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Physiological effects of pre-sowing seed treatment with gibberellic acid on *Nigella sativa* L.

SHOUKAT H. SHAH*

Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh-202 002, India

A pre-sowing treatment was applied to black cumin (*Nigella sativa* L.) seeds, which were surface-sterilized and soaked for 5, 10 or 15 h in 10^{-6}, 10^{-5}, or 10^{-4} M aqueous solution of gibberellic acid (GA). The potted plants were then analysed at 50, 70 and 90 days after sowing for leaf chlorophyll (Chl) content, stomatal conductance (g_s), carbonic anhydrase (CA) activity, nitrate reductase (NR) activity, total protein content, net photosynthetic rate (P_N), capsule number and seed yield at harvest (130 days after sowing). All these parameters were found to be appreciably enhanced by the hormone treatment. The most prominent results were obtained with 10^{-5} M GA following a pre-sowing treatment for 10 h, in which case the values for P_N, CA and NR activity, and seed yield were elevated by 44, 40, 30 and 40% respectively over the control at the 70 day stage.

Key words: seed, treatment, carbonic anhydrase, chlorophyll, *Nigella sativa*, photosynthetic rate, nitrate reductase, stomatal conductance, protein.

Abbreviations: GA – gibberellic acid, CA – carbonic anhydrase, NR – nitrate reductase, P_N – net photosynthetic rate, g_s – stomatal conductance, RuBPCO – ribulose,1–5, biphosphate carboxylase oxygenase

Introduction

Gibberellins are a class of endogenous plant growth substances that exert pleiotropic effects on plant developmental processes, such as seed germination, endosperm mobilization, stem elongation, leaf expansion, flower and fruit set (DAVIES 1995), and assimilate translocation (OUZOUNIDOU and ILIAS 2005), phloem loading (HAYES and PATRICK 1985), induce elongation and osmoregulation in internodes (AZUMA et al. 1997), dry mass and biomass production (GUPTA and DATTA, 2001), and the activities of key enzymes, i.e., carbonic anhydrase (CA) (SHAH et al. 2006), nitrate reductase (NR) (AFROZ et al. 2005), and α-amylase (GILROY and JONES 1992).

Although many studies have proved the efficacy of gibberellic acid (GA) in the promotion of germination (LEITE, et al. 2003), the study of the physiological development of plants grown from GA-treated seeds has rarely been carried out. Such a speculation of the possible effects of hormone presowing on later developmental processes arises from the

* Corresponding author: shoukathusseinee@gmail.com

opportunity of a plant to assimilate more nutrients following an early germination. Moreover, hormones, particularly GA, have been found to function via the basic processes of translation and transcription (MOORE 1989), which increases their potency for an appreciable period of time. The effects of pre-sowing hormone treatment may be reflected well into the vegetative stage, when the basic infrastructure of plant physiology is laid down.

Therefore, this study was aimed at investigating the effect of gibberellin, through a pre-sowing seed treatment, on the performance of *Nigella sativa* L., a Middle Eastern herb, greatly in demand on the domestic and international markets for its miraculous remedial properties and high aromatic value (SHAH et al. 2006).

