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EFFECT OF NaCl SALINITY ON CARBOHYDRATE METABOLISM IN GERMINATING SOYBEAN SEEDS

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The seeds of two varieties of soybean *(Glycine max* L. Merrill cv. Co-1 and cv. ADT-1) were soaked and germinated in 20, 40, 60 mM NaCl solutions. The germination percentage, sugar content, α and β amylase activity were studied during germination and seedling growth under NaCl stress at different time intervals. The increasing NaCI concentration had an increasing inhibitory effect on germination and carbohydrate metabolism of the seedlings.

Keywords: α and β amylase; NaCl strees; Seedlings, Soybean; Sugars.

Introduction

Salt stress is one of the major global issue in agricultural sector apparently is due to the salts of sodium. Soil salinity profoundly influence negatively the germination, the growth the yield and even very existence of crop plants.[1,2] Salinity mediated effects are further known to vary with the stage of plant growth at which the stress treatment is given.[3] According to Epstein *et al.*[4] besides an engineering approach, development of crops tolerant to salinity, is a better strategy for meeting the challenge of this problem. To achieve this a better understanding of physiology and mechanism of salt tolerance in plants is highly essential. In this present work it is proposed to study the changes in germination percentage, soluble sugars and the activity of hydrolytic enzymes like α and β amylase during seed germination and seedling growth in two varieties of soybean *(Glycine max* L. Merrill cv. Co-1 and cv. ADT-1) under different NaCl stress.

Materials and Methods

The seeds of *Glycine max* L. Merrill cv. Co-1 and cv. ADT-1 were obtained from Tamilnadu Agricultural University, Coimbatore. The seeds were surface sterilized with 0.1% $HgCl_2$ for two minutes, washed thoroughly with distilled water and soaked in 20, 40 and 60 mM NaCI solution for 12 hours. Distilled water was used for soaking control. After 12 hours the soaked seeds were sown on distilled water washed whatman No. 1 filter paper in 15 x 25 cm trays. Seven replicate were maintained for each concentration and irrigated with 100 ml respective solution daily. This setup was maintained upto 96 hours under $3.8 X 10^4$ lux light for 12 hours a day. The seedlings were randomly selected and used for analysis at 12, 24, 36, 48, 60, 72 and 96 hours after sowing.

The germination prcentage was calculated upto 96 hours, later no germination occured. Total sugars were hydrolysed with 6N sulphuric acid into reducing sugars and estimated using Nelson's[5] arsenomolybdate method. α amylase (EC 3.2.1.1) activity was assayed following the modified method of Tarrago and Nicolas[6] after heating the enyme extract at 70°C for 5 minutes in the presence of 3 mM $CaCl_2$ to inactivate β amylase. The β amylase (EC 3.2.1.2) activity was estimated at pH 3.4 and with 0.1 M EDTA to inactivate α amylase[6,7]

Results and Discussion

The germination percentage decreased with increasing NaCl concentration and it was 94, 76, 62 and 52 per cent in cv. CO-1 and 84, 76, 56, 48 per cent in cv. ADT-1 in control, 20, 40 and 60 mM NaCl treatments respectively (Table 1). NaCl salinity delayed the germination in both the varieties.

The total sugar content was 0.61 and 0.5 mg per seeds at 0 hours of germination in Co-1 and ADT-1 varieties of soybean respectively (Table 2). It increased rapidly till 12 hours of germination later it declined gradually till the 96th hour of germination in both the varieties of soybean. At 96 hours it was less by 2.59, 13.73 and 37.95 per cent over control in all the treatments in Co-1 variety. Similarly in the ADT-1 variety, the total sugar content was less by 17.25, 43.22 and 55.65 per cent over control. The total sugar content decreased with the increase of NaCl concentration in both the varieties of soybean.

α-amylase activity increased very rapidly upto 12 hours of germination later it declined gradually in both the varieties. This enzyme activity was less in NaCl treated seeds when compared to control (Table 3) In the Co-1 variety the amylase activity was less by 3.70, 8.88 and 21. 48 per cent over control in all NaCl concentrations at 72 hours of germination. The α amylase activity was also less by 4.90, 10.94 and 24.15 per cent over control in the ADT-1 variety. The loss of activity of α amylase due to increase in salinity was less in ADT-1 variety when compared to CO-1 variety of soybean.

The β amylase activity increased very rapidly upto 12 hours later it declined gradually in both the varieties in control and treatments (Table 4). The reduction in β amylase activity was 2.66, 30.00 and 43.33 per cent over control in all the treatments at 72 hours of germination in Co-1 variety of soybean. In the ADT-1 variety also, the reduction in the activity of β amylase was 12.90, 54.83 and 64.52 per cent over the control.

