

GIBBERELIC ACID INDUCED AMELIORATION OF SALT STRESS IN BLACK CUMIN (*Nigella sativa* L.)

Shah H. S.

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

Received: 07 June 2010 Accepted: 23 June 2011

Summary. The effect of salt stress (75 or 150 mM) on fresh mass (FM), dry mass (DM), leaf area (LA), leaf chlorophyll (Chl) content, stomatal conductance (g_s), net photosynthetic rate (P_N), capsule number and seed yield per plant were studied in black cumin (*Nigella sativa*) in a pot experiment. High salinity was found to significantly hamper all mentioned parameters, the higher concentration (150 mM) producing stronger inhibitory effects. However, amelioration of all these adverse effects caused by the higher concentration was noted upon treatment with foliar GA_3 with appreciable restoration of plant performance and productivity, more conspicuously in combination with 75 mM NaCl treatment.

Key words: Black cumin; chlorophyll; dry mass; fresh mass; net photosynthetic rate; salt stress; seed yield; stomatal conductance.

Abbreviations: Chl-chlorophyll content; DM – dry mass; FM – fresh mass; g_s – stomatal conductance; LA – leaf area; P_N – net photosynthetic rate.

INTRODUCTION

Salt stress is recognized as a major factor that limits plant growth, physiology and productivity (Murphy and Durako, 2003; Othman et al., 2006). It acts mainly by inducing osmotic and ion-specific effects and oxidative stress (Pitman and Lauchli, 2002) thereby hampering membrane stability, chlorophyll biosynthesis, carbon and nitrogen metabolism and rates of

photosynthesis and respiration (Marshner, 1986). Today, about 95 million hectares of arable soil worldwide are affected by high salinity (Szabolcs, 1994), which greatly discourages prospects for their fruitful cultivation.

It is therefore of relevant interest to devise methods aimed at counteracting the effects of salinity, and making possible

*Corresponding author: shoukathusseine@gmail.com

the productive exploitation of saline soils. One potential method may be the use of growth regulators, such as GA₃, which are involved in the regulation of plant responses to external environment (Ansari, 1996) and the control of a number of stress-induced genes (Itai, 1999). In a natural system, the ratio of various phytohormones is maintained by fine regulation of their synthesis, transport, and metabolism to ensure coordinated physiological functioning along a genetically defined pattern of growth and development. However, a limited desired deviation in this set pattern of growth and development may be achieved by their exogenous application to favorably manipulate various plant processes. Besides, it is known that the effect of endogenous plant growth regulators is suppressed during salt stress due to their degradation and lower rate of synthesis (Niazi et al., 2005). As such, their exogenous application during such conditions may be further justified as potentially being able to make up for this deficiency and thereby restore normal growth and productivity. In fact, studies on mustard (Ahmad, 2010), wheat ((Iqbal and Ashraf, 2010), barley (Tabur and Demir, 2010), and rice (Wen et al, 2010) have demonstrated the mitigation of salt stress by GA₃. However, the response of plant systems to stress differs considerably depending on species, and characterization of responses of various species is of fundamental importance for development of tolerance-enhancing strategies. In this context, the present study was designed to characterize the adverse effects of salt stress on growth, photosynthesis and productivity and their possible amelioration by GA₃ application,

using *Nigella sativa* L., a Middle Eastern herb, greatly demanded worldwide for its immense medicinal and aromatic value.

MATERIALS AND METHODS

A pot experiment was carried out to study the response of *Nigella sativa* L. to foliar GA₃ application during salt stress. The experiment was laid down on a completely randomized block design. Earthen pots (25cm in diameter) were lined with polythene sheets and filled with 9 kg of acid washed sand. Seeds of *Nigella sativa* L. were procured from the Regional Research Institute of Unani Medicine, Aligarh (U.P.) India. They were surface sterilized with 0.01% HgCl₂ solution and rinsed using double distilled water, and sown in pots. 5 plants per pot were maintained. Each pot was fed every week with 200 cm³ of full strength Hoagland's solution, containing 1M KNO₃, 1M Ca(NO₃)₂.4H₂O, 1M MgSO₄.7H₂O, 1M KH₂PO₄ and minor elements: H₃BO₃, MnCl₂. 4H₂O, ZnSO₄.7H₂O, MoO₃, CuSO₄.5H₂O. This was continued till germination, after which the salt treatment was initiated. Concentrations of 0, 75 or 150 mM of NaCl were maintained in the Hoagland's solution during the experimental period. The electrical conductivity of different salinity levels was adjusted by direct measurements with a conductivity meter. At 25 days after emergence (DAE)(i.e., vegetative stage), each plant was sprayed with 5 cm³ of 10⁻⁵ M GA₃ (Sigma Chemical Co., St. Louis, U.S.A). The concentration and stage of treatment with GA₃ were determined in previous experiments (Shah et al., 2006; Shah 2007). The control set was sprayed with double distilled water. Each

