EFFECTS OF SALT STRESS ON MUSTARD AS AFFECTED BY GIBBERELLIC ACID APPLICATION

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Summary. The effects of gibberellic acid (GA_3) on growth, physiology and yield of salt-stressed mustard (*Brassica juncea* L. Czern & Coss) cv. Varuna plants were studied. The stress imposed by 25 or 50 mM NaCl reduced substantially leaf area, dry mass, leaf chlorophyll content, stomatal conductance and net photosynthetic rate 50 days after emergence. At harvest, although other yield components were generally reduced, total seed protein content showed a significant increase. Furthermore, the response was more pronounced at the higher concentration NaCl (50 mM) applied. On the contrary, the application of 10^{-5} M GA_3 appeared to mitigate the adverse effects of salinity stress on the overall performance and productivity of mustard.

***Key words**:* Chlorophyll, gibberellic acid, growth, mustard, photosynthesis, salt stress, seed protein content, stomatal conductance.

INTRODUCTION

India ranks second in the world with regard to production of Brassicas (Afroz et al., 2005) and supplies nearly 7% of the world's edible oil (Khan et al., 2002). However, Indian mustard production still remains insufficient

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to meet even the daily requirement of its people (Khan et al., 2002), let alone fulfilling prospects of fruitful export. This low economic yield can be attributed to the crop's susceptibility to a number of biotic and abiotic stresses, among which of alarming concern, is the salt stress. High salinity afflicts about 95 million hectares of land worldwide (Szabolcs, 1994), and adversely affects germination, growth, physiology and productivity by causing ionic and osmotic stresses as well as oxidative damage (Iterbe-Ormaetxe et al., 1998). Moreover, salt stress has also been found responsible for an increased respiration rate, ion toxicity (Sudhir and Murthy, 2004), changes in C and N metabolism (Kim et al., 2004), mineral distribution, membrane instability (Marschner, 1986) and permeability (Gupta et al., 2002), decreased biosynthesis of chlorophyll (Khan, 2003) and inefficiency of photosynthesis (Munns, 2002), all of which ultimately leading to lowered economic productivity.

The role of an agronomist is, therefore, to manipulate the crop in order to counteract the influence of salt stress, and boost performance even under saline conditions. In this regard, attention has now come to be focused on the use of plant growth regulators, such as gibberellic acid (GA_3), which are known to be importantly concerned in the regulation of plant responses to the external environment and to control a number of stress-induced genes (Naqvi, 1999). Although innumerable works have confirmed the potential of GA_3 to synergistically improve crop performance under normal conditions, very little light has been thrown on the influence of GA_3 sprayed during salt stress. A few studies have, however, demonstrated the ability of foliar pretreatment with GA_3 to overcome adverse effects of NaCl stress (Chakraborti and Mukherji, 2003). GA_3 has also been shown to alleviate the effects of salt stress on pigment content, Hill activity (Aldesuquy and Gaber, 1993) and water use efficiency (Aldesuquy and Ibrahim, 2001).

The present study was therefore, designed as an attempt to characterize the influence of GA_3 on the adverse effects of salt stress in mustard with reference to basic growth, physiological and yield characters.

MATERIALS AND METHODS

A pot experiment to study the response of mustard to foliar GA_3

application during salt stress was carried out at the Department of Botany, Aligarh Muslim University, Aligarh, (U.P.) India. The experiment was laid down on a completely randomized block design. Earthen pots (25 cm in diameter), lined with polythene sheets were filled with 9 kg of acid washed sand. Seeds of *Brassica juncea* L. Czern & Coss, cv. Varuna were procured from the National Seed Corporation Ltd., New Delhi, India, sterilized with 0.01% $HgCl_2$ solution, rinsed using double distilled water, and then sown in the pots. Five plants per pot were maintained. Each pot was fed every week with 200 cm^3 of full strength Hoagland's solution, containing 6 mM of NO_3 as the sole Nitrogen source. This continued till germination, after which the salt treatment was initiated. Concentrations of 0, 25 or 50 mM NaCl were maintained in the Hoagland's solution supplied daily for 20 days following germination. Then, a daily supply of 500 cm^3 per pot of the usual nutrient solution was provided as growth progressed till harvest. At the age of 25 days after emergence (DAE), each plant was sprayed with 5 cm^3 of 10^{-5} M GA_3, obtained from Sigma Chemical Co., St. Louis, U.S.A. The concentration of GA_3 was determined in a previous experiment (Shah et al., 2006). The control plants were sprayed with double distilled water. Each treatment was done in triplicate.