In the present investigation both the varieties showed a reduction in germination percentage and delayed germination. When seeds were sown in saline environment, there was a significant decrease in the rate of germination and also delay in completion of germination which inturn fluctuated the germination percentage.[8-11] Many authors observed inhibition in germination and delay in germination due to sodium chloride salinity, in *Sorghum bicolor*[12] and mung bean[13]

The total sugar content was less in treatments when compared to control from the initial to final stages of germination in both the varieties of soybean. Iyengar and Pandya[14] observed a reduction in total sugars under saline conditions in ten varieties of sugar beet. Downton[15] observed a decrease in sugar content at 75 mM NaCl concentration in grapevines. The sugar content increased in 3 rice varieties which are salt tolerant but in the other six varieties of rice which are susceptible, it decreased.[16] Soybean was a salt susceptible species and our results coincides with some earlier reports.[14-16]

Table 1. Effect of NaCl on the germination percent of soybean seeds.
(Values are mean ± SD of 7 samples expressed in percentage of germinated seeds)

Germination Period in Hours	CV. CO-1 NaCl concentration				CV. ADT-1 NaCl concentration			
	Control	20mM	40 mM	60mM	Control	20mM	40mM	60mM
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	42.00	24.00	18.00	14.00	44.00	30.00	16.00	10.00
	±1.05	±1.20	±0.70	±0.40	±1.46	±0.76	±0.62	±0.47
36	60.00	36.00	28.00	22.00	56.00	48.00	24.00	18.00
	±1.46	±1.52	±1.02	±0.68	±0.94	±1.24	±0.88	±0.42
48	72.00	54.00	38.00	34.00	70.00	60.00	38.00	30.00
	±2.44	±1.36	±1.24	±1.46	±2.24	±1.66	±1.27	±0.75
60	94.00	66.00	58.00	48.00	84.00	68.00	45.00	40.00
	±2.35	±1.65	±0.96	±0.73	±2.10	±1.70	±1.13	±1.33
72	94.00	76.00	62.00	52.00	84.00	76.00	56.00	48.00
	±2.35	±1.90	±1.58	±0.87	±2.10	±1.96	±1.46	±1.28
96	94.00	76.00	62.00	52.00	84.00	76.00	56.00	48.00
	±2.35	±1.90	±1.58	±0.87	±2.10	±1.96	±1.46	±1.28

Table 2. Effect of NaCl on the total sugar content of the germinating soybean seeds.
(values are mean ± SD of 3 samples expressed in mg/seed)

Germination Period in Hours	CV. CO-1 NaCl concentration				CV. ADT-1 NaCl concentration			
	Control	20mM	40 mM	60mM	Control	20mM	40mM	60mM
0	0.610	0.610	0.610	0.610	0.500	0.500	0.500	0.500
	±0.018	±0.018	±0.018	±0.018	±0.013	±0.013	±0.013	±0.013
12	1.333	1.272	1.200	1.100	1.346	1.300	1.200	1.059
	±0.046	±00.038	±0.027	±0.028	±0.027	±0.033	±0.020	±0.026
24	1.279	1.226	1.100	1.013	1.200	1.146	1.000	0.839
	±0.021	±0.041	±0.026	±0.033	±0.024	±0.019	±0.066	±0.017
36	1.172	1.139	1.019	0.906	1.736	0.973	0.846	0.700
	±0.039	±0.043	±0.028	±0.033	±0.018	±0.024	±0.014	±0.012
48	1.100	1.052	0.946	0.800	0.946	0.800	0.700	0.573
	±0.026	±0.048	±0.037	±0.020	±0.024	±0.013	±0.012	±0.028
60	0.979	0.952	0.839	0.672	0.833	0.692	0.554	0.473
	±0.035	±0.040	±0.022	±0.031	±0.036	±0.023	±0.022	±0.012
72	0.879	0.846	0.752	0.579	0.666	0.566	0.426	0.339
	±0.021	±0.028	±0.018	±0.014	±0.016	±0.021	±0.018	±0.016
96	0.772	0.752	0.666	0.479	0.539	0.446	0.306	0.239
	±0.019	±0.020	±0.016	±0.007	±0.009	±0.018	±0.007	±0.006

Table 3. Effect of NaCl on the α -amylase activity in the germinating soybean seeds. (values are mean ± SD of 3 samples expressed in μg of maltose released/min./mg. Protein)

