri-germination phase, as achieved by the presowing treatment, might have favorably modulated the potential of the developing sink for growth and nutrient translocation, as evidenced by the 55% increase observed in DM of the test plants at 50 DAE (Table 2).

Consequent to the enhanced morphological and photosynthetic characteristics, at harvest, number of capsules and seed yield of *Nigella* plants grown from the treated seeds also increased by 27 and 23% (Table 2). Evidently, these plants were better equipped to harness the available nutrients and convert them into valuable photoassimilates. In addition, the effect of KIN on the delay of senescence could have further appended the aforementioned factors. Results similar to the present study have been reported by Iqbal and Ashraf (2005), for wheat under salt stress, wherein presowing treatment with KIN was postulated to mitigate the adverse effects on growth, photosynthetic activity and yield, by addressing the salinity-induced internal hormonal imbalance. However, in the light of the present findings, an inherent stimulatory role of exogenous KIN can be postulated, besides restoration of hormonal deficiency, which functions to optimize the internal metabolic environment, probably hence facilitating the acclimation processes to counter stress.

Conclusively, the present study confirms, in a previously uninvestigated species, the preceding reports of enhancement of growth and yield following presowing treatment with KIN, and further elucidates the enhancement in photosynthetic activity to have arisen from a favorable modulation of the levels of protein and activities of key enzymes. With the internal metabolic constraints hence overcome, the growing seedlings availed their resources to their potential, expressing better outcomes at growth and harvest.

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