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BRIEF COMMUNICATION

Salt stress induced changes in growth and enzyme activities in germinating *Phaseolus mungo* seeds

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Abstract

NaCl salt stress induced changes in growth and enzyme activities in blackgram (*Phaseolus mungo* L.) seeds during germination were studied. A decrease in germination percentage, root length, shoot length, and fresh mass was noticed with an increase in NaCl concentration. With the increase in NaCl concentration and duration of stress proline content increased and catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) activities decreased.

Additional key words: blackgram, catalase, peroxidase, polyphenol oxidase, proline.

Plants respond to salinity stress through morphological, physiological and metabolic modifications occurring in all plant organs. NaCl salinity is known to decrease seed germination, shoot and root length, hydrolytic enzyme activity during germination and also affect other metabolic processes (*e.g.* Shadad and Zidan 1989, Hampson and Simpson 1990, Chartzoulakis and Loupanak 1997, Mathew and Chandrasekhar 1998, Promila and Kumar 2000). As blackgram is a legume sensitive to salt stress, the present investigation was undertaken to study the growth and some oxidase enzymes activity of germinating seeds under NaCl salinity.

Seeds of blackgram (*Phaselous mungo* L.) were surface sterilized in 0.1 % $HgCl_2$ for 5 min and thoroughly washed in distilled water. Seeds were germinated in Petri plates containing *Whatman No. 1* filter paper moistened with distilled water or 1, 2, and 3 % NaCl solution and kept in dark at 25 °C. Emergence of 2 mm radicle was the operational definition of germination. Seeds were germinated upto 12 d in dark. Every 3 d, germination percentage was calculated from 3 samples of 100 seeds each and root lengths, shoot lengths, fresh matter and dry matter were measured.

For determination of proline content seeds were hand-homogenized in 3 % sulfosalicylic acid and centrifuged at 3 000 *g* at 4 °C for 10 min. The supernatants were used for proline estimation (Bates *et al.* 1973). For the extraction of catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) seeds were homogenized in 0.1 M phosphate buffer (pH 6.8) in a mortar and pestle and centrifuged at 17 000 *g* for 20 min at 4 °C. Assays to catalase (CAT) and peroxidase were done by the method of Chance and Maehly (1955) and that of polyphenol oxidase (PPO) by the method of Kar and Mishra (1976). Enzyme units are given as $\mu mol\ g^{-1}(f.m.)\ min^{-1}$.

Seed germination percentage decreased with increase in NaCl concentration. The shoot and root length decreased sharply and at 3 % NaCl concentration no emergence of root was noticed (Table 1). Similar results have been reported by Hajar *et al.* (1996), Ozturk *et al.* (1997) and Zidan and Elewa (1995). Dry matter production remained almost unaffected while fresh mass decreased.

Proline is an important parameter to measure the stress tolerance capacity of the plants (Delauney and Verma 1993, Yoshiba *et al.* 1995). In the present study proline content increased with increase in NaCl concentration as well as with increase in duration of stress. Similar trends have been reported in salt stressed barley (Maslenkova *et al.* 1992), soybean (Durgaprasad *et al.* 1996) and maize (Rodriguez *et al.* 1997) seedlings.

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Table 1. Effect of different NaCl concentrations on germination rate, root length, shoot length, fresh mass, dry mass, and proline content in germinating black gram seeds measured 3, 6 and 9 d after treatment. Means ± SE.

