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Research Article

Impact of Salt stress on Carbohydrate Metabolism in *Cicer arietinum* L.

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Abstract: Cereals are used as an important food of Indian diet. They are an important source of carbohydrates and proteins, so they are essential to a predominant diet. Chickpea (*Cicer arietinum* L.) is one of the important crops of India, mainly grown for their seeds which contain 20.6% protein, 2.2% fat and 61.2% carbohydrate, ranking next to wheat and barley both in acreage and in the production of grain. The major portion of crop production is consumed by the people in many forms like Dal, Besan, Crushed or whole grain, boiled or roasted gram or in sweet preparation. It is also used as a protein-rich diet for horses, and other cattle in the forms of soaked grain. Keeping the above views several *Cicer* varieties will be screened for their salt tolerance behaviour. Salt tolerant and salt susceptible variety will be selected for further comparative studies to expect that the findings of the present investigation would not only help with a better understanding of the mechanism of salt tolerant behaviour of *Cicer* at the physiological and biochemical levels and also suggest possibilities of selecting a variety suitable for salt stress condition.

Keywords: α-amylase, Salinity level, Electrical conductivity, Seedling age, Invertase activity, Sugars.

1. Introduction

Salinity caused due to accumulation of salt in the root zone of soil, resulting in partial or complete loss of soil productivity is a worldwide problem. Such soil salinity problem exists in arid, semi-arid areas. Where water deficit or drought is a major cause of salinity. An excess of salt interferes with the process of nutrient uptake and caused nutrient deficiency followed by low yield.

In the world, millions of hectares of land are saline, and more and more land is becoming saline/alkaline each year in India alone. In the northwestern province (now Uttar Pradesh) salinity affected area was estimated to cover altogether between 10,000 to 12,000 km. Salt-affected soils comprising mostly of saline and alkali type are scattered over a wide range of agro-climatic and soil texture zones. Based on the estimate prepared by (Singh, 1992), out of 8.373 million ha. of salt-affected land in India, 2.359 are alkali & 3.829 million ha. saline remaining are coastal saline. Salinity prevents crop production in an estimated area of about seven million hectares of marginal cultivated or potential cropland (Abrol *et al*., 1973).

Out of this, nearly 40% occur in the Indo-Gangetic plain of northern India. In Punjab state, about 0.6 million hectare land is affected with salts to different degrees. Out of this area, about one-third is completely barren (Singh, *et al*., 1980). In the state of Uttar Pradesh, it is reported that about 3.36 million acres of the land is affected by this malady. In general, salt-affected soils are extensive in the Northern alluvial plains, flanked by the Rann of Kutch and the Rajasthan desert in the west and the sub-humid, deltaic marshy and swampy lands of Sunder bans subject to tidal action in the east. The salt-affected soils also occur in the major river deltas in the east along the coastline, in major basins and local depressions in the semi-arid Deccan Plateau and its periphery, extending to the state of Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu.

The primary source of salt in the salt-affected areas is geomorphological and geophysiological. However, a human factor also plays an important role, in building the salty states of soils. The main cause of salinity in arid and semi-arid areas is due to high water table and the accumulation of salts on the surface by capillary movement of salts along with water and their deposition after the evaporation of water. Besides, continuous use of saline irrigation water and improper drainage, also adds to the soil salinity, in arid conditions.

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In agriculture, salinity problem denotes the soil condition, when the soil solution contains excessive amounts of neutral salts like chloride and sulphates of sodium. The electrical conductivity (EC) of the soil saturation extract of saline soil is more than 4 dS/m at 25°C, exchangeable sodium percentage is less than 15 and pH is not more than 8.5 (Richard, 1954).

2. Materials and Methods

Ten varieties of gram were obtained from Rajasthan Agricultural Research Station, Durgapura, Jaipur, for screening their salt tolerance variability. The screening was conducted according to Garrard's technique modified by Sarin and Rao (1961) and data were collected on the basis of percentage germination and early seedling growth as a percentage reduction in coleoptile length.

