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BRIEF COMMUNICATION

Salinity induced changes in α-amylase activity during germination and early cotton seedling growth

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Abstract

Salinity induced changes in α-amylase activity in three cotton cultivars (NIAB-Karishma, NIAB-86 and K-115) was studied during germination and early seedling growth under controlled conditions. The increase in NaCl concentration resulted in the decrease in α-amylase activity and break down of starch into reducing and non-reducing sugars in all cultivars, however, it was more pronounced in NIAB-86. K-115 showed highest germination followed by NIAB-Karishma and NIAB-86.

Additional key words: *Gossypium hirsutum*, reducing and non-reducing sugars.

Salinity is one of the major factors which contribute to the low cotton yield in Pakistan. At higher concentration, salinity increases influx of ions in the root as well as their translocation to the shoot which adversely affects the growth of plant (Jafri and Ahmad 1994). The salinity effect on other crops is also well documented (*e.g.* Ahraf *et al.* 1991, Promila and Kumar 2000).

The studies on α-amylase and associated changes in cotton under saline conditions may provide the useful information about the mechanism of salt tolerance in cotton at early germination and seedling stage which may be utilized in breeding for salt tolerance in cotton. The present study was therefore planned to investigate the effect of salinity on seed germination, seedling growth and changes in α-amylase activity and sugars in three cotton cultivars.

Seeds of cotton (*Gossypium hirsutum* L.) cultivars 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 transferred on filter papers in 14-cm Petri dishes, moistened with 10 cm^3 0, 50, and 100 mM NaCl. Five replications of each treatment were kept in a completely randomized design. The seed were allowed to germinate in dark at 28 °C in growth cabinets. Fresh samples were collected randomly at every 24 h after sowing for the assay of α-amylase. Ten seedlings were homogenized in a mortar and pestle, extracted with cold 1 % NaCl in 0.2 M phosphate buffer (pH 5.5), centrifuged at 10 000 *g* and supernatant used for assay of enzymes according to Chrispeel and Varner (1967). Parallel samples were dried at 70 °C in an oven. Reducing sugars were extracted in 80 % alcohol and were determined according to Nelson (1944) as modified by Somogyi (1952). The total sugars were determined in the same extracts after digestion with HCl. The non-reducing sugars were calculated according to the method of Loomis and Shull (1937).

Salinity significantly decreased the germination rate and fresh mass of seedlings in all cotton cultivars (Table 1). The maximum germination rate and fresh mass were noted in K-115 at all NaCl concentrations followed by NIAB-Karishma and NIAB-86. Similar trend was observed for shoot and root length (Table 1). The results are in accordance with Khan *et al.* (1995) and Rehman *et al.* (2000). K-115 was more resistant to salinity than other two cultivars of cotton for all growth parameters. The findings of Gupta (1991) also showed reduction in germination and seedling growth in different cotton cultivars when grown in salinity stress.

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Table 1. Effect of NaCl on germination and seedling fresh mass, shoot length and root length of cotton cultivars NIAB-Karishma, NIAB-86 and K-115. Means ± SE, $n = 4$.

Parameter	NaCl [mM]	NIAB-Karishma	NIAB-86	K-115
Germination [%]	0	70.00 ± 1.699	75.67 ± 1.841	77.67 ± 1.841
	50	65.67 ± 1.836	53.00 ± 1.578	72.00 ± 2.716
	100	53.67 ± 1.578	39.00 ± 1.412	64.00 ± 1.412
Fresh mass [g seedling^{-1}]	0	4.829 ± 0.326	4.655 ± 0.202	5.370 ± 0.114
	50	3.699 ± 0.202	3.405 ± 0.179	4.629 ± 0.158
	100	3.264 ± 0.141	2.256 ± 0.172	4.039 ± 0.184
Shoot length [cm]	0	9.18 ± 0.706	8.50 ± 0.706	10.29 ± 0.706
	50	5.76 ± 0.446	3.73 ± 0.391	8.33 ± 0.353
	100	4.68 ± 0.353	2.54 ± 0.353	5.15 ± 0.319
Root length [cm]	0	3.83 ± 0.404	3.63 ± 0.373	3.85 ± 0.352
	50	3.12 ± 0.318	2.07 ± 0.159	3.65 ± 0.376
	100	2.13 ± 0.163	1.07 ± 0.097	3.23 ± 0.174

Table 2. Effect of NaCl on changes in α-amylase activity [mg(starch digested) g^{-1}(f.m.) h^{-1}], and contents of reducing sugars and non-reducing sugars [mg g^{-1}(f.m.)] in cotton cultivars NIAB-Karishma, NIAB-86 and K-115 during germination. Means ± SE, $n = 5$.

