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Comparative effects of 4-Cl-IAA and kinetin on photosynthesis, nitrogen metabolism and yield of black cumin (*Nigella sativa* L.)

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Abstract – The leaves of 40-day old plants of black cumin (*Nigella sativa* L.) were sprayed with 10^{-7}, 10^{-6}, 10^{-5} M 4-Cl-IAA, and 10^{-6}, 10^{-5} and 10^{-4} M kinetin. Both the hormones improved vegetative growth, photosynthetic efficiency and seed yield of the test plants as compared to deionized water (control). However, 10^{-6} M 4-Cl-IAA was most prominent in its effect, generating 42, 30, 40, 41 and 51% higher values for carbonic anhydrase, nitrate reductase, net photosynthetic rate, leaf protein content and dry mass respectively, as compared to the control in 70-day old plants. Similarly, capsule number and seed yield per plant were elevated by 41 and 43% over the untreated control at harvest (130 days after sowing) following the same treatment. Overall, the auxin showed a higher efficiency than kinetin in all treatment concentrations.

Key words: 4-Chloroindole-3-acetic acid, photosynthesis, nitrate reductase, carbonic anhydrase, protein, kinetin, yield, *Nigella sativa*.

Abbreviations; CA – carbonic anhydrase, **4-Cl-IAA –** 4-chloroindole-3-acetic acid, **NR** – nitrate reductase, **PAR** – photosynthetic active radiation, **P_N**- net photosynthetic rate, **RuBPCO** – ribulose-1, 5-bisphosphate carboxylase/oxygenase

Introduction

Auxins are a class of naturally occurring plant growth substances found throughout the plant kingdom. They are known actively to influence a number of developmental processes such as stem elongation, ethylene biosynthesis and tissue vascularization (NORDSTROM et al. 1991). The first known ubiquitously occurring auxin is indole-3-acetic acid (IAA), but various other analogues such as 4-Cl-IAA have also been identified in certain species. This halogenated compound is characterized by very high auxin-like activity (REINECKE et al. 1995), demonstrated in the growth of excised tissue (KATEKAR and GEISSLER 1982), rooting

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in mung bean (STENLID et al. 1987), parthenocarpy and epinasty in tomato (SELL et al. 1953) and the synthesis/induction of specific enzymes in detached pea cotyledons (HIRASAWA 1989, AHMAD and HAYAT 1999, AHMAD et al. 2001a). In addition, acceleration of the rate of photosynthesis resulting in enhancement of the economic yield has also been reported (AHMAD et al. 2001b).

Closely associated in function with auxins are another group of endogenous plant growth substances, the cytokinins. These hormones have an equally potent effect on plant physiology and environmental responses, and are intimately involved in the regulation of cell division, apical dominance, chloroplast development, anthocyanin production and maintenance of the source-sink relationship (HUTCHKINSON and KIEBER 2002). Also, cytokinins are regarded as the most important senescence-retarding hormones and their exogenous application has been demonstrated to prevent the degradation of chlorophyll and photosynthetic proteins (WINGLER et al. 1998), as well as reverse leaf and fruit abscission (POSPÍŠILOVÁ et al. 2000). Owing to the interlinked nature of the action of these hormones and because of their potential benefits in relation to plant performance and productivity, the present study was designed to compare the effect of auxin (4-Cl-IAA) and cytokinin (kinetin) supplementation on growth, nitrogen metabolism and yield of black cumin (*Nigella sativa* L.). Though natively Middle Eastern, this herb is valued highly in domestic and international markets owing to its characteristic medicinal and aromatic properties. It is also a significant source of essential fatty acids, carbohydrates, vitamins (A, D, B_2 and niacin) and minerals (calcium, potassium, iron, magnesium, selenium and zinc) (SAEED et al. 1996).

Materials and methods

Seeds of black cumin (*Nigella sativa* L.) were obtained from the Regional Research Institute of Unani Medicine, Aligarh, and were surface sterilized by soaking in 0.01% mercuric chloride solution for 3 minutes, followed by repeated washing with double distilled water. The seeds were then sown in earthen pots (25 cm diameter), filled with 10 kg of a mixture of sandy loam soil and farmyard manure in a ratio of 9:1 (w/w). 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. The pots were lined in the Department's net house, according to simple randomized block design, with three replications. 5 uniform plants were maintained per pot.