Leaf area (LA), dry mass (DM), chlorophyll (Chl) content, stomatal conductance (g_s) and net photosynthetic rate (P_N) were measured at 50 DAE. The yield and its characteristics were recorded at harvest (90 DAE). Leaf area was calculated using leaf area meter-*Li-300*. Dry mass per plant was recorded by drying the plants at 80^o C for 24 h. Total Chl content was estimated following the method of Mackinney (1941). Photosynthetic rate and stomatal conductance were analysed using infrared gas analyzer (*LiCOR 6200*, Lincoln, NE, U.S.A.) on fully expanded uppermost leaves of 5 plants from each replicate. The atmospheric conditions during the experiment were as follows: photosynthetic active radiation (PAR) about 990 µmol $m^{-2}s^{-1}$, relative humidity 65% and temperature 22 ^{0}C. The method of Lowry et al. (1951) was followed for the colorimetric estimation of total protein content in the seeds. Fifty mg of oven dried seed powder 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 completely dissolved by

heating at 60 ^{0}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 an aliquot of 5 ml was used for final analysis after adding 0.5 ml Folin phenol reagent. The blue colour developed was measured colourimetrically at 660 nm against a standard solution of bovine serum albumin. Treatment means were compared by analysis of variance using statistical package SPSS 90.5 (SPSS 7.5. for windows, standard version, 1996). Least significant difference (LSD) was estimated at 0.05 level of probability.

RESULTS AND DISCUSSION

Exposure to high salinity was found to induce a general reduction in all parameters studied, except seed protein content (Tables 1-2). In addition, the higher concentration NaCl applied (50 mM) produced more deleterious effects. Meanwhile, hormone treatment with GA_3 clearly mitigated the adverse effects of salt stress based on the improved parameters studied as compared to the untreated plants, and there was a greater amelioration response in the 50 mM than the 25 mM salt treatment.

The reduction observed in LA and DM of the salt-treated plants (Table 1) can be attributed to the changes in plant water relations under salt stress, which cause a reduction in meristem activity as well as cell elongation (Dorgham, 1991), thereby inhibiting leaf expansion (Bernstein, 1993). Furthermore, high salinity is known to induce ionic stress, which causes premature abscission and senescence of adult leaves, thus reducing the available photosynthetic area (Munns, 2002). Thus, the observed decrease in DM of the salt-stressed plants can be traced to the scanty recovery of leaves following limited photosynthesis production. Moreover, these adverse effects of salinity were mitigated through treatment with foliar GA_3 (Table 1). Aldesuquy and Ibrahim (2001) proposed that hormones used during salt stress may reduce water loss rates and cause a concomitant increase in leaf water potential and carbon gain rates. In the present study, GA_3 application might have de-repressed the LA expansion and caused increased DM production in the salt-treated plants.

Leaf chlorophyll content, g_s and P_N were significantly reduced in the

Parameters	-GA_3			+GA_3			LSD at 5%
	0	25	50	0	25	50	
LA (cm^2 $plant^{-1}$)	319.21	275.21	249.12	340.02	302.11	281.20	18.2
DM (g $plant^{-1}$)	2.51	2.16	1.93	2.74	2.35	2.23	0.19
Chl (g kg^{-1} FM)	1.475	1.218	1.070	1.554	1.421	1.264	0.10
g_s (mol $m^{-2}s^{-1}$)	2.62	1.92	1.02	2.81	2.51	2.23	0.11
P_N(µmol (CO_2) kg^{-1} s^{-1})	16.85	13.39	12.15	18.61	15.21	14.94	1.02

Table 1. Effect of foliar spray with 10^{-5} M GA_3 on growth and physiological parameters of mustard (*Brassica juncea*) subjected to 25 or 50 mM NaCl treatment. Determinations were done at 50 DAE. –GA_3 - without GA_3 spray; +GA_3 - with GA_3 spray.

salt-treated plants (Table 1). The observed chlorophyll depletion may be considered to be a result of the inhibition of chlorophyll biosynthesis following an increase in ethylene production brought about by the elevated NaCl content (Khan, 2003). Further, chlorophylase activity increases during stress conditions (Singh and Jain, 1981), suggesting that the observed low chlorophyll content could be a result of both decreased synthesis and increased degradation under salt stress. However, treatment of the salt-stressed plants with GA_3 was found to restore normal chlorophyll levels (Table 1). This may well be attributed to the GA_3–generated enhancement of ultra structural morphogenesis of plastids coupled with retention of chlorophyll and delay of senescence caused by the hormone treatment (Arteca, 1997).

