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Protease Activity and Associated Changes During Germination and Early Seedling Stages of Cotton Grown under Saline Conditions

[1]Sara Zafar, [2]M. Yasin Ashraf and [3]M. Ashraf
[1]Department of Botany, GC University, Faisalabad, Pakistan
[2]Nuclear Institute for Agriculture and Biology, P.O. Box 128, Jhang Road, Faisalabad, Pakistan.
[3]Deptartment of Botany, University of Agriculture, Faisalabad, Pakistan

Abstract: Salinity (NaCl) induced changes in protease activities and associated metabolic changes in three cotton varieties (NIAB-Karishma, NIAB-86 and K-115) were studied during germination and early seedling stages under controlled conditions. The increase in salinity of the medium resulted in the decrease of protease activities in all varieties, however it was more pronounced in NIAB-86. Decrease in mobilization of reserve protein and reduced concentrations of total free amino acids with increasing salinity were recorded in all the cotton varieties. Variety K-115 showed better performance than others. It showed highest germination followed by NIAB-Karishma and NIAB-86. The variety K-115 also had higher to mobilization capacity and had higher levels of total free amino acids and less reserve protein during germination and early seedling growth stages.

Key words: Protease activity, protein, amino acids, seedling growth and salinity

INTRODUCTION

Plants are generally most sensitive to salinity during germination and early seedling growth[1,2]. Even in obligate halophytes reduced salinity is necessary for germination[3]. Most of the work on the effect of salinity on cotton is restricted to germination and fresh weight of plants whereas studies on salinity induced metabolic changes during germination is scarce. Genotypic differences between salt sensitive and tolerant plants may be studied with respect to a number of physiological and biochemical parameters to develop rapid screening method for salt tolerance/ stress tolerance[4-6]. Varietal differences in relation to salinity have been reported by Khan and Ashraf[7] in sorghum and cotton.

Enzymes, the organic compounds are the reaction catalysts of biological systems; they are produced by living cells and are capable of catalyzing chemical reactions yield specific products. Alpha amylase is an important enzyme, which has an active role in the hydrolysis of starch just before a seed germinates[4,8]. By the breakdown of starch, sugars are produced which provide readily available energy to the growing embryo and also play an important role in osmotic adjustment during early germinating and seedling stages.

Proteases play an important role especially during germination, in mobilization of stored protein in seed as free amino acids, which are utilized in building necessary protein and enzymes required for growing embryo[9]. The present study describes the salinity induced metabolic changes in the protease activity and mobilization of protein during germination and early seedling growth in three cotton varieties with the objective whether cotton varieties can be isolated on the basis of physiological parameters/traits for salt tolerance.

MATERIALS AND METHODS

Seeds of three varieties of cotton viz. NIAB-Karishma, NIAB-86 and K-115 were surface sterilized with 0.1% solution of $HgCl_2$ for 5 min washed thoroughly in sterile distilled water and placed on filter papers in 14 cm petri-dishes. Ten cm^{-3} of saline solutions were used containing 0, 50 and 100 mM (NaCl) dm^{-3}. Five replications of each treatment were kept in a completely randomized design. The seed were allowed to germinate in dark at 28±2°C in growth cabinets. Fresh samples were collected randomly at every 24 h after sowing for the estimation of fresh weight and for the assay of protease, protein and total amino acids.

Protease activity: Protease activity was determined according to method of Ainouz *et al.*[10] for which five seedlings were homogenized in a mortar and pestle, extracted with cold 1% NaCl in 0.2 M dm^{-3} phosphate buffer (pH 7.0) and centrifuged at 12000 xg for 30 min. One mililiter of supernatant was incubated at 50°C with 5 cm^{-3} of 1% casein solution in 0.2 M dm^{-3} phosphate buffer

Corresponding Author: Sara Zafar, Department of Botany, GC University, Faisalabad, Pakistan

(pH 6.0). The reaction was terminated after 60 min with 1cm^{-3} of 40% TCA (Trichloroacetic acid). The proteolytic activity was measured at 570 nm in TCA soluble fraction after reaction with Folin phenol reagent[11].