Name of Varieties: RSG-865; RSG-895; RSG-959; RSG-811; RSG-807; RSG-823; RSG-256; RSG-974; RSG-931; RSG-945.

The influence of salinity levels of 2, 4, 6, 8 dS/m, EC (electrical conductivity), salt solutions (containing equimolar concentrations of $CaC1_2$ and NaCl on early seedling growth of 10 varieties of gram) was investigated following Garrard's technique as modified by Sarin and Rao (1958). Test tubes of 30ml were filled with rolls of filter folded on top into cones to support the seeds. The seeds were surface sterilized with 0.1% $HgC1_2$. The test tubes were filled to one-third with the test solution so that the salt solution could be supplied to the roots through the capillary action of filter paper. Five replications were maintained for each treatment, including control (distilled water). Three seeds were allowed to germinate between the paper roll and glass wall of each tube. The tubes were kept in dark at 35°C ± 2°C temp. Observations on the total length of the root and coleoptile were recorded at 24 hrs. intervals up to 96 hrs under green, safe light.

The data were analyzed statistically on factorial basis, following the analysis of variance. To eliminate, such differences that might be attributed to seed size, water absorption capacity and seed vigour, the growth of each variety was recorded as percent over the control. Two varieties, one tolerant and other susceptible were selected for further comparative studies.

The selected varieties were raised in Petri plates for various physiological and biochemical studies, after sterilizing and thorough washing, the seeds were transferred on moist filter paper in sterilized Petri plates. Deionised water was used as a control and for treatments of seeds were irrigated with salt solutions of 2, 4, 6 and 8 dS/m (EC) levels using the same salt as in seedling growth. The replicates were placed in a dark growth chamber at 28±2°C. Fresh samples of seedlings were drawn at every 24 hrs after sowing, for the assay of various enzymes. Parallel samples were dried at 70±2°C in an oven till constant weight was reached. The dry matter thus obtained was maintained in desiccators, for various biochemical determinations. All estimations and assays were repeated thrice. All samples for various metabolic studies were drawn at random.

For extraction of α-amylase, the seedlings were collected randomly and homogenized in chilled mortar and pestle, using 0.2M Citrate buffer of 5.5 pH. The buffer was used in the ratio of 1:10 with the fresh material. The homogenized samples were centrifuged at 4°C in a centrifuge (International centrifuge model PR-2) at 10,000xg for 15 minutes. The supernatant thus obtained was used as crude enzymes.

The enzyme assay was carried out according to Chrispeel and Varner (1967). The reaction was carried out in a test tube containing a suitable aliquot of enzymes (0.02-0.2ml) and enough water to make 1.0ml. The reaction was started by adding 1ml of starch solution and allowed to proceed at room temperature for a suitable time (1-5 minutes). The reaction was stopped by adding 1ml of iodine reagent. 5ml of distilled water was added to each tube and after thorough mixing, the optical density (OD) was read at 620nm. All colorimetric observations were recorded on a Carl Zeiss (Spikol). The initial OD of starch solution was also recorded. The decrease in OD at 620nm caused by the action of enzyme is proportional to the quantity of α-amylase present, and the length of reaction time over the range of 30-75% decrease in OD. The activity is expressed as mg of starch utilized per hour per gram fresh weight.

Invertase activity was determined according to the method of Kaufman *et al.*, (1968). The weighed material was homogenized in a chilled mortar in 10ml of 0.2m phosphate-citrate buffer pH 5.6. The homogenate was centrifuged at 2,000 rpm for 10 minutes. The reaction mixture consisted of 0.1ml enzyme preparation, 30µ moles of 0.2M phosphate-citrate buffer pH 5.6 and 60µ moles of sucrose in total volume of 1.0ml. The enzyme reaction was started with the addition of enzyme and was stopped after 10 minutes of incubation period by the addition 1.0ml of alkaline reducing sugar reagent (Nelson, 1944). The reducing content was determined by standard methods of Somogyi (1952) was arsenomolybdate reagent. Protein was determined according to the method of Lowry *et al.*, (1951). The specific activity was expressed as 1gm glucose liberated/h/mg protein.