Material and methods

Homogenous *Nigella sativa* seeds (obtained from the Regional Research Institute of Unani Medicine, Aligarh (U.P.), India) were surface-sterilized by soaking in 1mol m^{-3} $HgCl_2$ solution for 3 min, washed thoroughly with distilled water, and divided into twelve sets which were soaked in distilled water containing 10^{-6}, 10^{-5} or 10^{-4} M GA respectively, for 5, 10 or 15 h. After soaking, the thoroughly washed seeds were planted (15 per pot) in earthenware pots (25cm diameter) filled with 10 kg sandy loam soil and farmyard manure (ratio 9:1 v/v). The pots were kept in a green house and were subject to natural day/night conditions. Maximum/minimum temperature and relative humidity were 23.2/ 17.4°C and 52/64%. The pots were then irrigated with tap water as required. After germination only 5 uniform seedlings were left in each pot. Each treatment was replicated five times. Net photosynthetic rate (P_N), stomatal conductance (g_s), leaf chlorophyll (Chl) content, carbonic anhydrase (CA) (E.C. 4.2.1.1) and nitrate reductase (NR) (E.C. 1.6.6.1) activities and protein content were analysed in the leaves of 50, 70 and 90 day old plants. Seed yield was recorded at harvest (130 days after sowing). P_N and g_s were measured using infrared gas analyzer (LICOR 6200, LINCOLN, NE) on fully expanded uppermost leaves, at atmospheric conditions between (1100–1200h): photosynthetic active radiation (PAR) about 990 μmol $m^{-2}s^{-1}$, relative humidity 63%, temperature 23°C. Chlorophyll (Chl) content was estimated according to MACKINNEY (1941). CA activity was estimated using the procedure of DWIVEDI and RANDHAWA (1974). Nitrate reductase activity was determined in the fresh leaves of the plants by the method of JAWORSKI (1971). Protein content of the leaves was estimated by the method of LOWRY et al. (1951). Statistical analysis of the data was carried out using *SPSS* (*SPSS 7.5.1 for Windows*, standard version 1996). The results were subjected to an analysis of variance (ANOVA).

Results

Pre-sowing treatment with GA was found to appreciably enhance all parameters studied, especially at the 10^{-5} M concentration, following a 10 h soaking period. All other treatment combinations proved either mild, or supra-optimal. A harmonious enhancement was recorded in the Chl content, g_s, P_N, CA and NR activity and capsule number and seed yield at harvest. P_N in the test plants after 10h soaking was found to be 34, 44 and 33% enhanced at 50, 70, and 90 days after sowing stages respectively as compared to the control (Tab. 1). Activity of CA registered an increase of 38, 40 and 33%, whereas that of NR was elevated

Tab. 1. Leaf carbonic anhydrase (CA) activity [mol (CO_2) kg^{-1} s^{-1}], nitrate reductase (NR) activity [n mol (NO_2) g^{-1} min^{-1}], protein content [% (DM)], chlorophyll (Chl) content [g kg^{-1}], stomatal conductance (g_s) [mol m^{-2} s^{-1}], and net photosynthetic rate (P_N) [µmol (CO_2) m^{-2} s^{-1}], in *Nigella sativa,* raised from seeds soaked in water (control), 10^{-6}, 10^{-5}, or 10^{-4}M GA for 5, 10, or 15 h and sampled at 50, 70, and 90 days after sowing. The least significant difference at 0.5% level between different soaking periods (S) and hormone concentrations (GA), respectively. NS – not significant.