4-Chloroindole-3-acetic acid (4-Cl-IAA) and kinetin were obtained from *Sigma Chemicals* Co., St. Louis, USA. The plants of 40-days age (vegetative stage) were sprayed with 10^{-7}, 10^{-6} or 10^{-5} M aqueous solution of 4-Cl-IAA or 10^{-6}, 10^{-5} or 10^{-4} M that of kinetin at the rate of 5 cm^3 per plant. Control plants were sprayed with double distilled water only. The stage of spraying was determined through an earlier experiment (SHAH 2007).

Five plants from each replicate were randomly selected and carbonic anhydrase activity (CA), dry mass, nitrate reductase activity, net photosynthetic rate (P_N), and protein content were assessed at 50 and 70 days after sowing, corresponding to vegetative and flowering stage respectively. The CA activity in the leaves was measured following the procedure described by DWIVEDI and RANDHAWA (1974). Nitrate reductase (NR) activity was determined in fresh leaf samples, followed the method of JAWORSKI (1971). The net photosynthetic rate was measured using a LI-6200 portable photosynthetic system (LI-COR 6200, Lincoln, NE, USA) on fully expanded uppermost leaves, at atmospheric conditions (between 1100–1200 h): photosynthetic active radiation (PAR) about 990 µmol m^{-2} s^{-1}, relative humidity 63%, temperature 23 °C. Protein content of the leaves was estimated by the method of LOWRY et al. (1951). Shoot dry mass was determined after dehydrating the plants at 80 °C for 24 h. Capsule number and seed yield per plant were estimated at harvest (130 days after sowing). Treatment means were compared by analysis of variance using the statistical package SPSS (SPSS 7.5.1 for Windows, standard version 1996). Least significant difference was estimated at 0.05 level of probability.

Results

Foliar treatment of either hormone was found appreciably to enhance all the studied parameters; however, a higher degree of stimulation was noted following the application of 4-Cl-IAA, especially at the 10^{-6} M concentration. Among different concentrations of kinetin, 10^{-5} M was found to elicit the maximal response (Tabs. 1, 2). The activities of enzymes CA and NR were significantly elevated in the hormone-treated test plants as compared to the water sprayed control. A maximum enhancement of 35 and 42% was noted in the activity of CA at the 50 and 70 days after sowing sampling stages respectively, following treatment with 10^{-6} M 4-Cl-IAA. On the same sampling days, treatment with kinetin was found to cause an enhancement of 28 and 37%, respectively (Tab. 1). Concurrently, the activity of NR was optimized by 30 and 39% following the auxin treatment as noted at 50 and 70 days after sowing, respectively. On the other hand, corresponding enhancements of 24 and 32 % in enzyme activity were achieved at the respective samplings from treatment with kinetin. In correspondence with the increase in NR activity, protein content was also found to be enhanced in the leaves of the test plants as compared to the control; there was a maximum enhancement of 27 and 38% with the auxin treatment in the samplings, respectively. Analogous enhancements of 21 and 29% were recorded subsequent to treatment with kinetin. P_N of the treated plants was also appreciably elevated as compared to the control, registering a maximum increase of 26 and 38% at the 50 and 70 days after sowing stages following 4-Cl-IAA spray. Comparatively, an enhancement of 20 and 30% was brought about by the kinetin treatment at respective samplings. Consequent to the increased P_N, the test plants also exhibited an increased dry mass production, the maximum enhancement being by 35 and 50% with 4-Cl-IAA, whereas that with kinetin was 26 and 42% over the control at respective sampling stages.

Finally, at harvest, the hormone treated plants showed an appreciable improvement of the yield parameters. Number of capsules and seed yield $plant^{-1}$ were increased by 39 and 41% following the auxin treatment. Correspondingly, treatment with kinetin achieved an increase of 31 and 32%, respectively in the mentioned parameters as compared to the control (Tab. 2).