3. Results and Discussion

3.1 α-amylase activity

The ANOVA for α-amylase activity indicated that the treatment, duration and the interaction of variety with treatment was found to be significant.

Table 1.1a showed that varietal behaviour under salt-stressed condition irrespective of salinity levels and seedling age, it was found that variety RSG-807 had

higher levels of enzyme activity as compared to RSG-865.

The overall effect of different salinity level is represented in (Table 1.1b). It is evident that enzyme activity decreases significantly with increasing salinity level, while the reverse trend was observed with the seedling age (Table 1.1c).

The interaction of varieties with the salinity levels clearly indicate (Table 1.1d) that the enzyme activity decreases in both the varieties, however, the degree of enzyme inhibition differs. A remarkable decrease was observed in susceptible variety RSG-807 from a minimum level of salinity to maximum (2EC to 8EC), whereas variety RSG-865 showed an approximate effect only at 8EC. Table 1.1e clearly demonstrated an increase in enzyme activity with increasing seedling age. The optimum was recorded at 96 hrs; thereafter it tends to decline of the behaviour of the two cultivars remains more or less the same. It is interesting to note that the effect of salinity minimizes with the advancing of seedling age (Table 1.1f). The final interaction (Table 1.1) depicts the overall effect of salinity at different stages at seedling growth in different varieties with respect to salinity. It was observed that salinity caused an inhibition in both the varieties, however, the degree of inhibition being more in salt-sensitive variety RSG-807 as compared to tolerant variety RSG-865.

Table 1.1. Effect of Salinity on α-amylase Activity of two Gram Varieties.

(a) Main Factor: Varieties

RSG-807	RSG-865	SEM ±	C.D. at 5% P
7.89	7.02	0.47	N.S.

(b) Main Factor: Salinity Level

Control	2EC	4EC	6EC	8EC	SEM ±	C.D. at 5% P
9.54	7.53	6.56	5.26	3.86	0.27	0.66

(c) Main Factor: Seedling Age

24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	120 HRs.	SEM ±	C.D. at 5% P
5.61	6.46	8.02	9.48	8.17	0.39	0.96

(d) Interaction V × T

S. No.	Variety	Control	2EC	4EC	6EC	8EC
1.	RS4-807	11.71	9.21	7.13	5.91	2.58
2.	RS4-865	9.41	8.07	7.02	5.89	5.12

S.E.M. ± 0.36; C.D. at 5% P 0.89

(e) Interaction V × D

S. No.	Variety	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	120 Hrs.
1.	RS4-807	5.36	7.01	8.46	9.66	9.73
2.	RS4-865	4.80	6.21	7.91	9.81	9.97

S.E.M. ± 0.38; C.D. at 5% P 0.94

(f) Interaction "Seedling Age × Salinity Level"

Seedling Age	Control	2EC	4EC	6EC	8EC
24 Hrs.	7.14	5.54	5.21	4.81	3.27
48 Hrs.	8.45	7.43	6.43	5.75	5.52
72 Hrs.	11.41	9.32	7.67	6.79	5.93
96 Hrs.	12.13	10.68	9.33	8.54	6.6
120 Hrs.	12.45	11.39	9.91	7.97	5.91

S.E.M. ± 0.187; C.D. at 5% P.N.S.

3.2 Invertase activity

The ANOVA for invertase activity showed that main effects, i.e. varieties, treatment, and duration were found to be statistically significant. The interaction of variety with seedling age and seedling age with salinity levels were also significant. However, the interaction of varieties with salinity levels was found to be no significant.

Table 1.2a represents the overall varietal behaviour irrespective of salinity and seedling age. Variety RSG-807 showed a better performance as compared to RSG-865. Both the varieties differ significantly as far as their varietal behaviour is concerned.