Germination Period in Hours	CV. CO-1 NaCl concentration Control	20mM	40 mM	60mM	CV. ADT-1 NaCl concentration Control	20mM	40mM	60mM
12	1.329 ±0.022	1.254 ±0.024	1.145 ±0.022	1.014 ±0.020	1.936 ±0.048	1.743 ±0.044	1.555 ±0.038	1.465 ±0.036
24	0.850 ±0.017	0.832 ±0.016	0.810 ±0.016	0.785 ±0.014	1.550 ±0.051	1.350 ±0.045	1.222 ±0.041	1.130 ±0.038
36	0.688 ±0.017	0.653 ±0.021	0.594 ±0.012	0.532 ±0.011	1.159 ±0.042	0.374 ±0.038	0.888 ±0.033	0.786 ±0.029
48	0.588 ±0.013	0.542 ±0.014	0.459 ±0.015	0.406 ±0.008	0.410 ±0.014	0.382 ±0.016	0.342 ±0.015	0.305 ±0.012
60	0.494 ±0.016	0.464 ±0.015	0.370 ±0.009	0.297 ±0.010	0.376 ±0.016	0.344 ±0.013	0.301 ±0.010	0.280 ±0.007
72	0.270 ±0.006	0.260 ±0.005	0.246 ±0.003	0.212 ±0.002	0.265 ±0.009	0.252 ±0.006	0.236 ±0.005	0.201 ±0.008
96	0.265 ±0.011	0.252 ±0.010	0.232 ±0.005	0.198 ±0.003	0.180 ±0.005	0.242 ±0.012	0.226 ±0.007	0.176 ±0.008

Table 4. Effect of NaCl on the β-amylase activity in the germinating soybean seeds. (values are mean ± SD of 3 samples expressed in μg of maltose released/min./mg. Protein)

Germination Period in Hours	CV. CO-1 NaCl concentration Control	20mM	40 mM	60mM	CV. ADT-1 NaCl concentration Control	20mM	40mM	60mM
12	1.057 ±0.026	0.990 ±0.025	0.944 ±0.023	0.865 ±0.022	1.235 ±0.052	1.198 ±0.049	1.178 ±0.046	1.145 ±0.044
24	0.902 ±0.023	0.890 ±0.030	0.865 ±0.028	0.849 ±0.028	1.030 ±0.048	0.974 ±0.036	0.900 ±0.035	0.834 ±0.031
36	0.625 ±0.015	0.580 ±0.019	0.537 ±0.010	0.500 ±0.001	0.770 ±0.029	0.645 ±0.022	0.576 ±0.018	0.522 ±0.016
48	0.550 ±0.014	0.510 ±0.013	0.460 ±0.017	0.425 ±0.014	0.620 ±0.021	0.550 ±0.022	0.460 ±0.017	0.400 ±0.013
60	0.475 ±0.018	0.420 ±0.015	0.350 ±0.009	0.285 ±0.006	0.477 ±0.016	0.410 ±0.014	0.340 ±0.009	0.250 ±0.005
72	0.300 ±0.007	0.292 ±0.010	0.210 ±0.012	0.170 ±0.004	0.310 ±0.010	0.270 ±0.012	0.140 ±0.003	0.110 ±0.006
96	0.265 ±0.005	0.250 ±0.003	0.190 ±0.006	0.146 ±0.004	0.290 ±0.003	0.246 ±0.006	0.122 ±0.004	0.096 ±0.005

The α and β amylase activity increased upto 12 hours after sowing later it declined in the control and treatments of both the varieties. The reduction in the activity of α and β amylase due to increase in salinity was less in ADT-1 variety when compared to CO-1 variety of soybean. There were reports on inhibition of hydrolytic enzymes like α amylase and protease in various parts of germinating susceptible rice seeds.[17-18] Sheoran[19] reported a decrease in amylase activity in the cotyledons of germinating mung bean seeds under salt stress. A decrease in amylase activity in the maize endosperm under stress during early period of germination was reported.[20] Sarin and Narayanan [21] reported that salinization decreased the amylase activity by inhibition. A reduction in the soluble sugar content and α and β amylase activity with increase of sodium salt stress can be well correlated in both the varieties of soybean.

The higher germination percentage can be well correlated with higher sugar content α and β amylase activity in CO-1 variety. The ADT-1 variety showed a lesser germination percentage with lower sugar content and lower activity of α and β amylase during germination under NaCl stress when compared with the CO-1 variety of soybean.

Based on this results both the varieties of soybean seems to be saline susceptible. When these two verieties are compared the CO-1 variety seems to be more stable in its metabolism by having higher germination percentage α and β amylase activity under NaCl stress.

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