Discussion

Test plants receiving either of the hormone treatments exhibited an increase in net photosynthetic rate (P_N). This effect could be a consequence of the direct stimulation of the process itself by the hormones, as well as of the optimization of other parameters to facili-

Tab 1. Carbonic anhydrase (CA) activity [mol (CO_2) kg^{-1} s^{-1}], nitrate reductase (NR) activity [nmol (NO_2) g^{-1} min^{-1}], net photosynthetic rate (P_N) [µmol (CO_2) m^{-2} s^{-1}], protein content [% (DM)] and dry mass (DM) [g $plant^{-1}$], in *Nigella sativa* leaves, sprayed with water (control), 4-Cl-IAA or kinetin (KIN) at 40-d after sowing and sampled at 50 (vegetative stage) and 70 DAS (flowering stage). Each value is mean ± S.E, (n = 3) LSD for $P = 0.05$.

Parameter	Treatment	[M]	50 DAS	% increase over control	70 DAS	% increase over control
CA	control		2.17±0.20	–	2.64±0.18	–
	KIN	10^{-6}	2.51±0.22	16	3.19±0.23	21
		10^{-5}	2.79±0.17	28	3.61±0.24	37
		10^{-4}	2.73±0.22	26	3.56±0.21	35
	4-Cl-IAA	10^{-7}	2.58±0.15	19	3.30±0.20	25
		10^{-6}	2.93±0.25	35	3.74±0.25	42
		10^{-5}	2.88±0.22	33	3.70±0.23	40
LSD			0.068		0.057	
NR	control		7.11±0.65	–	7.56±0.81	–
	KIN	10^{-6}	7.97±0.55	12	8.93±0.54	18
		10^{-5}	8.82±0.65	24	9.97±0.75	32
		10^{-4}	8.89±0.81	25	9.90±0.81	31
	4-Cl-IAA	10^{-7}	8.32±0.64	17	9.30±0.71	23
		10^{-6}	9.25±0.61	30	10.50±0.71	39
		10^{-5}	9.17±0.71	29	10.36±0.81	37
LSD			0.17		0.18	
P_N	control		15.01±1.3	–	16.05±1.6	–
	KIN	10^{-6}	16.66±1.6	11	18.65±1.9	16
		10^{-5}	18.01±1.7	20	20.88±1.4	30
		10^{-4}	17.71±1.5	18	20.38±1.7	27
	4-Cl-IAA	10^{-7}	17.10±1.6	14	19.75±1.4	23
		10^{-6}	18.95±1.9	26	22.15±1.6	38
		10^{-5}	18.61±1.7	24	21.66±1.7	35
LSD			0.48		0.58	
Protein	control		11.35±1.1	–	12.75±1.1	
	KIN	10^{-6}	12.83±1.2	13	14.65±1.4	15
		10^{-5}	13.74±1.4	21	16.46±1.3	29
		10^{-4}	13.63±1.3	20	16.34±1.2	28
	4-Cl-IAA	10^{-7}	13.30±1.5	17	15.31±1.5	20
		10^{-6}	14.42±1.6	27	17.62±1.8	38
		10^{-5}	14.21±1.1	25	17.48±1.5	37
LSD			0.32		0.78	
DM	control		1.15±0.11		1.85±0.17	
	KIN	10^{-6}	1.29±0.12	12	2.18±0.18	18
		10^{-5}	1.45±0.13	26	2.63±0.16	42
		10^{-4}	1.44±0.10	25	2.61±0.19	41
	4-Cl-IAA	10^{-7}	1.35±0.09	17	2.31±0.16	24
		10^{-6}	1.55±0.16	35	2.79±0.20	50
		10^{-5}	1.53±0.15	33	2.74±0.21	48
LSD			0.06		0.08	

Tab 2. Number of capsules and seed yield plant^{-1} in *Nigella sativa* L. plants, sprayed with water (control), 4-Cl-IAA or kinetin (KIN) at 40-d after sowing and sampled at harvest (130 DAS), LSD for *P* = 0.05. Each value is mean ± S.E, (*n=3*).

