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PHOSPHATASES AND PEROXIDASE ACTIVITIES IN THE GERMINATING SEEDS OF *CROTALARIA STRIATA* DC. UNDER NaCl STRESS

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The effect of NaCl on the activities of acid and alkaline phosphatases and peroxidase was determined during the germination of *Crotalaria striata* seeds. The increased acid and alkaline phosphatases activity in the NaCl treated seeds in the initial stages of germination was accompanied with the increase in the inorganic phosphate content. At the later stages of germination NaCl inhibited the activities of phosphatases resulting in the reduced liberation of inorganic phosphates in the treated ones. The peroxidase activity increased with increase in concentration of NaCl during the initial stages of germination, later on it decreased with increase in salinity.

Keywords : *Crotalaria striata*; Germinating seeds; NaCl stress.

Introduction

The information on the biochemical events occur during germination is scattered[1]. The imbibition of water triggers the metabolism of seeds which involves the hydrolytic activity. Among the various hydrolytic enzymes, the alkaline phosphatase is reported to be associated with the anabolic processes while the acid phosphatase participates in the catabolic processes[2]. The significance of peroxidase in the regulation of growth and differentiation has frequently been suggested by various workers.

The influence of NaCl on amylase and protease activities during the germination of the seeds of *Crotalaria striata* DC was investigated[3]. The present paper reports the influence of NaCl on the activities of acid and alkaline phosphatases and peroxidase during the germination of *Crotalaria striata* DC. seeds.

Materials and Methods

Seeds of *Crotalaria striata* DC. were collected from non-saline habitat and surface sterilized with 0.2% and 0.4% solution of NaCl for 24 hr. The seeds were germinated on the blotters moistened with respective concentrations of NaCl solution following standard blotter paper method (ISTA 1966) and incubated at room temperature. The seeds soaked in 100 ml of distilled water and germinated in distilled water served as control.

The activities of acid and alkaline phosphatases were estimated by following the procedure of Sadasivam and Manikam[4]. The acid and alkaline phosphatases were extracted in 50 mM citrate buffer (pH 5.3) and centrifuged at 8000 rpm for 10 min. The substrate for acid phosphatase was prepared by dissolving 1.49 g EDTA, 0.84 g citric acid and 0.03 g p-nitrophenyl phosphate in 100 ml distilled water (pH adjusted to 5.3) and for alkaline phosphatase by dissolving 375 mg glycine, 10 mg magnesium chloride, 165 mg p-nitrophenyl phosphate in 42 ml of 0.1 N NaOH and diluted to 100 ml and pH was adjusted to 10.4.

For assay, 3 ml of the respective substrate was incubated at 37°C for 5 min and 0.5 ml of enzyme extract was added and mixed. From this, 0.5 ml was removed and 9.5 ml of 0.85 N NaOH was added which served as blank and the remaining solution was incubated at 37°C for 15 min. After incubation, 0.5 ml of the solution was mixed with 9.5 ml of 0.85 N NaOH and the absorbance was measured at 405 nm using p-nitrophenol as the standard. Phosphatase activity was expressed as μ moles of p-nitrophenol released/ min / mg of protein.

Peroxidase activity was assayed using the procedure of Sadasivam and Manikam[4]. Peroxidase was extracted in 0.2 M phosphate buffer and centrifuged at 8000 rpm for 10 min. Guaiacol (20 mM) was used as substrate for the assay of peroxidase. For the assay, 3 ml of phosphate buffer, 0.05 ml substrate 0.1 ml enzyme extract and 0.03 ml of 0.042% H_2O_2 solutions were taken in cuvette, mixed and placed the cuvette in the spectrophotometer and the time taken for the absorbance to increase by 0.1 nm was noted. Peroxidase activity was expressed as O.D. changes /min / mg protein.

Inorganic phosphate was estimated by following the procedure of Ames[5] using potassium dihydrogen phosphate standards. Soluble proteins were estimated according to the method of Lowry *et al.*[6] using BSA standards. The data were statistically analysed by preparing ANOVA tables.[7] The significant difference between treatments, days and interaction was recorded at 5% and 1% levels.

Results and Discussions

Acid phosphatase activity increased during germination both in control and salt stressed seeds up to 9th day and decreased thereafter (Table 1). A two fold increase in the acid phosphatase activity in all the cases was observed in first day compared to the activity observed immediately after soaking for 24 hr. Acid phosphatase activity was higher in salinity stressed seedlings than in control up to 3rd day. On the 3rd day maximum activity was seen in 0.4% salinity stressed seedlings. From 4th day onwards a slight reduction in the acid phosphatase activity was observed in salt stressed seedlings when compared to the control and the extent of inhibition was more in 0.4% salt stressed seedlings. Statistical analysis revealed a significant difference between the treatments, period and interactions at 1% level.