It is evident from Table 1.2b, the enzyme activity decreases with increasing salinity level, which was found to be significant, whereas, the invertase activity

shows an upward trend with the advancement of seedling age (Table 1.2c).

The interaction of variety with salinity level (Table 1.2d) decreases, however, variety RSG-807 showed comparatively more reduction than the variety RSG-865.

Table 1.2e showed the interaction of varieties with seedling age, irrespective of salinity levels. Both the varieties i.e. RSG-807 and RSG-865 registered an increase in enzyme activity till 96 hrs after which it declined. Maximum enhancement was observed in variety RSG-807.

The interaction of seedling age with salinity levels (Table 1.2f) portrayed that after 72 hrs the effect of salinity was not prominent as up to 48 hrs of duration.

Table 1.2 depicts the overall effect of salinity at different stages of seedling growth in the varieties with respect to salinity. It was observed that salinity caused an inhibition of the invertase activity in both the varieties; however, the degree of inhibition was less intolerant variety.

Table 1.2. Effect of Salinity on Invertase Activity of two Gram Varieties (µg glucose liberated/mg protein/hrs).

(a) Main Factor: Varieties

RSG-807	RSG-865	SEM ±	C.D. at 5% P
2.78	1.93	0.16	0.40

(b) Main Factor: Salinity Level

Control	2EC	4EC	6EC	8EC	SEM ±	C.D. at 5% P
3.23	2.93	2.60	2.14	0.97	0.16	0.39

(c) Main Factor: Seedling Age

24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	120 HRs.	SEM ±	C.D. at 5% P
0.714	1.73	2.81	2.97	1.16	0.19	0.46

(d) Interaction V × T

S. No.	Variety	Control	2EC	4EC	6EC	8EC
1.	RS4-807	3.15	2.30	1.83	1.43	1.13
2.	RS4-865	2.03	1.93	1.72	0.98	0.67

S.E.M. ± 0.07; C.D. at 5% P 0.73 (N.S.)

(e) Interaction V × D

S. No.	Variety	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	120 Hrs.
1.	RS4-807	0.87	1.75	2.96	2.70	1.80
2.	RS4-865	0.75	1.51	1.66	1.93	1.09

S.E.M. ± 0.52; C.D. at 5% P 0.150

(f) Interaction "Seedling Age × Salinity Level"

Seedling Age	Control	2EC	4EC	6EC	8EC
24 Hrs.	0.81	0.71	0.63	0.54	0.47
48 Hrs.	2.31	1.78	1.43	1.07	0.56
72 Hrs.	3.62	3.06	2.60	1.82	0.79
96 Hrs.	2.93	3.00	2.99	2.10	1.56
120 Hrs.	1.51	1.19	1.08	1.03	0.67

S.E.M. ± 0.001; C.D. at 5% P 0.003

3.3 Total Sugars

ANOVA for total sugars indicates that main effects, varieties, salinity level (EC) and seedling age, as well as the interaction of variety with salinity level, was found to be significant. The interaction of variety with seedling age and seedling age with salinity levels was found to be not significant.

The varietal behaviour under salt stress, irrespective of seedling age and salinity level is shown in Table 1.3a and was found to be significant. RSG-807 has more amounts of total sugars than the variety RSG-865.

With increasing salinity levels of the medium, the number of total sugars decreased, irrespective of seedling age and varieties (Table 1.3b). A significant increase in the total sugars was observed with an increase in the seedling age of *Cicer*, however, it is declined at 120 hrs (Table 1.3c).

The interaction of varieties with salinity levels is presented in Table 1.3d, irrespective of seedling age. The varieties registered a significant decrease in the

total sugar contents with increasing salinity level, however, they differ in degree. Variety RSG-807 showed higher amounts of total sugars than RSG-865. The interaction of varieties with seedling age is presented. In Table 1.3e with the advancement of seedling age, the total sugar content increased varieties and a sharp increase was recorded at 96 hrs. RSG-807 sugar than RSG-865 right from the beginning.