The decrease in g_s (Table 1) can be explained based on the fact that accumulation of salts triggers a transient water deficit which induces an increase in ABA accumulation and causes stomatal closure (Aldesuquy and Ibrahim, 2001). Our results showed that the application of GA_3 restored g_s (Table 1) which can be accounted for by an inhibition of ABA through conjugation (Arteca, 1997) leading to a decline in ABA levels (Younis et

al., 1991).

As indicated by the lowered levels of leaf chlorophyll and g_s (Table 1), the P_N of the salt-treated plants was clearly reduced as compared to the untreated control. This can be proposed to be a consequence of the oxidative damage to important photosynthetic cells (Iterbe-Ormaetxe et al., 1998) which contain an array of photosensitizing pigments that produce and consume oxygen (Kim et al., 2004). Besides, salt stress is known to enhance the oxygenase activity of Rubisco and reduce its carboxylase activity (Sivakumar et al. 2000) which may lead to the observed decrease in CO_2 fixation rate (Table 1). On the contrary, GA_3 is known to promote P_N through enhancement of not only the carboxylase activity of Rubisco (Yuan and Xu, 2001), but also the rates of cyclic and non-cyclic phosphorylations (Naidu and Swamy, 1995). This is probably why the GA_3-treated plants recovered efficiently from salt stress and exhibited greatly enhanced P_N (Table 1).

All yield parameters, except seed protein content, were substantially lowered due to salt treatment (Table 2). These results are consistent with those of Aldesuquy and Ibrahim (2001) and Afroz et al. (2005). It has been proposed that under salt stress conditions the thickness of the assimilate conducting pathway 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, which might be the reason for the observed decrease in pod number, seeds per pod, 1000-seed weight and seed yield per plant (Table 2). On the other hand, these adverse effects of high salinity were alleviated by the hormone treatment, primarily by rejuvenation of the sink potential and enhancement of the duration or rate of dry mass accumulation in developing reproductive organs (Davies, 1995).

Our results showed that in contrast to the other yield parameters, seed protein content increased significantly under salinity stress, especially at 50 mM NaCl (Table 2). Similar results reported by Dorgham (1991) have led to the suggestion that salinity promotes the fixation of inorganic nitrogen into protein, thus favouring protein synthesis. Application of GA_3 under such conditions was found to synergistically increase seed protein content, being in accordance with the results of Singh and Sharma (1996) and Aldesuquy

Parameters	-GA_3			+GA_3			LSD at 5%
	0	25	50	0	25	50	
Pod number plant^{-1}	186.91	172.52	169.01	192.01	183.21	179.11	7.51
Seeds pod^{-1}	18.01	16.21	13.85	21.31	18.02	15.41	1.12
1000-seed weight(g)	4.50	3.95	3.09	4.64	4.10	3.19	0.32
Seed yield (g plant^{-1})	14.68	11.04	7.23	18.99	13.52	8.80	1.65
Protein (mg g^{-1} seed weight)	246.12	267.11	300.11	275.46	321.36	351.41	15.21

Table 2. Effect of foliar spray with 10^{-5} M GA3 on yield characteristics of mustard (Brassica juncea) subjected to 25 or 50 mM NaCl treatment. Determinations were done at 90 DAE. –GA_3 - without GA_3 spray; +GA_3 - with GA_3 spray.

and Ibrahim (2001). GA_3 is known to have a secondary enhancement effect on protein content through the intensification of nitrate reductase activity (Shah, 2004). Stimulation of the enzyme protein synthesis by GA_3 stimulates the overall protein synthesis (Premabatidevi, 1998).

In conclusion, the results of the present study indicate the ability of GA_3 to ameliorate all adverse effects of salt stress and rescue the productivity of mustard.

References

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, absicisic acid concentration, and yield of wheat plants. J. Agron. Crop. Sci., 187, 185-193.

Aldesuquy, H.S., and 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.

Arbona, V., A.J. Marco, D.J. Ijlesias, M.F. Lopez-Climent, M. Talon, A. Gómez-Coudenas, 2005. Carbohydrate depletion in roots and leavers of salt stressed potted *Citrus clemtina* L. Plant Growth Regul., 46, 153-160.

