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## Exogenous Application of Gibberellic Acid Counteracts the Ill Effect of Sodium Chloride in Mustard

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**Abstract:** The response of mustard (*Brassica juncea* (L.) Czern & Coss cv. Varuna) to presowing seed treatment with sodium chloride (NaCl) was investigated. The plants raised from treated seeds were sprayed with water or  $10^{-6}$  M  $GA_3$  at the 30-day stage. The seeds imbibed in 1 or 10 mM of NaCl resulted in a decrease in dry mass, leaf chlorophyll content, carbonic anhydrase activity (E.C. 4.2.1.1), nitrate reductase activity (E.C. 1.6.6.1) and net photosynthetic rate at the 60-day stage, and pod number and seed yield at harvest. However, spray application of  $GA_3$  neutralized the ill effect of soaking treatment in NaCl (1 or 10 mM).

**Key Words:** *Brassica juncea*, carbonic anhydrase, chlorophyll, nitrate reductase, salinity, seed yield.

**Abbreviations:** CA-carbonic anhydrase; DAS-days after sowing; DH- dry mass; NR-nitrate reductase;  $P_N$ -net photosynthetic rate.

### Introduction

*Brassica juncea* (Indian mustard or brown sarson) is an important oil yielding crop, accounting for approximately 80% of the total production of rape seed and mustard in India (1). India is second in the world with regard to the production of Brassicas. The susceptibility of different varieties to salinity, drought, diseases and pests is the major cause of their low productivity. Salinity affects at least 20% of the world's arable land and more than 40% of irrigated land to various degrees (2). Salinity influences adversely several aspects of reproductive growth, including flowering, pollination, fruit development, yield and quality and seed production (3-6). It also inhibits the activity of many enzymes (7-10), photosynthesis (10,11), absorption of minerals (12) and respiration (13).

Plant growth regulators are known to affect growth, and assimilate translocation, flowering on transport (14-16). Although the effect of gibberellic acid on growth, photosynthesis, enzyme activities and productivity have been well studied (16-18) and its effect on salinity-induced inhibition was studied on various crops (6,19-21) but pertinent information with regard to mustard is not available. Therefore, the present experiment was carried out to investigate whether  $GA_3$  could ameliorate the

influence of salt stress on mustard, thereby increasing its salt tolerance.

### Materials and Methods

Seeds of *Brassica juncea* (L.) Czern & Coss cv. Varuna were purchased from the National Seed Corporation Ltd., New Delhi, India. Healthy seeds of uniform size after surface sterilization with 5% NaOCl solution for 5 min were soaked in 0 (water), 1 and 10 mM NaCl for 6 h (durations were selected on the basis of a preliminary experiment). The treated seeds were then sown in earthen pots (25 cm diameter) filled with soil plus farmyard manure in the ratio of 9:1. The pots were kept in rows in a net house and irrigated regularly with tap water as and when required. The raised plants at 30 days after sowing (vegetative stage) were sprayed with water or  $10^{-6}$  M of aqueous solution of  $GA_3$ . The concentration of  $GA_3$  is based on our earlier experiment (16). A constant volume (50 ml) was sprayed in all cases with a manual pump. The plants were divided into 4 groups: (i) control (water soaked), (ii) plants received NaCl solution at 1 and 10 mM, (iii) plants treated with  $GA_3$  ( $10^{-6}$ M), (iv) plants treated with  $GA_3$  plus each of the salinity levels. Each treatment was replicated 5 times. DM (at 80 °C), photosynthetic rate ( $P_N$ ), leaf chlorophyll (a+b) content,

and carbonic anhydrase (CA) and nitrate reductase (NR) activities were assessed in the leaves of 60-day-old plants (flowering stage). The pod number and seed yield were recorded at harvest Chlorophyll content was estimated following the method described by Mackinney (22). The CA and NR activities were determined by adopting the standard procedures (23,24).  $P_N$  in intact leaves was measured with the help of an LI-6200 portable photosynthesis system (LI-COR, Lincoln, NE, USA). The data were statistically analyzed using the least significance difference (LSD) test (25).

## Results and Discussion

The application of NaCl to *Brassica* plants adversely influenced the dry mass, chlorophyll, NR, CA and  $P_N$  compared with the control plants. However, GA<sub>3</sub>-treated plants exhibited an increase in tolerance to salt treatment (Table). This improvement in salt tolerance was reflected in the above parameters where all the parameters have higher values compared with plants receiving only NaCl treatment (Table).

