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Research Note

Effects of Nitrogen Fertilisation on Nitrate Reductase Activity, Protein, and Oil Yields of *Nigella sativa* L. as Affected by Foliar GA_3 Application

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Abstract: The influence of foliar GA_3 application (0, 10^{-4}, 10^{-5}, or 10^{-6} M) on the responses of *Nigella sativa* L. to various levels of N fertilisation (0, 176, 264, 352, or 442 mg N pot^{-1}) was analysed through 2 pot experiments conducted at the Department of Botany, Aligarh Muslim University, Aligarh, India. The N fed plants showed a significant enhancement of capsule number and seed yield $plant^{-1}$, and nitrate reductase activity, as well as protein and oil yields $plant^{-1}$, especially upon application of 352 mg N pot^{-1}. Moreover, the effect of basal N was further potentiated following an additional treatment with various GA_3 concentrations. However, in contrast to all other parameters, seed oil content decreased with either treatment. Nonetheless, the combined application of 352 mg N pot^{-1} and 10^{-5} M GA_3 proved to be maximally stimulative.

Key Words: *Nigella sativa*, nitrate reductase, nitrogen, GA_3, seed oil content, seed protein content, seed yield

Introduction

Nitrogen (N) is the single most important growth limiting factor for crops and, when supplied in the form of urea, has proved to be most instrumental among all major elements in boosting the yield of numerous plants (Kumar et al., 2004; Ashraf & Noman, 2006; Ashraf et al., 2006). The success of N fertilisation mainly arises from the indispensability of N as a plant nutrient, wherein it forms an integral part of biologically critical molecules, such as nucleic acids, structural and catalytic proteins, and chlorophyll, that regulate photosynthesis and crop production (Wu Feibo et al., 1998). Evidently, ample availability of N liberates a plant from impairing nutrient deficiencies and provides a sound platform for superlative growth. However, toxicity and cost concerns limit the use of increasing amounts of inorganic fertilisers, and therefore implicate the use of additional supplementary treatments that can manipulate a crop to utilise maximum possible available resources and potentiate the performance and productivity to obtain a superior yield at harvest. In this respect, the use of phytohormones such as GA_3 has been found to be sufficiently effectual in enhancing the growth and productivity of various plants by promoting the uptake of nutrients (Khan et al., 1998; Shah et al., 2006) and their incorporation to result in enhanced growth, dry mass, and biomass production as well as photosynthetic rate (Khan & Samiullah, 2003; Shah & Samiullah, 2006; Shah et al., 2007).

Nigella sativa L., native to the eastern Mediterranean region, has been used since antiquity for culinary and pharmacological purposes. It is also a valuable source of carbohydrates, proteins, essential fatty acids, vitamins, and minerals (Saeed et al., 1996). Because of its characteristics properties, there is an increasing demand for this herb in the domestic and international markets (Shah & Samiullah, 2006). Therefore, this investigation was designed to study the effects of various N fertilisation rates as influenced by foliar application of GA_3, in relation to N metabolism, protein, seed, and oil yields of black cumin (*Nigella sativa* L.).

Materials and Methods

Two pot experiments were carried out at the Department of Botany, Aligarh Muslim University, Aligarh, India, on *Nigella sativa* L. in the winter seasons of 2000-2001 and 2001-2002, respectively. The soil of the experimental pots was of sandy loam type, with available

N for the 2 years being 195 and 198 kg N ha^{-1}, respectively. The basal application of N in the form of urea was done at the time of sowing at the rate of 0, 176, 264, 352, or 442 mg N pot^{-1}. Spray concentrations of GA_3 at the 10^{-6}, 10^{-5}, or 10^{-4} M were administered 40 days after sowing (vegetative stage), at the rate of 5 cm^3 per plant. Water was used as a control treatment. A randomised complete block design with 3 replications was used. Seeds were obtained from the Regional Research Institute of Unani Medicine, Aligarh, India, and were surface sterilised with 0.01% mercuric chloride solution, followed by repeated washings with double distilled water. They were then sown in earthen pots (25 cm in diam.) filled with soil and farmyard manure, mixed in a ratio of 9:1 (v/v).

Nitrate reductase (E.C. 1.6.6.1.) activity was estimated in fresh leaves of the plants as described by Jaworski (1971). Then 500 mg fresh leaf pieces were weighed and placed in polythene vials. To each, 2.5 ml of phosphate buffer (pH 7.5) and potassium nitrate (0.02 M) solution were added followed by addition of 2.5 ml of 5% isopropanol. Lastly, 2 drops of chloramphenicol solution were added to prevent bacterial growth in the medium. The vials were incubated for 2 h in the dark at 28 ºC. Then 0.4 ml of incubated mixture was taken in a test tube to which 0.3 ml each of 1% sulphanilamide and 0.02% naphthylethylene diamine hydrochloride (NED-HCl) had been added. The solution was left for 20 min for maximum colour development. It was diluted to 5 ml with a sufficient amount of DDW and optical density was read at 540 nm using a spectrophotometer. A blank consisting of 4.4 ml of DDW and 0.3 ml each of sulphanilamide and NED-HCl was used simultaneously for comparison. A standard curve was plotted by taking known graded dilutions of potassium nitrate from a standard aqueous solution of this salt. The optical density of the samples was compared with this calibrated curve and NRA was expressed as η mol $NO_2^- g^{-1} h^{-1}$ fresh leaf tissue.