Arteca, R.N. 1997. Plant Growth Substances. Principles and Applications. CBS Publishers, New Delhi.

Bernstein, N., Silk, W.K., Läuchli, A. 1993. Growth and development of Sorghum leaves under conditions of NaCl stress. Planta, 191, 433-439.

Chakraborti, N., and S. Mukherji, 2003. Effect of phytohormone pretreatment on nitrogen metabolism in *Vigna radiata* under salt stress. Biol.Plant., 46, 63-66.

Davies, P.J., 1995. The plant hormones: their nature, occurrence and functions. In: Plant Hormones. Ed. P.T. Davies, Kluwer Academic Publishers, Dordrech, 1-2.

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.

Gupta, N.K., S.K. Meena, S. Gupta, S.K. Khandelwal, 2002. Gas exchange, membrane permeability, and ion uptake in two Species of indian jujube differing in salt tolerance. Photosyntetica, 40, 535-539.

Iterbe-Ormaetxe, I., P.R. Escuredo, C. Arrese-Igor, M. Becana, 1998. Oxidative damage in pea plants exposed to water deficit of paraquat. Plant Physiol., 161, 173-181.

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.

Khan, N.A., H.R. Ansari, M. Khan, R. Mir, Samiullah, 2002. Effect of phytohormones on growth and yield of Indian mustard. Indian J. Plant Physiol., 7, 75-78.

Kim, Y., J. Arihara, T. Nakayama, N. Nakayama, S. Shimada, K. Usui, 2004. Antioxidative responses and their relation to salt tolerance in *Echinochloa oryzicola* vasing and *Steraia virdis* (L.) Beauv. Plant Growth Regul., 44, 87-92.

Lowry, O.H., N.J. Resbrough, A.L. Farr, R.J. Randall, 1951. Protein

measurement with folin-phenol reagent. J. Biol. Chem., 193, 265-275.

Mackinney, G., 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem., 140, 315-322.

Marschner, H., 1986. Mineral nutrition in higher plants. Academic Press, London, 477-542.

Munns, R., 2002. Comparative physiology of salt and water stress. Plant cell Environ., 25, 239-250.

Naidu, C.V., P. M. Swamy, 1995. Effect of gibberllic acid in growth, biomass production and associated physiological parameters in some selected tree species. Indian J. Plant Physiol., 38, 15-17.

Naqvi, S.S.M., 1999. Plant hormones and stress phenomena. In: Handbook of Plant and Crop Stress, Ed. M. Pessarakli, Marcel Dakker, New York, 709-730.

Premabatidevi, R.K., 1998. Effect of IAA, GA_3, and kinetin on nitrate reductase and nitrite reductase in the leaves of a tree legume. Indian J. Plant Physiol., 3**,** 97-101.

Shah, S.H., 2004. Morphophysiological response of black cumin (*Nigella sativa*) to nitrogen, gibberellic acid and kinetin application. PhD Thesis, Aligarh Muslim University, Aligarh, India.

Shah, S.H., I. Ahmad, 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 Scis., 5, 881-884.

Singh, D.B., T.V.R. Sharma, 1996. Effect of GA_3, NAA and 2,4-D on growth and yield of cowpea (*Vigna unguiculata* (L.) walf) variety arka garima. Flora Fauna Jhansi, 2, 5-6.

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.

Sivakumar, P., P. Sharmila, S.P Pardha, 2000. Proline alleviates salt-stress-induced enhancement in ribulose-1,5-biphosphate oxygenase activity. Biochem. biophys. Res. Commun., 279, 512-515.

Sudhir, P., S.D.S. Murthy, 2004. Effects of salt stress on basic processes of photosynthesis. Photosynthetica, 42**,** 481- 486.

Szabolcs, I., 1994. soils and salinization. Handbook of Plant and Crop Stress. Ed. M. Pessarakli, Marcel and Dekker, New York, 3-11.

Younis, M.E., O.A. El-Shahaby, S.A.A. Bo - Hamed, S.A. Haroun, 1991. Plant growth, metabolism and adaptation in relation to stress conditions. XI. Modifications of osmotic stress-induced metabolic effects by GA_3 or IAA in *Pisum Sativum* L. plants. Acta Agron. Hung., 40, 367-375.

Yuan, L., D.Q. Xu, 2001. Stimulation effect of gibberellic acid short-term treatment on leaf photosynthesis related to the increase in Rubisco content in broad bean and soyabean. Photosynth. Res., 68, 39-47.