The interaction of seedling age with salinity level (Table 1.3f) irrespective of varieties was found to be non-significant. Hereby showing that there has been no deviation due to the interaction of the two factors and they behaved in the same manner as the main effect. Total sugars increased up to 6EC salinity levels and then declined at 8 dS/m however, with seedling age a gradual reduction was recorded.

The final interaction in Table 1.3 portrayed the overall effect of salinity at different stages of seedling growth in the two varieties with respect to different salinity levels. It was observed that in both the varieties with increasing salinity levels total sugars decreased, whereas, with age, they increased up to 96 hrs of seedling age followed by a sharp decline in 120 hrs duration.

Table 1.3. Effect of Salinity on Total Sugars of two Gram Varieties (% dry weight).

(a) Main Factor: Varieties

RSG-807	RSG-865	SEM ±	C.D. at 5% P
0.503	0.284	0.070	0.173

(b) Main Factor: Salinity Level

Control	2EC	4EC	6EC	8EC	SEM ±	C.D. at 5% P
0.651	0.506	0.350	0.271	0.193	0.003	0.009

(c) Main Factor: Seedling Age

24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	120 HRs.	SEM ±	C.D. at 5% P
0.212	0.299	0.403	0.679	0.376	0.002	0.006

(d) Interaction V × T

S. No.	Variety	Control	2EC	4EC	6EC	8EC
1.	RS4-807	0.858	0.680	0.444	0.326	0.208
2.	RS4-865	0.444	0.332	0.256	0.214	0.176

S.E.M. ± 0.033; C.D. at 5% P (N.S.)

(e) Interaction V × D

S. No.	Variety	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	120 Hrs.
1.	RS4-807	0.282	0.434	0.552	0.734	0.514
2.	RS4-865	0.142	0.164	0.254	0.624	0.238

S.E.M. ± 0.031; C.D. at 5% P (N.S.)

(f) Interaction "Seedling Age × Salinity Level"

Seedling Age	Control	2EC	4EC	6EC	8EC
24 Hrs.	0.377	0.472	0.673	0.1041	0.643
48 Hrs.	0.242	0.402	0.503	0.871	0.514
72 Hrs.	0.182	0.257	0342	0.616	0.351
96 Hrs.	0.152	0.162	0.291	0.514	0.226
120 Hrs.	0.107	0.152	0.204	0343	0.146

S.E.M. ± 0.019; C.D. at 5% P (N.S.)

3.4 Reducing Sugars

ANOVA for reducing sugars, clearly demonstrated that main effects, i.e. varieties, salinity levels, and seedling age were significant, whereas their interactions were not significant.

The varietal behavior irrespective of salinity levels and seedling age, depicted in Table 1.4a showed that variety RSG-807 (salt-sensitive) possesses more amount of reducing sugars as compared to variety RSG-865 (salt tolerant). In general, with an increase in salinity level, reducing sugar decreases. Tile degree of inhibition was recorded right from 2EC salinity levels onwards (Table 1.4b).

Table 1.4c shows the main effect, i.e. seedling age. It was observed that with the advancement of seedling age the amount of reducing sugars also increases.

The interaction of varieties with salinity level is presented in Table 1.4d. It is evident that in both the varieties, the reducing sugars decreased with increasing salinity level of the medium; however, the degree of

reduction differs. RSG-807 (salt-sensitive) registered more reducing sugar content than RSG-865 right from the beginning.

Table 1.4e depicts the interactions of variety with seedling age. An increasing trend of reducing sugars was observed in both the varieties, whereas, the increase was not so prominent in the salt-sensitive variety RSG-807.

The interaction of seedling age with different salinity levels is portrayed in Table 1.4f. The overall pattern shows an increase in reducing sugar contents with increasing salinity level in one hand and reduction with the advancement of seedling age on the other.