treatment was replicated three times. The plants were grown under natural day/night conditions with a 12 h photoperiod (maximum and minimum temperatures and relative humidity of 23.2 and 17.4°C, respectively, 64 and 52%, respectively, at midday during the experimental period). The photosynthetic photon flux density (PPFD) at maximum plant height was approximately 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Fresh mass (FM), dry mass (DM), leaf area (LA), chlorophyll (Chl) content, stomatal conductance (g_s) and net photosynthetic rate (P_N) were analyzed at 35 DAE. Capsule number and seed yield per plant were recorded at harvest (90 DAE). Leaf area was measured by a portable leaf area meter, Li-300. Dry mass was measured by drying the plants at 80°C for 48 h. Total Chl content was estimated following the method of Metzner et al. (1965). P_N and g_s were measured on fully expanded leaves by a portable photosynthetic system (Li-COR 6200, Lincoln, NE, USA). The means were compared by analysis 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.

RESULTS AND DISCUSSION

Salt stress was found to induce a reduction in all the considered parameters (Table 1), with the higher concentration (150 mM) of NaCl producing more deleterious effects. However, hormone treatment with GA_3 clearly mitigated the adverse effects of salt stress and improved all considered parameters, there being a greater response in the 75 mM than the 150 mM salt treatment.

High salinity is known to adversely affect water absorption (Azaizeh et al., 1992) by inducing a decrease in cellular permeability of water (Mansour and Stadelmann, 1994). The ensuing reduction in plant water potential, in turn, depresses meristematic activity and cell elongation (Dorgham, 1991). These effects were evidenced in the present study as the reduction in fresh mass and

Table 1. Fresh mass (FM) [g plant^{-1}], dry mass (DM) [g plant^{-1}], leaf area (LA) [$\text{cm}^2 \text{plant}^{-1}$], chlorophyll (Chl) content [g kg^{-1}], net photosynthetic rate (P_N) [$\mu\text{mol CO}_2 \text{m}^{-2}\text{s}^{-1}$], and stomatal conductance (g_s) [$\text{mol m}^{-2}\text{s}^{-1}$] in *Nigella sativa* leaves treated with NaCl (75 or 150 mM) and sprayed with water (control) or gibberellic acid (GA_3) at 25 d after emergence (DAE) and sampled at 35 DAE. LSD for $P = 0.05$, mean \pm SE.

Parameters	– GA_3			+ GA_3			LSD
	0 mM	75 mM	150 mM	0 mM	75 mM	150 mM	
FM	3.36 \pm 0.27	2.65 \pm 0.21	2.47 \pm 0.21	4.08 \pm 0.51	3.59 \pm 0.28	3.21 \pm 0.25	0.49
DM	2.41 \pm 0.21	1.81 \pm 0.29	1.57 \pm 0.19	3.08 \pm 0.21	2.52 \pm 0.23	2.12 \pm 0.17	0.32
LA	225.9 \pm 31	170.6 \pm 21	144.2 \pm 16	291.7 \pm 23	241.8 \pm 18	197.7 \pm 22	29.0
Chl (a+b)	1.21 \pm 0.06	0.92 \pm 0.06	0.80 \pm 0.04	1.56 \pm 0.08	1.36 \pm 0.05	0.98 \pm 0.04	0.17
P_N	15.12 \pm 1.40	11.51 \pm 1.5	9.86 \pm 1.28	19.54 \pm 1.20	15.91 \pm 1.33	12.92 \pm 1.39	1.61
g_s	2.18 \pm 1.18	1.84 \pm 0.23	1.39 \pm 0.21	2.63 \pm 0.28	2.44 \pm 0.17	1.73 \pm 0.29	0.15