	Treatment [h]	50 DAS			70 DAS			90 DAS		
		5	10	15	5	10	15	5	10	15
CA	Control	1.82	1.86	1.85	2.51	2.62	2.54	1.34	1.32	1.35
	10^{-6} M	2.11	2.29	2.22	2.83	3.15	2.97	1.47	1.59	1.54
	10^{-5} M	2.40	2.57	2.52	3.34	3.66	3.57	1.59	1.76	1.67
	10^{-4} M	2.24	2.41	2.48	3.21	3.52	3.38	1.52	1.67	1.62
		S = 0.11			S = 0.11			S = 0.09		
		GA = 0.16			GA = 0.19			GA = 0.13		
NRA	Control	7.04	7.06	7.10	7.45	7.61	7.54	4.76	4.81	4.83
	10^{-6} M	7.45	7.61	7.55	8.40	8.62	8.52	5.15	5.52	5.36
	10^{-5} M	8.28	8.56	8.49	9.51	9.89	9.74	5.72	6.09	5.95
	10^{-4} M	7.92	8.11	8.01	9.21	9.46	9.32	5.06	5.45	5.25
		S = 0.16			S = 0.19			S = 0.15		
		GA = 0.19			GA = 0.23			GA = 0.27		
Protein	Control	11.35	11.42	11.46	12.35	12.41	12.52	10.50	10.45	10.41
	10^{-6} M	12.16	12.90	12.45	14.65	15.16	14.85	11.89	12.55	12.04
	10^{-5} M	14.90	15.51	15.16	17.91	18.74	18.17	13.79	14.31	13.95
	10^{-4} M	13.82	14.26	14.02	17.15	17.64	17.45	12.92	13.26	13.01
		S = 0.18			S = 0.34			S = 0.23		
		GA = 0.35			GA = 0.72			GA = 0.41		
Chl	Control	1.02	1.05	1.08	1.20	1.24	1.19	0.85	0.82	0.78
	10^{-6} M	1.07	1.25	1.19	1.37	1.45	1.42	1.01	1.16	1.02
	10^{-5} M	1.22	1.41	1.35	1.56	1.78	1.72	1.12	1.31	1.25
	10^{-4} M	1.15	1.38	1.24	1.44	1.52	1.51	1.06	1.16	1.10
		S = 0.12			S = 0.09			S = 0.06		
		GA=0.17			GA=0.15			GA=0.09		
g_s	Control	0.302	0.296	0.291	0.352	0.346	0.351	0.259	0.252	0.252
	10^{-6} M	0.323	0.342	0.332	0.391	0.413	0.406	0.265	0.272	0.273
	10^{-5} M	0.383	0.418	0.409	0.448	0.474	0.463	0.313	0.362	0.332
	10^{-4} M	0.376	0.402	0.395	0.432	0.442	0.443	0.316	0.322	0.301
		S = NS			S = NS			S = NS		
		GA=0.022			GA=0.032			GA=0.014		
P_N	Control	10.97	11.06	11.13	12.25	12.51	12.42	10.02	10.22	10.27
	10^{-6} M	12.01	13.95	12.46	14.72	15.97	15.26	10.76	11.71	11.51
	10^{-5} M	14.17	15.41	14.92	16.55	18.01	17.78	12.35	13.65	13.21
	10^{-4} M	14.11	14.81	14.35	15.95	17.11	17.01	12.01	12.26	13.01
		S = 0.95			S = 0.84			S = 0.76		
		GA=1.15			GA=1.25			GA=0.92		

by 21, 30 and 27% over the untreated control at similar samplings. Further, seed yield was also enhanced by 40% by the GA treatment as compared to the water-treated seeds.

Discussion

Net photosynthetic rate was found to be remarkably elevated in plants deriving from the GA treated seeds (Tab. 1). However, in the present study, GA was found to function at multiple levels to bring about such an influence. One such aspect is the content of Chl in the leaf tissues, which is detrimental for the light-harvesting efficiency of plants. Chl levels were found to be significantly higher in the test plants than in the control (Tab. 1), which may be attributed primarily to the GA-generated enhancement of ultrastructural morphogenesis of plastids (ARTECA 1997), and secondarily to the retention of Chl and delay of senescence caused by the hormone treatment (OUZOUNIDOU and ILIAS 2005).This factor may have been further enhanced by the increase in the rates of cyclic and non-cyclic phosphorylations induced by GA (NAIDU and SWAMY 1995). Another level of action for the phytohormone might have been the availability of CO_2, which was found to be highly optimized because of enhancement of g_s and CA activity, brought about by the GA treatment (Tab. 1). GA is known to induce an influx of Ca^{2+} into the endoplasmic reticulum of guard cells, thereby initiating a process that leads to increase in stomatal activity (ASSMANN and ARMSTRONG 1999). A lesser stomatal resistance, in turn facilitates greater $g_{s,}$ and enables a freer exchange of gases (ATRECA and DONG 1981). Diffusion of CO_2 into the stomata is followed by its transport across a membrane into the chloroplast cavity, wherein it is duly reduced by ribulose,1–5, biphosphate carboxylase oxygenase (RuBPCO). The former function is performed by CA, which is responsible for the provision of CO_2 for RuBPCO by catalyzing the dehydration of HCO_3^- to CO_2 in close proximity to this CO_2-fixing enzyme (JEBANATHIRAJAH and COLEMAN 1998). Enhancement in the activity of CA (Tab.1) and RuBPCO (YUAN and XU 2001) by GA might have efficiently linked both the processes, and hence have resulted in an enhanced rate of CO_2-fixation, as implied by our results. With reference to the CA activity, the observed increase probably may have been due to some influence of GA on the *de novo* synthesis of CA, which involves transcription and / or transla-