The method described by Lowry et al. (1951) was followed for the colorimetric estimation of total protein content in the seeds. Oven dried seed powder (50 mg) was ground and dissolved in trichloroacetic acid to make a final volume of 5 ml. Protein content was then precipitated by allowing the sample to stand for 1 h, after which it was centrifuged. The precipitated proteins were then completely dissolved by heating at 60 °C in a water bath for 30 min. The sample was then again centrifuged and the supernatant containing protein fraction was collected in 25 ml of 1 N NaOH. Out of this solution 5 ml was used for final analysis after adding 0.5 ml of Folin phenol reagent. The blue colour that developed was measured colorimetrically at 660 nm against a standard solution of bovine serum albumin.

For determination of seed oil content, oil was extracted 3 times with a chloroform/methanol (2:1, v/v) mixture, according to the method outlined by Kates (1972). The percent oil and protein contents in seeds were then multiplied by seed yield to obtain oil and protein yields. At harvest, 5 plants from each treatment were selected and capsule number $plant^{-1}$ was recorded. The seed yield from 5 randomly selected plants was recorded after threshing the seeds.

Analysis of variance was carried out on the data obtained and LSD (P = 0.05) was calculated (Gomez & Gomez, 1984).

Results and Discussion

From results presented in Tables 1 & 2, it is evident that the application of basal N, especially at the rate of 352 mg N pot^{-1}, had the most favourable effect on all the parameters studied. Furthermore, this improvement was synergistically enhanced by the additional application of GA_3 to the N fed plants. Although GA_3 improved the plant responses to various N dosages in all concentrations, the combined application of 352 mg N pot^{-1} and 10^{-5} M GA_3 brought about the maximum stimulation.

The enhancement of capsule number and seed yield $plant^{-1}$ (Table 1) can be explained based on the fact that under optimal N nutrition CO_2 assimilation and P_N are favourably upregulated (Reddy et al., 1996). This results in an adequate supply of photoassimilates to the developing meristems, which enhances and maintains their growth. Thus, more reproductive structures (capsules) are produced per plant and area, as is the case with grains (Lowlor, 2002). Moreover, the capacity of these grains to grow is substantially increased probably because more cells with greater enzyme capacity are produced (Lowlor, 2002). Under such an enhanced sink potential, the availability of ample nutrients can be expected to cause more grain filling and thereby increase overall yield at harvest.

With reference to the additional GA_3 treatment, the accentuation of all the positive effects of N on yield

Table 1. Effects of nitrogen fertilisation and GA_3 application on nitrate reductase activity (analysed at 70 DAS), capsule number, and seed yield plant^{-1} of *Nigella sativa* L. (recorded at 130 DAS).

N rate (mg pot^{-1})	Spray treatments (M)				
	0	10^{-6}	10^{-5}	10^{-4}	
	NRA [nmol (NO_2) g^{-1} min^{-1}]				Mean
0	4.75	5.46	6.85	6.90	5.99
176	5.61	6.86	7.54	7.61	6.90
244	6.30	7.10	8.61	8.50	7.63
352	7.10	8.21	10.15	10.25	8.93
442	7.01	8.10	10.03	10.11	8.81
Mean	6.15	7.14	8.63	8.67	
LSD		F = 0.79	H = 0.34	F × H = 1.5	
	Capsule number plant^{-1}				
0	15.25	17.61	19.31	20.01	18.05
176	19.21	21.20	24.50	23.95	22.25
244	21.84	25.21	29.91	28.79	26.44
352	26.10	30.51	36.05	35.74	32.1
442	25.05	29.94	35.52	35.04	31.38
Mean	21.50	24.90	29.05	28.70	
LSD		F = 2.5	H = 1.3	F × H = 3.7	
	Seed yield (g plant^{-1})				
0	1.23	1.41	1.85	1.91	1.60
176	1.46	1.85	2.15	2.22	1.92
244	1.92	2.39	2.79	2.65	2.43
352	2.31	2.75	3.18	3.05	2.82
442	2.77	2.71	3.05	2.91	2.84
Mean	1.94	2.22	2.60	3.18	
LSD		F = 0.19	H = 0.21	F × H = 0.3	

F - Fertilisation rates.
H - Hormone concentrations.
LSD - Least significant difference.

(Tables 1 & 2) might have resulted from an increase in vascular capacity (Kuang et al., 1991a, 1991b) brought about by GA_3 under an enhanced sink potential, thereby facilitating increased translocation of photoassimilates to the developing reproductive organs. Moreover, the capacity of GA_3 to regulate the induction of flower/fruit set (Arteca, 1996) could also have supplemented the other causes to result in an overall enhancement of yield.