leaf area of the salt stressed plants by 21 and 24% respectively as compared to the control, following treatment with 75 mM NaCl (Table 1). Moreover, dry mass of the salinized plants was also lowered by 25%, probably as a result of the limited photosynthate production due to a curtailment of photosynthetic area and premature senescence and abscission of adult leaves following the salt stress (Munns, 2002). On the other hand, treatment of the salinized plants with GA₃ was found to somewhat restrict these adverse effects of salt stress with reduction in fresh mass, leaf area and dry mass brought down at most in 75mM NaCl stressed plants to 12, 17 and 18 %, respectively, as compared to the hormone-treated unstressed control (Table 1). The action of the hormone probably might have been mediated by optimization of plant water relations under stress as proposed by Aldesuquy and Ibrahim (2001). The resultant restoration of cellular division and elongation could therefore restore leaf area expansion, and subsequently optimize photosynthates production and dry matter accumulation.

A decrease of 24, 24 and 16 % was also noted in the leaf Chl content, g_s and P_N of the salinized (75 mM NaCl) plants as compared to the unstressed water sprayed control (Table 1). Elevated NaCl contents are known to increase ethylene production, thereby inhibiting chlorophyll biosynthesis (Khan, 2003). Further, based on evidences that suggest an increase in chlorophyllase activity during stress conditions (Singh and Jain, 1981), it is possible to suggest that the lowered Chl contents may have resulted from decreased synthesis, and increased degradation under salinity stress. Meanwhile, treatment of the

salt-stressed plants with GA₃ was found to restore the depleted Chl level, with the minimum decrease in treated plants of 13 % as compared to the respective hormone-treated unstressed control (Table 1). This may well be attributed to the GA₃ generated ultrastructural morphogenesis of plastids (Arteca, 1997) coupled with the retention of chlorophyll and delay of senescence caused by the hormone treatment (Ouzounidau and Ilias, 2005). Further, the reduction (by 16%) in g_s under salt stress observed in our study (75 mM NaCl stressed plants) (Table 1), can be attributed to the accumulation of salts in the substrate under high salinity, which triggers a transient water deficit. This induces an increase in ABA accumulation and results in stomatal closure (Aldesuquy and Gaber, 1993; Gomez-Cdenas et al., 2002). GA₃ counteracted this effect of ABA through inhibition by conjugation (Arteca, 1997) and thus restored g_s to a minimum decrease by 7% as compared to the respective control (Table 1).

As indicated by the lowered levels of leaf Chl and g_s and duly observed (Table 1), P_N of the salt-treated plants was reduced as compared to the untreated control, by 24% in 75 mM NaCl treated plants as compared to the unstressed water sprayed control. A similar inhibition of photosynthesis as a result of salinity stress has been reported by various researchers (Ashraf and Shahbaz, 2003) and can be proposed to have been a consequence, besides the decrease in the mentioned determinants, of the enhancement of the oxygenase activity of RuBPCO coupled with curtailment of its carboxylase activity due to salt stress. On the contrary, GA₃ is known to promote photosynthesis through enhancement of not only the carboxylase

Table 2. Number of capsules and seed yield plant⁻¹ in *Nigella sativa* treated with NaCl (75 or 150 mM) and sprayed with water (control) or gibberellic acid (GA₃) at 25 d after emergence and sampled at harvest (90 DAE). LSD for *P* = 0.05, mean±SE.

Parameter	Treatment [mM]	90 DAE	
		- GA ₃	+ GA ₃
Number of capsules [plant ⁻¹]	0	16.45±1.20	20.71±1.61
	75	12.05±1.21	16.56±1.40
	150	9.76±0.85	13.25±1.11
LSD		2.16	
Seed yeild [g plant ⁻¹]	0	1.14±0.17	1.45±0.12
	75	0.83±0.09	1.18±0.11
	150	0.70±0.51	0.96±0.06
LSD		0.16	

activity of RuBPCO (Mansour et al., 2005), but also the rates of cyclic and non-cyclic phosphorylations (Naidu and Swami, 1995). This is probably why the GA₃ treated plants recuperated efficiently from salt stress and displayed a restoration of P_N, with the minimum decrease by 18% as compared to the hormone-treated unstressed control (Table 1). Moreover, the observed enhancement in Chl content and g_s resulting from the hormone treatment may have further supplemented the ameliorative effects of GA₃ and collaboratively elevated the rate of photosynthesis, even in the salt-stressed plants.