Protein and total amino acids: Five seedlings were ground in 0.1 M (NaCl) dm^{-3} solution[12] in the ratio of 1:10 (w/v) in a mortar and pestle and filtered through nylon cloth. The filtrate was precipitated with equal volume of 10% TCA and centrifuged at 1000 x g for 5 min. The pellet was re-suspended in 0.1 M (NaOH) dm^{-3} and protein was then estimated by the method of Lowry *et. al.*[11] The supernatant was assayed for total amino acid by the method of Moor and Stein[13].

All the collected data were statistically analyzed and treatment and varietal means were compared using DMR test Steel and Torrey[14].

RESULTS

Salinity significantly affected the germination rate in all cotton varieties. The maximum germination was recorded in the plants grown under control conditions, followed by 50 and 100 mM dm^{-3} of NaCl (Table 1 and Fig. 1a). The varietal means showed that maximum germination rate was noted in K-115 followed by NIAB-Karishma and NIAB-86. All the varieties responded in a similar way under control conditions. As the salinity increased, the germination rate significantly reduced in all the varieties. However, variety K-115 performed better under 50 and 100 mM (NaCl) dm^{-3} salinity levels. Maximum effect of salinity was recorded in NIAB-86 at highest salinity level.

Salinity significantly affected fresh weight (Fig. 1b) of seedlings in all the cotton varieties. Maximum seedling fresh weight was recorded in the plant grown under control conditions followed by 50 and 100 mM dm^{-3} of NaCl salinity (Fig. 1b). Varietal means showed that highest seedling fresh weight was noted in cotton variety K-115, NIAB-Karishma and NIAB-86. K-115 performed better under all salinity treatments. Similar trend was observed for shoot and root length (Fig. 1c and d).

The proteolytic activity increased with the passage of time under all treatments in all cotton varieties, however, it significantly decreased with the increase in salinity (Table 1 and Fig. 2). The protease activity showed a sharp increase after 48 h and reached a maximum value at 96 h. The protease activity was maximum in cotton variety K-115, followed by NIAB-Karishma and NIAB-86 under all treatments except control (Fig 2a-c), where pattern was same at all time intervals. Pronounced reduction in protease activity in NIAB-86 was recorded under saline condition (Table 1 and Fig. 2a).

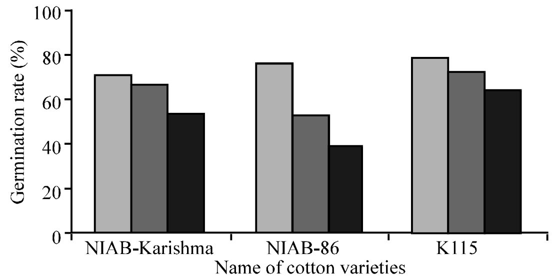

Fig. 1a: Effect of NaCl salinity on seed germination of three cotton varieties

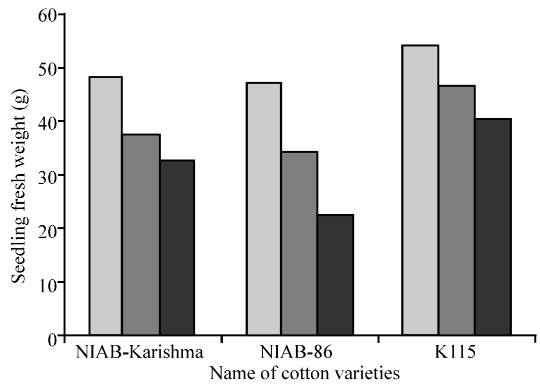

Fig. 1b: Effect of NaCl salinity on seeding fresh weight of three cotton varieties