Treatment	[M]	Number of capsules [plant^{-1}]	% increase over control	Seed yield [g plant^{-1}]	% increase over control
Control		16.15±1.4	–	1.20±0.17	–
KIN	10^{-6}	18.50±1.7	14	1.35±0.20	13
	10^{-5}	21.28±1.8	31	1.58±0.21	32
	10^{-4}	20.98±1.6	30	1.57±0.19	31
4-Cl-IAA	10^{-7}	19.57±1.9	21	1.43±0.17	19
	10^{-6}	22.46±2.2	39	1.69±0.20	41
	10^{-5}	22.15±1.8	37	1.65±0.11	38
LSD		0.65		0.05	

tate efficient photosynthetic activity. The elevation of the activities of enzymes CA and NR can be said to have been of significant consequence. After the substrate for carbon fixation, CO_2, diffuses into the stomata and is transported into the chloroplast, it is reduced by RuBPCO, the main C fixing enzyme. However, this supply of CO_2 to RuBPCO depends on the activity of CA, which catalyses the reversible hydration of HCO_3 to CO_2 in close proximity to RuBPCO. This entire mechanism could have been augmented by the enhancement in CA activity, as achieved in the present study through hormone supplementation. In context of the 4-Cl-IAA treatment, such results can be ascribed to the action of auxins in de-repressing certain genes and in activating the process of translation and transcription (KEY 1969, HOPKINS 1995). Similarly, the enhancement effect of kinetin on CA activity can be said to have arisen at the level of transcription and / or stabilization of the transcripts while they increased the content of CA-mRNA through translation (SUGIHARTO et al. 1992). Our hypothesis that enhanced CA activity brought about an optimization of CO_2 availability, consequently to affect P_N, is strongly supported by the positive correlation observed between CA and P_N of the hormone treated plants (r = 0.875).

Another level of influence for the hormones on P_N was the protein content in leaves of the test plants, which was enhanced following an increase of NR activity (Tab. 1). NR regulates the major rate limiting stage in the reduction of nitrate to ammonium, which is then incorporated in amino acids (HOPKINS 1995).The hormone treatment resulted in an increase in NR activity and thus elevated the useable form of nitrogen (ammonia) to produce a larger pool of amino acids/amides, in addition to stimulating their incorporation to polypeptide synthesis (by activating transcription and/or translation) and optimizing protein formation. This conjecture is supported by the correlation obtained between NR activity and the level of protein in leaves of the test plants (r = 0.821) (SINGH and SINGH 1985). In the context of NR, similar enhancements have been reported following auxin and kinetin treatment, although not from the modulation of the activity of the existing enzyme, but by induction of its *de novo* synthesis (PREMABATIDEVI 1998). Hence in the present study, the influence of auxins and cytokinins on translation/transcription mechanisms, as aforementioned, can be referred to in the context of increased NR activity and protein content of the test plants.

In the present study, leaves of plants receiving either of the hormone treatments not only photosynthesized at a faster rate, but also possessed an extended period of metabolic activity, because of the delayed senescence caused by the phytohormones (DAVIES 1995). As a result, these test plants accumulated a large quantity of metabolites, which is evident from the increase observed in their dry mass (Tab. 1). A similar positive relation between P_N and dry mass production has been observed by KHAN (1994).

The sufficient availability of nutrient facilitated ample vegetative growth in the treated plants, thus resulting in an increase in the number and size of reproductive sink (number of capsules per plant) (Tab. 2). This, when coupled with the 4-Cl-IAA-enhanced mobilization of photoassimilates to the developing capsules (DAVIES 1995), may have further stimulated their growth and hence caused an increase in seed yield, as observed herein (Tab. 2). Likewise, the effect of kinetin on economic yield, though relatively mild, may be ascribed to the increased opportunity for formation of new buds (BRUINSMA 1977), and successful reversal of flower and fruit abscission (NAGEL et al. 2001).

Conclusively, the efficacy of auxin (4-Cl-IAA) was found to be superior to that of kinetin with regard to performance and productivity of *Nigella sativa*. The relatively subdued effect of kinetin may have been because endogenous cytokinin is seldom limiting in plants, and hence exogenously applied hormone seems less effective (MOORE 1989).

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