Further, capsule number and seed yield per plant were substantially lowered by 26 and 27%, respectively under the influence of the 75 mM NaCl salt treatment (Table 1). These results are in harmony with those of Aldesuquy and Ibrahim (2001) and Afroz et al. (2005). It is proposed that under the adverse conditions of salt stress, thickness of the assimilate conducting canal is reduced

(Aldesuquy and Ibrahim, 2001) and leaves start behaving as sinks rather than sources (Arbona et al., 2005). This causes inhibition of assimilate movement towards the developing reproductive organs and thus, can be held responsible for the observed decrease in the yield attributing characters (Table 2). On the other hand, these adverse effects of high salinity were alleviated by the hormone treatment, primarily by causing an increased duration or rate of dry mass accumulation in developing reproductive organs (Davies, 1995), with the decrease in 75mM NaCl treated plants by 20 and 19% as compared to the respective unstressed control (Table 1).

The efficient counteraction of all adverse effects of salt stress by application of foliar GA₃, observed in the present study, suggests that this particular hormone treatment holds considerable relevance for further investigations with reference to efficient exploitation of saline soils for fruitful commercial cultivation.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. A. Ridhal for meticulous suggestions concerning refinement of the manuscript.

REFERENCES

- Ahmad P, 2010. Growth and antioxidant responses in mustard (*Brassica juncea* L.) plants subjected to combined effect of gibberellic acid and salinity. Arch. Agro. Soil Sci., 56(5): 575–588.
- Afroz, S., M. Firoz, S. Hayat, M. H. Siddiqui, 2005. Exogenous application of gibberellic acid counteracts the ill effect of sodium chloride in mustard. Turk J. Biol., 29: 233–236.
- Aldesuquy, H. S., A. H. Ibrahim, 2001. Interactive effect of seawater and growth bio-regulators on water relations, abscisic acid concentration and yield of wheat plants. J. Agron. Crop Sci., 187:185–193.
- Aldesuquy, H. S., A. M. Gaber, 1993. Effect of growth regulators on *Vicia faba* plants irrigated by seawater, leaf area, pigment content and photosynthetic activity. Biol Plant, 35: 519–527.
- Ansari, H. R. 1996. Effect of Some Phytohormones and NPK on Growth and Metabolism of Mustard. Ph.D Thesis, India: Aligarh Muslim University, Aligarh.
- Arbona, V., A. J. Marco, D. J. Ijlesias, T. M. Lopez-Climent, A. Gomez-Couendas, 2005. Carbohydrate depletion in roots and leaves of salt stressed potted *Citrus clementina* L. Plant Growth Regul., 46: 153–160.
- Arteca, R. N., 1997. Plant Growth Substances: Principles and Applications. CBS Publishers, New Delhi.
- Ashraf. M. M. Shahbaz, 2003. Assessment of genotypic variation in salt tolerance of early CIMMYT hexaploid wheat germplasm using photosynthetic capacity and water relations as selection criteria. Photosynthetica, 41: 273–280.
- Azaizeh, H., B. Gunse, E. Steudle, 1992. Effects of NaCl and CaCl₂ on water transport across root cells of maize (*Zea mays* L.) seedlings. Plant Physiol., 99: 886–894.
- Davies, P. J. 1995. The plant hormones: their nature, occurrence and functions. In: Plant Hormones. Ed. P. T. Davies, Kluwer Academic Publishers, Dordrech, 1–12.
- Dorgham, E.A., 1991. Effect of water stress, irradiation and nitrogen fertilization on grain filling, yield and quality of certain wheat cultivars. Ph.D. Thesis. Ain Shams University of Cairo, Egypt.
- Gomez-Cadenas, A., V. Arbona, J. Jaca, E. Primo-Millo, M. Talon, 2002. Abscisic acid reduces leaf abscission and increases salt tolerance in citrus plants. J. Plant Growth Regul., 21: 234–240.
- Iqbal, M., Ashraf, M, 2010. Gibberellic acid mediated induction of salt tolerance in wheat plants: Growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. Environ Exp. Bot., (in press).
- Itai C., 1999. Role of phytohormones in plant responses to stress. In: Plant Responses to Environmental Stress. Ed. H. R. Lerner, (From