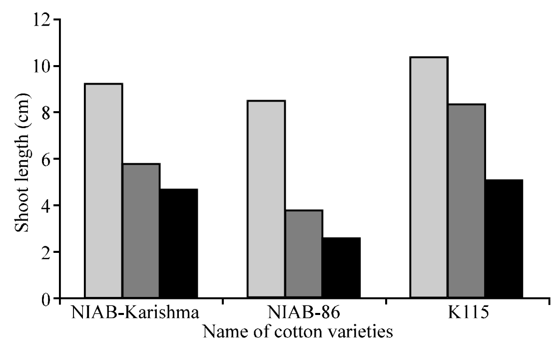

Fig. 1c: Effect of NaCl salinity on shoot length of three cotton varieties

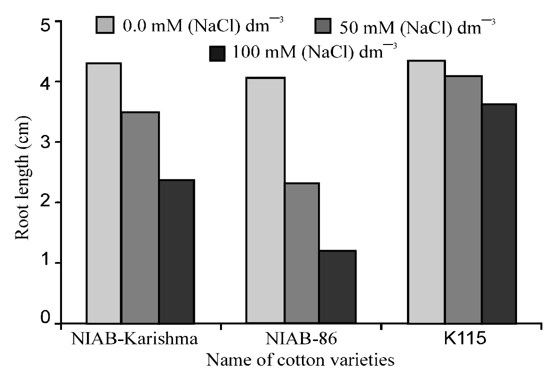

Fig. 1d: Effect of NaCl salinity on root length of three cotton varieties

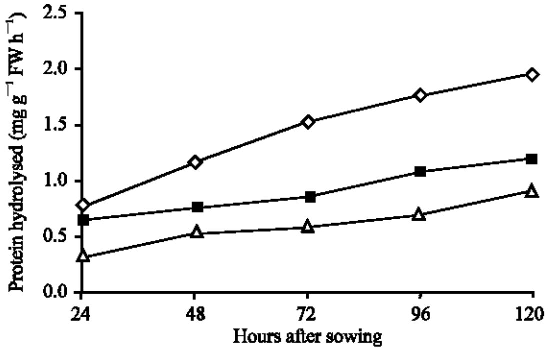

Fig. 2a: Protease activity in cotton variety NIAB-Karishma grown under saline conditions

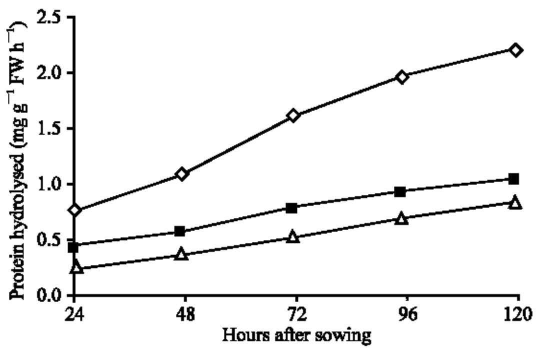

Fig. 2b: Protease activity in cotton variety NIAB-86 grown under saline condition

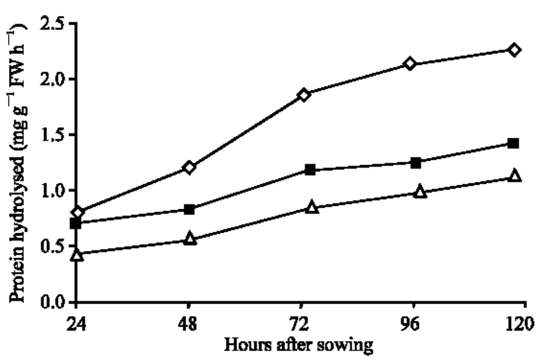

Fig. 2c: Protease activity in cotton variety K-115 grown under saline conditions

The seedlings of cotton variety NIAB-86 had significantly higher level of protein than NIAB-Karishma and K-115 under all salinity treatments (Table 1 and Fig. 3a-c) at all time intervals. The minimum reduction in protein content in the seedling of NIAB-86 showed that the protease activity was slow in this variety (Fig. 2b and 3b). The salinity significantly reduced the protease activity; as a result decrease in protein was less as compared to control in all the varieties. The protein

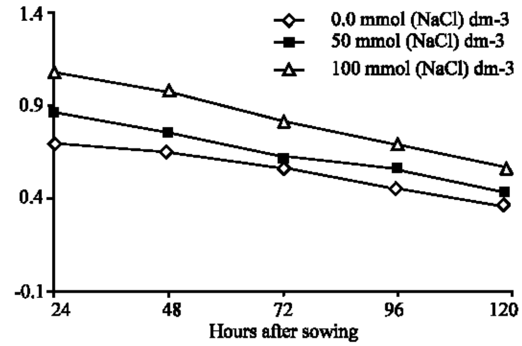

Fig. 3a: Effect of salinity on the changes in protein in cotton variety NIAB-Karishma during germination