N fertilisation was also found to result in enhanced seed protein content and yield plant^{-1} (Tables 1 & 2). This is expected, as N is vital in basic protein structure as well as being an active constituent of RNA and DNA, which are essential for protein synthesis (Marschner, 1995). In addition, an equally potent effect of N on protein content was found to arise through an enhancement of NR activity (Table 1). NR is the regulatory enzyme in the N metabolism and is responsible for the reduction of nitrate to ammoniacal N, which is then incorporated in the production of amino acids (Hopkins, 1995). It is therefore understood that an increase in activity of NR will obviously enhance the production of amino acids thereby optimising their condensation into proteins. Furthermore, NR is known to be highly substrate inducible and hence any factor may change NR activity by influencing the nitrate availability (Khan & Srivastava, 1998). As such, the increased NRA observed after N application may well be attributed to the optimised availability of substrate. However, besides this, various other factors also regulate the activity of NR. These include the presence or absence of irradiation (Knypl & Krystyna, 1979), and presence of hormones, such as gibberellins, cytokinins (Roth-Bejerano & Lips, 1970),

Table 2. Effects of nitrogen fertilisation and GA_3 application on protein and oil contents and yields plant^{-1} of *Nigella sativa* L. (recorded at 130 DAS).

N rate (mg pot^{-1})	Spray treatments (M)				
	0	10^{-6}	10^{-5}	10^{-4}	Mean
	Oil Content (%)				
0	37.34	37.45	37.54	37.50	37.45
176	36.82	36.91	36.96	36.91	36.90
244	36.66	36.71	36.79	36.81	36.74
352	36.55	36.62	36.72	36.70	36.64
442	36.41	36.44	36.51	36.49	36.46
Mean	36.75	36.82	36.90	36.88	
LSD		F = NS	H = NS	F × H = NS	
	Oil yield (g plant^{-1})				
0	0.46	0.53	0.70	0.72	0.60
176	0.54	0.69	0.80	0.82	0.71
244	0.70	0.88	1.03	0.98	0.89
352	0.84	1.01	1.17	1.12	1.03
442	1.01	0.99	1.11	1.06	1.04
Mean	0.71	0.82	0.96	0.94	
LSD		F = 0.08	H = 0.05	F × H = 0.1	
	Protein content (%)				
0	19.75	20.42	21.89	21.71	20.94
176	22.11	22.81	23.41	23.35	22.92
244	23.35	24.55	25.21	25.51	24.65
352	25.48	26.41	27.85	27.65	20.84
442	26.04	26.11	27.25	27.01	21.60
Mean	23.35	24.06	25.12	25.05	
LSD		F = 0.85	H = 0.65	F × H = 1.6	
	Protein yield (g plant^{-1})				
0	0.24	0.29	0.40	0.41	0.33
176	0.32	0.44	0.50	0.51	0.44
244	0.45	0.58	0.72	0.67	0.60
352	0.59	0.72	0.89	0.84	0.76
442	0.59	0.70	0.83	0.78	0.72
Mean	0.44	0.55	0.67	0.51	
LSD		F = 0.09	H = 0.06	F × H = 0.1	

F - Fertilisation rates.
H - Hormone concentrations.
NS - Not significant.
LSD - Least significant difference.

auxins (Ahmad & Hayat, 1999), and monochloro-indole acetic acids (Ahmad et al., 2001). In the present study GA_3 was found to potentiate the response of NR to N fertilisation (Table 1), which may be ascribed to the influence of gibberellins on the basic processes of translation/transcription (Huttly & Phillips, 1995) thereby causing some effect on enzyme and protein synthesis.

In contrast to all other parameters, seed oil content was found to decrease with increasing levels of basal N (Table 2), which may be because the sink became more voluminous due to the application of fertiliser, resulting in dilution with growth, and hence decreasing the oil content of the seeds. Moreover, it is known that available N stimulates the accumulation of protein in the seed rather than oil (Sawan et al., 2001) by causing the preferential utilisation of "C" skeletons at the time of seed filling towards protein synthesis, instead of oil formation. However, due to enhanced production of seeds, the oil yield plant^{-1} was so spectacular that it outbalanced the

lowered oil content (Table 2). This is an obvious commercial advantage for the herb. These results are on expected lines and corroborate the findings reported by Pandrangi et al. (1992), Krishnamoorthy et al. (2000), and Sawan et al. (2001). Likewise, application of GA_3 to the N-treated plants elicited a similar pattern of response, with the oil yield plant^{-1} being enhanced appreciably enough to compensate for decreases in the seed oil content (Table 2). Similar findings have been reported by Mahmoud (1996).

In conclusion, the results of the present study suggest that combined application of 352 mg N pot^{-1} and 10^{-5} M spray of GA_3 holds promising potential for practical use in order to ensure maximal productivity without excessive use of inorganic fertiliser.

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