	NaCl [%]	3 d	6 d	9 d
Germination [%]	0	88.5 ± 6.60	96.0 ± 5.85	98.0 ± 6.35
	1	72.0 ± 9.89	82.0 ± 4.12	85.0 ± 6.60
	2	46.5 ± 11.50	51.8 ± 3.28	60.0 ± 3.54
	3	19.0 ± 2.40	27.0 ± 2.48	28.5 ± 2.56
Root length [cm]	0	1.90 ± 0.23	4.55 ± 0.26	6.35 ± 0.56
	1	1.54 ± 0.26	2.82 ± 0.34	4.12 ± 0.22
	2	0.20 ± 0.09	0.32 ± 0.11	0.41 ± 0.21
	3	-	-	-
Shoot length [cm]	0	3.75 ± 1.20	13.10 ± 2.50	24.21 ± 2.80
	1	3.40 ± 1.18	7.24 ± 2.20	10.24 ± 0.36
	2	2.13 ± 0.98	3.22 ± 1.28	4.12 ± 1.80
	3	1.10 ± 0.68	2.35 ± 1.18	2.62 ± 1.71
Fresh mass [mg plant^{-1}]	0	0.09 ± 0.04	0.79 ± 0.68	0.80 ± 0.07
	1	0.09 ± 0.02	0.42 ± 0.04	0.46 ± 0.04
	2	0.08 ± 0.06	0.32 ± 0.03	0.34 ± 0.03
	3	0.05 ± 0.01	0.18 ± 0.05	0.22 ± 0.02
Dry mass [μg plant^{-1}]	0	13.0 ± 4.00	22.0 ± 6.00	25.0 ± 5.00
	1	22.0 ± 5.00	24.0 ± 6.00	38.0 ± 8.00
	2	21.0 ± 2.50	23.0 ± 5.00	32.0 ± 6.00
	3	21.0 ± 2.50	22.0 ± 5.00	31.0 ± 6.00
Proline [pg g^{-1}(f.m)]	0	1.92 ± 0.21	3.81 ± 0.16	5.80 ± 0.36
	1	1.98 ± 0.24	3.92 ± 0.34	6.00 ± 0.42
	2	2.25 ± 0.46	4.25 ± 0.37	6.25 ± 0.60
	3	2.42 ± 0.34	4.35 ± 0.49	6.50 ± 0.58

Table 2. Effect of different NaCl concentrations on changes in CAT, POX and PPO activities in germinating black gram seeds measured 3, 6, 9, and 12 d after treatment. Means ± SE.

	NaCl [%]	3 d	6 d	9 d	12 d
CAT [U g^{-1}(f.m.)]	0	152.0 ± 5.21	185.0 ± 18.26	217.0 ± 15.41	234.0 ± 26.12
	1	125.0 ± 10.11	150.0 ± 15.21	180.0 ± 20.18	225.0 ± 15.14
	2	95.0 ± 10.21	136.0 ± 10.34	150.0 ± 10.41	176.0 ± 16.26
	3	89.0 ± 7.81	124.0 ± 10.12	131.0 ± 10.18	149.0 ± 19.21
POX [U g^{-1}(f.m.)]	0	38.0 ± 2.5	40.0 ± 6.10	67.0 ± 4.51	77.0 ± 5.10
	1	35.0 ± 2.51	39.0 ± 2.52	49.0 ± 5.08	67.5 ± 7.21
	2	33.5 ± 2.41	37.5 ± 2.12	39.0 ± 2.01	44.0 ± 2.50
	3	27.0 ± 1.28	36.5 ± 3.75	36.5 ± 2.10	47.0 ± 4.10
PPO [U g^{-1}(f.m.)]	0	2.6 ± 0.48	4.0 ± 0.51	8.5 ± 0.98	22.0 ± 1.14
	1	2.2 ± 0.45	2.5 ± 0.46	7.0 ± 0.86	12.6 ± 1.10
	2	1.5 ± 0.48	2.0 ± 0.91	6.4 ± 1.01	10.5 ± 1.21
	3	1.2 ± 0.41	1.5 ± 0.62	4.5 ± 1.08	0.5 ± 0.21

A gradual decline in peroxidase activity was marked at higher NaCl concentrations. Similar results have been reported by Saha and Gupta (1997) in salt stressed sunflower seedlings whereas no significant inhibition of POX activity was reported by Thiyagarajah *et al.* (1996) and increased activity of POX by Subhasini and Reddy (1990). Catalase (CAT) and polyphenol oxidase (PPO) activity also showed a decreasing trend with the increase in NaCl concentration. Decrease in CAT activity has also been suggested by Saha and Gupta (1997) and Bishnoi and Singh (1997). The decrease in POX and CAT activity, suggest a greater accumulation of H_2O_2. This

might also be responsible for degradative reactions in the germinating seeds (Bhattacharjee and Mukherjee 1997) and decreased activity of PPO might be linked with accumulation of free radicals which are often generated in stressed plants (Elstner 1982).

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