Alkaline phosphatase activity increased during germination up to 9th day and decreased thereafter both in control and salt stressed seeds (Table 2). During the initial stages of germination, higher alkaline phosphatase activity was observed in treated seedlings over control. From the 2nd day onwards a slight reduction in the alkaline phosphatase activity was seen in salt stressed seedlings compared to control. The rate of inhibition of the enzyme activity increased with increasing salt concentration. Statistical analysis revealed a significant difference between the treatments and period at 1% level. The difference was not significant between the interactions even at 5% level.

Peroxidase activity increased during germination both in control and salt stressed seeds till 6th day and thereafter decreased (Table 3). The higher enzyme activity was observed in the seedlings grown in 0.4% salinity levels up to 5th day when compared to the seedlings treated with 0.2% NaCl and control. From 6th day onwards the higher enzyme activity was observed in the seedlings treated with 0.2% NaCl. Statistical analysis revealed a significant difference between the treatments, period and interactions at 1% level.

An increase in acid phosphatase activity with increasing salinity was reported in spinach leaves.[8] An initial increase in the activities of both acid and alkaline phosphatases in tolerant varieties of rice seedlings during the early period of growth was reported, maximum being on the 10th day and decreased thereafter.[9] They have also reported that the acid phosphatase was 10 to 15 times more active than alkaline phosphatase. In the present investigation an increase in the activity of phosphatases was observed till 9th day and thereafter it decreased. However, the activity of acid phosphatase was only 5-6 times more when compared to alkaline phos-

Table 1. Effect of NaCl on acid Phosphatase activity* in the germinating seeds of *Crotalaria striata* DC.

Treatment-> Days	Control	0.2% NaCl	0.4% NaCl
0	2.19±0.005	2.39±0.017	2.59±0.018
1	5.03±0.046	5.51±0.056	5.99±0.084
2	6.30±0.012	7.58±0.024	7.83±0.011
3	7.67±0.008	7.83±0.020	8.17±0.003
4	8.93±0.013	8.79±0.027	8.25±0.026
5	10.3±10.011	9.41±0.021	8.64±0.007
6	13.31±0.032	11.38±0.019	9.20±0.009
7	13.73±0.021	12.97±0.025	10.68±0.041
8	13.94±0.034	13.43±0.031	11.98±0.007
9	14.37±0.029	13.64±0.017	12.02±0.035
10	13.57±0.017	12.55±0.106	11.87±0.061
11	11.86±0.003	10.56±0.003	9.36±0.003

	S.E.	C.D.5%	C.D1%
for treatment	0.643	1.49	2.12
for days	3.384	7.87	11.13
for interaction	0.897	2.09	2.95

± indicates SEM

(*Enzyme activity expressed as µ moles of p-nitrophenol released / min / mg of protein)

Table 2. Effect of NaCl on alkaline Phosphatase activity* in the germinating seeds of *Crotalaria striata* DC.

Treatment-> Days	Control	0.2% NaCl	0.4% NaCl
0	1.28±0.005	1.31±0.003	1.34±0.011
1	1.36±0.006	1.39±0.002	1.45±0.003
2	1.70±0.002	1.64±0.002	1.60±0.003
3	1.72±0.001	1.67±0.002	1.63±0.001
4	1.99±0.004	1.74±0.004	1.70±0.008
5	2.16±0.008	1.91±0.003	1.74±0.020
6	2.71±0.012	2.41±0.004	1.81±0.004
7	2.74±0.011	2.51±0.009	2.07±0.007
8	2.96±0.008	2.92±0.005	2.47±0.005
9	3.42±0.005	3.32±0.052	2.84±0.002
10	3.33±0.005	3.27±0.018	2.28±0.016
11	3.21±0.003	3.19±0.003	2.16±0.029

	S.E.	C.D. 5%	C.D. 1%
for treatment	0.236	0.549	0.777
for days	0.648	1.509	2.133
for interaction	0.235	N.S.	N.S.

± indicates SEM

(* Enzyme activity expressed as µ moles of p-nitrophenol released / min / mg of protein).

Table 3. Effect of NaCl on Peroxidase activity* in the germinating seeds of *Crotalaria striata* DC.

Treatment-> Days	Control	0.2% NaCl	0.4% NaCl
0	0.105±0.001	0.109±0.005	0.112±0.007
1	0.131±0.001	0.136±0.001	0.145±0.005
2	0.157±0.002	0.171±0.002	0.193±0.002
3	0.186±0.001	0.249±0.002	0.264±0.003
4	0.256±0.002	0.277±0.003	0.322±0.006
5	0.408±0.008	0.465±0.007	0.474±0.006
6	0.672±0.007	0.725±0.009	0.572±0.007
7	0.643±0.004	0.682±0.007	0.479±0.004
8	0.601±0.007	0.643±0.008	0.455±0.003
9	0.562±0.006	0.615±0.006	0.418±0.002
10	0.526±0.005	0.532±0.004	0.376±0.002

	S.E.	C.D. 5%	C.D. 1%
for treatment	0.033	0.076	0.107
for days	0.201	0.467	0.661
for interaction	0.058	0.136	0.192

± indicates SEM

(* Enzyme activity expressed as O.D. changes / min / mg of protein).