Tab. 2. Number of capsules and seed yield per plant in *Nigella sativa* raised from seeds soaked in water (control), 10^{-6}, 10^{-5}, or 10^{-4} M GA for 5, 10 or 15 h and sampled at harvest (130 days after sowing). The least significant difference at 0.5% level between different soaking periods (S) and hormone concentrations (GA), respectively.

Treatment [h]	Number of capsules per plant				Seed yield [g per plant]			
	5	10	15	Mean	5	10	15	Mean
Control	16.25±1.7	16.35±1.4	16.41±1.5	16.33	1.20±0.17	1.27±0.12	1.24±0.15	1.23
10^{-6} M	18.25±1.6	19.80±1.8	18.85±1.7	18.96	1.19±0.15	1.41±0.14	1.34±0.13	1.31
10^{-5} M	22.01±1.9	23.89±2.1	22.45±1.9	22.70	1.46±0.14	1.78±0.17	1.67±0.15	1.63
10^{-4} M	20.65±1.7	21.46±1.8	20.81±1.5	20.97	1.31±0.16	1.50±0.14	1.43±0.13	1.41
Mean	19.29	20.37	19.62		1.29	1.49	1.42	
		S = 0.45				S = 0.12		
		GA = 0.72				GA = 0.22		

tion (OKABE et al. 1980), as also observed by HAYAT et al. 2001. GA is in fact known to affect these processes and hence may have some control over enzyme and protein synthesis (HUTTLY and PHILLIPS 1995). This conjecture is further supported by the observed enhancement in the activity of NR and level of total protein in the test plants (Tab. 1). NR is the key enzyme in nitrogen metabolism and is responsible for the initiation of nitrate assimilation and hence, protein synthesis. As such an increase in NR activity and thereby in protein content bought about by the hormone application, may have exerted a pivotal role in enhancement of P_N. Moreover, as with CA, GA is also known to stimulate NRA through stimulation of the enzyme protein synthesis (PREMABATIDEVI 1998).

The ultimate culmination of enhancement in the above mentioned parameters was the increased overall yield (Tab. 2) of *Nigella* plants given forth by the GA treated seeds. Evidently, because of enhancement in all the basic photosynthetic attributes, these plants could more efficiently harvest the available light energy and subsequently fix it into valuable photoassimilate. In addition, the GA treatment may have also strengthened the sink potential of the developing pods (PERETÓ and BELTRÁN 1987) and through enhancement of the duration rate of assimilate translocation to these reproductive structures (DAVIES 1995), caused the observed increase in seed yield and capsule number per plant at harvest.

In the present study, although hormone supplementation was at the pre-sowing stage, the physiology and yield of *Nigella* test plants was significantly enhanced by GA. Such an effect can be hypothesized to have arisen from two major factors, the first being the mechanism of action of GA. It is known that induction of various metabolic processes by GA is through stimulation of *de novo* synthesis of specific enzymes, in turn brought about via the enhancement of basic translational/ transcriptional mechanism (MOORE 1989). It is this »relay mechanism« which causes a delay in both the appearance as well as the decline of the hormone effects. With reference to this experiment, it can be said that action of the hormone (GA) applied before sowing could have sustained itself till the plants were well into the vegetative stage. And it is during this critical growth phase that the basic infrastructure of the plant functioning is laid down, the effective dividends of which are reaped when the plant reaches harvest (SHAH et al. 2006).

The second major factor can be said to be the nature of enzymes stimulated by GA. These include various proteases, which cause hydrolysis of stored proteins, to liberate amino acids for new protein synthesis (MOORE 1989). Pre-sowing supplementation of GA as such might have triggered enhanced protein synthesis, which in turn could have contributed additionally to the amino acid rescue and protein turnover during active metabolism, later in plant life.

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