The data presented in the Table show that CA, NR and  $P_N$  were significantly reduced in salinized mustard plants compared with the control plants. As it is well known that salinity-stressed plants have a consistently lower photosynthetic rate in other plants (10,11,26). The reduction in photosynthesis of plants raised from NaCl-soaked seeds might have resulted from the inhibition of leaf formation and expansion as well as early leaf abscission or due to ion toxicity, membrane disruption

and complete stomatal closure (27,28). The decrease in CA and NR activities and  $P_N$  was maximum in 10 mM of NaCl. However, GA<sub>3</sub> alone or in combination with NaCl levels significantly stimulated NR, CA and  $P_N$ . The increase in CA might be due to its *de novo* synthesis by involving transcription and/or translation (29). CA is found in abundance in the photosynthesizing tissue of both C<sub>3</sub> and C<sub>4</sub> plants and regulates the availability of CO<sub>2</sub> to RuBPCO by catalyzing the reversible hydration of CO<sub>2</sub> (30). Khan et al. (31) and Hayat et al. (16) also demonstrated that GA<sub>3</sub> enhanced the  $P_N$  and CA activity in mustard leaves.

The elevated leaf NR activity by GA<sub>3</sub> alone or in combination with NaCl might additionally support its photosynthetic efficiency. NR is responsible for the initiation of the nitrate metabolism and consequently for the protein synthesis at various levels of the plant body. The activity of NR is very much unsteady and depends on the presence or absence of irradiation (32), and the presence of hormones (16,33). In the present investigation also NR is increased by the application of GA<sub>3</sub> (Table).

The total chlorophyll content of NaCl-treated plants was significantly decreased below that of the control plants (Table). Similarly, Dela-Rosa and Maiti (34) and Khodary (10) also reported inhibition in chlorophyll biosynthesis and reduced chlorophyll content in salt-stressed sorghum and maize plants, respectively. GA<sub>3</sub>-treated plants exhibited higher values of chlorophyll than did the control or salinity-treated samples (Table). The enhancing effect of GA<sub>3</sub> on  $P_N$  could be attributed to its stimulatory effect on CA activity and chlorophyll content

Table. Effect of foliar spray of GA<sub>3</sub> on various parameters of mustard grown from NaCl treated seeds (f.m. = fresh mass).

Gibberellic Acid (M)	NaCl (mM)	DM plant <sup>-1</sup> [g]	Chl (a+b) [g kg <sup>-1</sup> f.m.]	NR [n mol NO <sub>2</sub> h <sup>-1</sup> g <sup>-1</sup> f.m.]	CA [mol (CO <sub>2</sub> ) kg <sup>-1</sup> f.m. s <sup>-1</sup> ]	$P_N$ [ $\mu$ mol (CO <sub>2</sub> )m <sup>-2</sup> s <sup>-1</sup> ]	Pods plant <sup>-1</sup>	Seed yield plant <sup>-1</sup> [g]
0	0	2.35	1.316	292.54	1.68	14.92	205.10	7.25
	1	2.04	1.113	268.50	1.32	13.10	196.80	6.73
	10	1.83	0.965	249.68	1.15	11.86	189.41	6.56
10 <sup>-6</sup>	0	2.65	1.429	329.30	1.95	18.31	219.17	7.70
	1	2.41	1.330	297.31	1.71	15.73	207.05	7.29
	10	2.13	1.169	283.45	1.43	14.65	201.35	7.10
L.S.D. at P = 0.05		0.22	0.13	16.20	0.12	1.14	8.71	0.31

in this study. An increase in chlorophyll content with GA<sub>3</sub> was also reported earlier (16,31). The decrease in photosynthesis in the NaCl-stressed plants of mustard was further reflected in reduced vegetative growth (data not included) and dry matter production (Table). Therefore, the availability of photosynthates decreased during the reproductive phase, which finally resulted in decreased pod number and seed yield at harvest. However, this decrease was partially overcome by the application of GA<sub>3</sub> in stressed plants.

In summary, it might be concluded that GA<sub>3</sub> treatment of salt-stressed mustard plants could stimulate their salt tolerance by accelerating their photosynthetic performance and nitrogen metabolism.

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