Table 4. Inorganic phosphate content (mg/g d.wt.) in the germinating seeds of *Crotalaria striata* DC.

Treatment-> Days	Control	0.2% NaCl	0.4% NaCl
0	0.745 ± 0.132	0.841 ± .013	0.858 ± 0.006
1	1.063 ± 0.008	1.154 ± 0.210	1.338 ± 0.005
2	1.941 ± 0.018	2.019 ± 0.022	2.511 ± 0.013
3	3.760 ± 0.016	3.558 ± 0.015	3.277 ± 0.48
4	4.506 ± 0.008	4.035 ± 0.056	3.787 ± 0.299
5	5.407 ± 0.026	5.210 ± 0.022	4.328 ± 0.036
6	6.980 ± 0.012	6.448 ± 0.055	4.602 ± 0.040
7	7.178 ± 0.054	6.734 ± 0.066	4.834 ± 0.013
8	7.361 ± 0.086	6.850 ± 0.017	4.973 ± 0.017
9	7.916 ± 0.056	7.384 ± 0.063	5.713 ± 0.105
10	7.366 ± 0.046	7.166 ± 0.023	4.938 ± 0.035
11	6.806 ± 0.065	6.456 ± 0.015	4.311 ± 0.020

	S.E.	C.D. 5%	C.D. 1%
for treatment	0.679	1.57	2.23
for days	2.192	5.09	7.21
for interaction	0.599	1.39	1.97

± Indicates SEM

phatase. The activities of acid and alkaline phosphatases were slightly higher in the treated seedlings during initial days of germination. A decrease in acid phosphatase activity under salt stress was also reported[10,11].

In the present study the inorganic phosphate content was higher in the seedlings grown under saline conditions up to 2nd day which was accompanied by the higher activities of both acid and alkaline phosphatases and thereafter the trend was reversed. From the 3rd day of germination the inorganic phosphate was higher in the control over the treated seedlings, but it increased gradually in all the cases till 9th day and again declined. This trend of increase in inorganic phosphate from 3rd day till 9th day was accompanied by increased activity of phosphatases. The increased activity of phosphatases appears to release more of inorganic phosphate. It was stated that moderate levels of salinity increases the activities of both acid and alkaline phosphatases enabling higher metabolic status of cells by providing higher rate of inorganic phosphate liberation[9]. They also reported the inhibitory activities of the enzymes at higher concentration of NaCl stress in rice seedlings. In the present investigation NaCl did enhance the inorganic phosphate content in the initial stages later on it adversely affected.

In the present study, the peroxidase activity was higher in the seedlings grown in 0.4% salinity till 5th day and thereafter the maximum peroxidase activity was observed in the seedlings grown in 0.2% salinity. In general, salinity induced peroxidase activity. However, the higher concentration of NaCl inhibited peroxidase activity with lapse of time from 5th day onwards. The increase in peroxidase activity during germination has been earlier reported by various workers in different plants under salt stress conditions.[12-15] An inhibition in the peroxidase activity during germination of *Arachis hypogea* L. and *Triticum aestivum* L. cv. *Chanab 70* respectively under saline conditions was reported.[16,13] The increased peroxidase activity was attributed to the breakdown of toxic substances like peroxide, phenols etc. produced by the seedlings during germination in response to salt stress.[17]

References

1. Khan A A 1977, *The Physiology and Biochemistry of seed Dormancy and Germination.* North Holland Publishing Co., New York.
2. Rauser W E 1971, *Can. J. Bot.* **49** 311
3. Roy Mathew and Chandrashekar K R 1998, *Egyptian J. Botany* (Communicated).
4. Sadasivam S and Manikam A 1992, *Biochemical methods for Agricultural sciences,* Eastern Ltd., New Delhi, pp. 246
5. Ames B N, Garry B and Herzenberg L H 1960, *J. Gen. Microbiol.* **22** 369
6. Lowary O H, Rosenbrough N J, Farr A L and Randall R J 1951, *J. Biol. Chem.* **193** 265
7. Cochran G W and Cox M G 1957, *Experimental Design.* John Wiley & Sons Inc. New York pp. 611
8. Pan S M 1987, *Aust. J. Plant Phsiol.* **14** 117
9. Dubey R S and Sharma K N 1989, *Indian J. Plant Physiol.* **33** 217
10. Pan S M and Chen Y R 1986, *Taiwania*
11. Mittal R and Dubey R S 1991, *Plant Physiol. Biochem.* **29** 31
12. Kady E L, Mansour M M and Sheweikh A A 1982, *J. Agr. Research* **6** 1
13. Malik K A and Shavkat S S 1986, *Pakistan J. Bot.* **18** 29
14. Dhingra H R and Varghese T M 1990, *Indian J. Plant Physiol.* **33** 262
15. Kaphina V and Foudouli A 1991, *Fiziologiya na Rasteniyata.* **17** 35
16. Satakopan V N, Abitha Devi N and Srinivasan R 1990, *Indian J. Plant. Physiol.* **33** 85
17. Subhashini K and Redy G M 1990, *Indian J. expt. Biol.* **28** 277.