- Phytohormones to Genome Reorganisation. Marcel Dekker, New York-basel, 287–301.
- Khan, N. A., 2003. NaCl inhibited chlorophyll synthesis and associated changes in ethylene evolution and antioxidative enzyme activities in wheat. *Biol. Plant*, 47: 437–440.
- Metzner, H., H., Rau, H., Singer, 1965. Untersuchungen zur synchronisierbarkeit Einzelner pigment mangel Mutanten von *Chlorella*. *Planta*, 186–199.
- Mansour, M. M. F., E. J. Stadelmann, 1994. NaCl induced changes in protoplasmic characteristics of *Hordeum vulgare* cultivars differing in salt tolerance. *Physiol. Plant.*, 31: 29–41.
- Mansour, M. M. F., K. H. A. Salama, F. Z. M. Ali, A. F. Abou Hadid, 2005. Cell and plant responses to NaCl in *Zea mays* L. cultivars differing in salt tolerance. *Gen. Applied Plant Physiol.*, 31: 29–41.
- Marschner, H., 1986. Mineral nutrition in higher plants. 2nd Edn., Academic Press, London, U.K.
- Munns, R., 2002. Comparative physiology of salt and water stress. *Plant Cell Environ* 25: 239–250.
- Murphy, K. S. T., M. J. Durako, 2003. Physiological effects of short-term salinity changes on *Ruppia maritima*. *Aquat. Bot.*, 75: 293–309.
- Naidu, C. V., P. M. Swamy, 1995. Effect of gibberellic acid in growth, biomass production and associated physiological parameters in some selected tree species. *Ind. J. Plant Physiol.*, 38: 15–17.
- Naizi, B. H., J. Rozema, M. Atahr, 2005. Effect of pre-germination and post-germination treatment with growth hormones (kinetine and abscisic acid) on ion concentration and biochemical content of fodderbeet and seabet under saline conditions. *Gen. Applied Plant Physiol.*, 31: 89–104.
- Ouzounidou, G., I. Ilias, 2005. Hormone induced protection of sunflower photosynthetic apparatus against copper toxicity. *Biol. Plant.*, 49: 223–228.
- Othman, Y., G. Al-Karak, A. R. Al-Tawaha, A. Al-Horani, 2006. Variation in germination and ion uptake in barley genotypes under salinity condition. *World J. Agric. Scis.*, 2: 11–15.
- Pitman, M.G., A. Läuchli, 2002. Global impact of salinity and agricultural ecosystems. In: Ed .A. Läuchli, U. Lüttge ,Salinity: Environment, plants, molecules. Kluwer Academic, Dordrecht, the Netherlands. 3–20.
- Shah, S.H., Ahmad, I., Samiullah, 2006. Effect gibberellic acid spray on growth, nutrient uptake and yield attributes during various growth stages of black cumin (*Nigella sativa*). *Asian J. Plant Sci.*, 5: 881–884.
- Shah, S.H., Ahmad, I., Samiullah, 2007. Responses of *Nigella sativa* L. to foliar application of gibberellic acid and kinetin. *Biol. Plant*. 51: 563–566.
- Singh, G., S. Jain, 1981. Effect of some growth regulators on certain biochemical parameters during seed development in chickpea under salinity. *Indian J. Plant Physiol.*, 20: 167–179.
- Szabolics, I., 1994. Soils and salinization. In: Handbook of plant and crop stress. Ed. M. Pessarkli, Marcel and Dekker,

- New York, 3–11.
- Tabur, S., K. Demir, 2010. Role of some growth regulators on cytogenetic activity of barley under salt stress. *Plant Growth Regulation*. 60: 99–104.
- Wen, F., Zhang, Z., Bai, T., Xu, Q., Pan, Y., 2010. Proteomics reveals the effects of gibberellic acid (GA₃) on salt-stressed rice (*Oryza sativa* L.) shoots. *Plant Sci.*, 178 (2): 170–175.