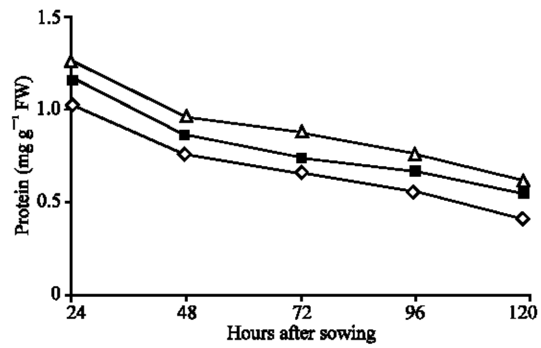

Fig. 3 b: Effect of salinity on the changes in protein in cotton variety NIAB-86 during germination

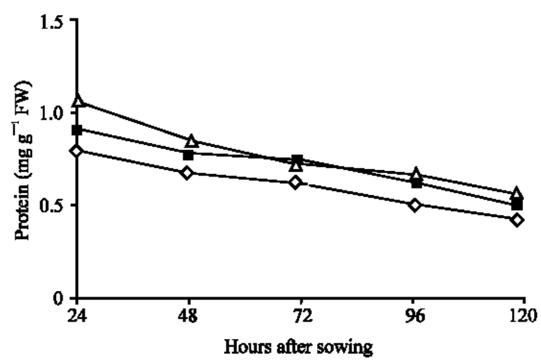

Fig. 3c: Effect of salinity on the changes in protein in cotton variety K-115 during germination

content in seedlings of all the varieties was progressively decreased with time interval or growth period.

The total amino acids increased with parallel increase in protease activity in all the cotton varieties under all treatments (Fig. 4a-c). The amount of amino acids increased gradually with time and reached maximum after 120 h of sowing. The maximum increase was recorded in K-115 (Fig. 4c) followed by NIAB-Karishma (Fig. 4a) and NIAB-86 (Fig. 4b). The total amino acids were

Table 1: Mean square for different parameters of three cotton varieties grown under saline conditions

	Source of variation			
Parameters	Treatment	Variety	Variety X Treatment	Error
Degree of Freedom	2	2	4	18
Protease activity after				
24 h	0.444**	0.127**	0.002^{NS}	0.004
48 h	1.085**	0.072**	0.003^{NS}	0.003
72 h	4.173**	0.801**	0.147*	0.042
96 h	4.766**	0.328**	0.004^{NS}	0.003
120 h	5.938**	0.587**	0.111**	0.002
Protein after				
24 h	0.228**	0.314**	0.006^{NS}	0.023
48 h	0.117**	0.087**	0.006^{NS}	0.005
72 h	0.081**	0.062**	0.008^{NS}	0.005
96 h	0.088**	0.048**	0.003^{NS}	0.003
120 h	0.063**	0.020**	0.003^{NS}	0.001
Total amino acids after				
24 h	0.019**	0.808**	0.004^{NS}	0.004
48 h	0.718**	0.004^{NS}	0.003^{NS}	0.006
72 h	1.354**	0.095**	0.003^{NS}	0.011
96 h	2.147**	0.142**	0.005^{NS}	0.005
120 h	1.709**	0.477**	0.007^{NS}	0.007
Germination rate	3032.150**	169.150**	120.930**	12.481
Seedling weight of 10 seedlings	413.640**	138.630**	7.680*	2.568
Shoot length	32.554**	11.814**	3.240**	0.208
Root length	0.836**	0.199**	0.056**	0.010

**= highly significant, *= Significant, NS=Non-significant

significantly reduced by the application of salinity (Table 1 and Fig. 4) in all the varieties under all time intervals; however, decrease was more pronounced in NIAB-86.