	Cultivar	NaCl [mM]	24 h	48 h	72 h	96 h	120 h
α-Amylase activity	NIAB-Karshima	0	6.77±0.313	10.08±0.316	12.81±0.310	16.85±0.301	26.46±0.347
		50	3.60±0.255	7.06±0.158	9.89±0.446	12.81±0.118	17.00±0.297
		100	2.71±0.103	5.80±0.282	8.28±0.280	10.32±0.101	13.78±0.246
	NIAB-86	0	7.34±0.116	10.14±0.164	13.14±0.131	18.36±0.161	27.72±0.227
		50	3.60±0.199	5.06±0.158	6.88±0.441	10.02±0.164	12.65±0.376
		100	2.71±0.118	3.47±0.152	5.70±0.200	7.53±0.230	9.86±0.290
	K-115	0	7.36±0.114	11.02±0.158	13.91±0.510	18.19±0.322	28.19±0.067
		50	4.27±0.114	8.20±0.200	10.55±0.550	14.48±0.219	22.02±0.315
		100	3.48±0.138	6.35±0.120	8.55±0.506	10.86±0.314	17.39±0.172
Reducing sugars	NIAB-Karishma	0	0.87±0.063	1.30±0.096	1.35±0.071	2.30±0.099	2.55±0.141
		50	0.52±0.032	0.89±0.044	1.06±0.026	1.41±0.070	1.70±0.113
		100	0.34±0.014	0.72±0.071	0.80±0.070	1.02±0.075	1.20±0.071
	NIAB-86	0	0.87±0.079	1.26±0.104	1.40±0.141	2.12±0.038	2.47±0.176
		50	0.45±0.032	0.62±0.049	0.77±0.078	0.89±0.071	1.21±0.092
		100	0.21±0.009	0.43±0.033	0.62±0.049	0.76±0.070	0.87±0.071
	K-115	0	0.82±0.071	1.35±0.045	1.56±0.095	2.24±0.085	2.63±0.114
		50	0.50±0.035	0.84±0.071	1.08±0.068	1.45±0.075	1.88±0.071
		100	0.42±0.035	0.74±0.054	0.86±0.071	1.10±0.084	1.42±1.149
Non-reducing sugars	NIAB-Karishma	0	0.19±0.009	1.13±0.042	3.92±0.339	7.13±0.318	10.16±0.319
		50	0.12±0.006	0.68±0.040	1.27±0.088	6.11±0.035	7.10±0.192
		100	0.13±0.007	0.20±0.016	0.97±0.022	3.90±0.155	5.52±0.071
	NIAB-86	0	0.17±0.011	0.88±0.046	3.84±0.114	6.86±0.126	9.80±0.292
		50	0.09±0.007	0.35±0.032	1.44±0.127	5.81±0.141	7.12±0.317
		100	0.08±0.007	0.13±0.071	0.61±0.071	2.45±0.127	3.09±0.028
	K-115	0	0.13±0.006	1.41±0.072	4.65±0.095	7.20±0.322	10.08±0.317
		50	0.09±0.009	0.57±0.038	1.61±0.072	5.95±0.223	7.28±0.093
		100	0.10±0.003	0.30±0.013	1.62±0.071	4.67±0.063	5.47±0.161

Salinity had pronounced effect on α-amylase activity and associated changes in content of reducing and non-reducing sugars of all the cotton cultivars (Table 2). There was a progressive increase in α-amylase activity upto 120 h after germination. The activity decreased with the increase in salinity. The effect of salinity was more pronounced in seedlings of cultivar NIAB-86. There are many reports which indicate that salinity reduced the α-amylase activities (*e.g.* Khan *et al.* 1989, Gupta 1991, Ashraf *et al.* 1995, Promila and Kumar 2000).

The release of both reducing and non-reducing sugars followed the trend of α-amylase activity: sugars content gradually increased with passage of time, but decreased by increasing NaCl concentrations. In the seedlings of all the cultivars non-reducing sugar contents were higher than reducing sugar contents at all NaCl concentrations after 72 h.

The results showed that reduced α-amylase activity in all cotton cultivars under salinity was sufficient to break the starch into sugars to fulfill the necessary requirements of the plants, during germination stage, which suggested that the cotton cultivars have ability to maintain the integrity of their enzyme systems. In addition, ability to maintain requirements of reducing and non-reducing sugars allows the growing seedling osmotic adjustment to salinity. Salt tolerance in K-115 during germination, as a result of lesser effect on α-amylase activity and higher contents of sugars, has also been reported by Khan *et al.* (1989) in sorghum resistant cultivar. But Promila and Kumar (2000) pointed out that in case of mungbean, complete inhibition of growth of the embryo under salinity was responsible for reduced or complete inhibition of mobilization of reserve sugars from the cotyledons to the embryo rather than hydrolysis of starch to sugars. Reducing and non-reducing sugar contents increased with the age of seedling but less under salinity. Bhardwaj (1985) and Ashraf *et al.* (1995) observed similar results in gram and wheat.

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