DISCUSSIONS

Protease plays an important role during germination in the mobilization of storage proteins (Fig.2). The results showed that the proteolytic activity increased gradually during germination and showed a rapid increase only after 48 h, which is similar to the findings of Ramana and Radhakrishanan[15] in Pearl millet. The activity, however, decreased with increase in salinity. The decrease in proteolysis caused by salinity resulted in slower mobilization of reserve protein during germination of seeds(Fig. 3), due to which the residual protein level was more with increasing salinity. Prisco and Viera[16] reported that NaCl caused delay in the breakdown of protein in the cotyledon of Vigna seeds. They ascribed it to the inhibition of translocation of hydrolyzed products than to the protease activity. However, in our experiments a definite inhibition of protease activity was evident. Sheoran and Gray[17] and Khan *et al.*[2] reported that salinity stress reduced protease activity. The reserve protein decreased gradually with the passage of time, which may be interpreted as the result of protein hydrolysis[2,9].

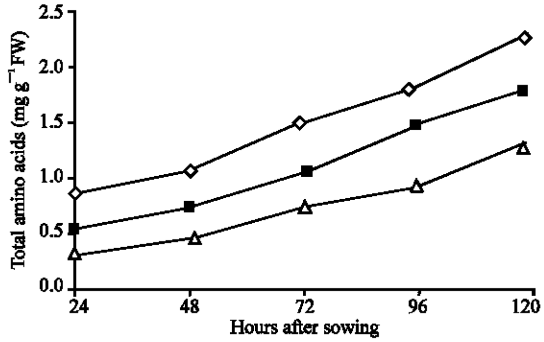

Fig. 4a: Effect of salinity on the changes in total amino acid of cotton variety NIAB-Karishma during germination

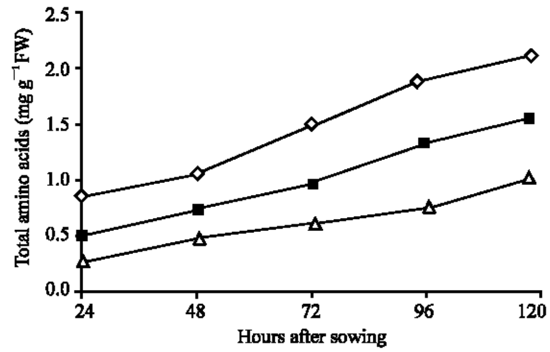

Fig. 4b: Effect of salinity on the changes in total amino acids in cotton variety NIAB-86 during germination

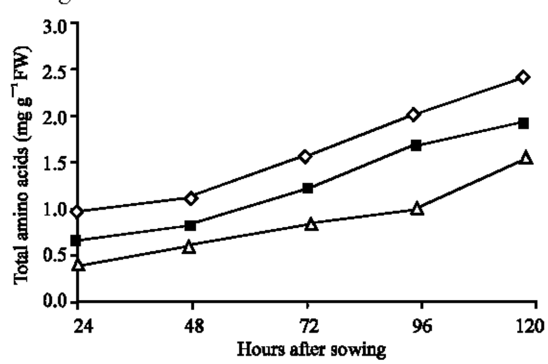

Fig. 4c: Effect of salinity on the changes in total amino acids of cotton variety K-115 during germination

Amino acids are derived from degradation of intracellular proteins. The amounts of amino acids in plant tissues are carefully regulated to just meet the requirements for biosynthesis of proteins and a few other molecules needed to support growth[6]. Amino acids catabolism does occur in plants, but it is generally concerned with the production of metabolites for other biosynthetic pathways. The results showed that the level of amino acids increased gradually with the passage of time and decreased with increase in salt concentration (Fig. 4). Ashraf and Khan[18] reported that NaCl delayed biosynthesis of proteins required for the plant growth. The results suggest that the initiation of germination, activity of the protease enzyme and the metabolic sequence induced by it are delayed under salt stress.

The salinity significantly affected germination, emergence, fresh weight of seedling, shoot length and root length (Fig. 1). The results showed that addition of salt progressively reduced germination, shoot length and root length. The present results are in accordance with Khan *et al.*[4] and Rahman *et al.*[19]. Salinity in general exposes the plants to inimical situations such as an unfavourable water balance that leads to physiological drought which reduces shoot length[1]. The results obtained showed that the salinity affected the shoot length. Cultural techniques and selection of salt tolerant cultivars appear to be the most important considerations when undertaking saline agriculture. An understanding of varietal differences to salt tolerance can aid in identifying cultivars, which have the potential to produce a reasonably high yield and to identify the physiological markers/traits for salt tolerance. By using these traits in the breeding programme, salt tolerant and high yielding varieties can be introduced.

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