Table 1.4 portrayed an overall effect of salinity at different varieties of *Cicer* with respect to salinity levels. It was observed that increasing salinity caused decreases in total reducing sugar at different seedling age. RSG-807 (salt-sensitive) showed more reducing sugar contents than RS4-865 (salt tolerant).

It is an established fact that salinity retards plant growth and this inhibition in growth is related to variable physiological and biochemical activities. In the present work, the experimental material is *Cicer*, which is rich in carbohydrates. The key enzymes regulating carbohydrate metabolism at seed germination is α-amylase and invertase. During early seedling growth, there is hardly any possibility of photosynthetic activity, and the main source of energy comes directly from the stored reserve food in endosperm, any adverse effect on enzyme activity at this stage ultimately affects the plant growth.

The α-amylase hydrolyses the starch, breaking it into free sugars necessary for plant growth. As described in the results the α-amylase activity decreases under saline condition, however, varietal differences existed. The susceptible variety RSG-807 showed maximum inhibition in amylase activity as compared to salt-tolerant RSG-865 variety, the degree of inhibition increases with increase in salinity levels.

There are numerous reports regarding inhibition of an amylase activity under saline media (Sarin and Narayanan, 1968). Almansouri *et al.,* (2004) and Ansari *et al.,* (1977) has also reported decrease in α-amylase activity in wheat. Amylase is synthesized in the aleurone layer in the presence of GA3 Jacobson *et al*., (1970). Present findings also corroborate with the earlier parts. Invertase activity has also shown a direct correlation with plant growth, under salt stress conditions like an amylase. Its activity also decreases with increase in salinity. Such trend is also reported by various workers such as Parihar and Baijal (1983) and Jain (2002) in different crops. Hayashi *et al.,* (1956) suggested that the enhanced activity of amylase and invertase parallels increases plant growth. Hatch and Glaszion (1963) have reported a close relationship between growth rate and invertase activity. The same is true here also, with depressed plant growth due to salinity, the activity of invertase also decreases. Several workers have also reported that salt causes decreases in invertase activity.

The activity of α-amylase and invertase is closely related to the release of sugars, and they are the direct energy source for the growing seedling. The enzyme activity decreases the hydrolysis of starch, thereby releasing less sugar available for plant growth. In the present findings, however, no correlation was observed between amylase and sugars, as the level of sugars does not seen to be a limiting factor for reduced plant growth directly. The cause of growth inhibition, during seed germination, could lie somewhere else. This hypothesis finds support from the reports of Sarin and Narayanan (1968) demonstrated that the supply of GA3 is successful in completing ameliorating the adverse effects of salts on an amylase activity, but fails to do so in the release of sugars in wheat and *Cicer*.

The α-amylase activity increases with advancement of seedling age up to 96 hrs. in both salts sensitive and salt tolerant varieties products in the *Cicer* varieties. However, susceptible variety showed more accumulation of sugars as compared to salt-tolerant variety.

Thus, the main causes for growth inhibition are due to high accumulation of sugars which were not utilized as per arrested supply. Further, no direct correlation was found between enzyme activities and their hydrolyzed product i.e. sugars.

Table 1.4. Effect of Salinity on Reducing Sugars of two Gram Varieties (% dry weight).

(a) Main Factor: Varieties

RSG-807	RSG-865	SEM±	C.D. at 5% P
0.218	0.167	0.00839	0.0236

(b) Main Factor: Salinity Level

Control	2EC	4EC	6EC	8EC	SEM ±	C.D. at 5% P
0.243	0.208	0.200	0.172	0.152	0.005	0.015

(c) Main Factor: Seedling Age

24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	120 HRs.	SEM ±	C.D. at 5% P
0.144	0.174	0.195	0.208	0.244	0.003	0.009

(d) Interaction V × T

S. No.	Variety	Control	2EC	4EC	6EC	8EC
1.	RS4-807	0.264	0.234	0.220	0.196	0.180
2.	RS4-865	0.222	0.182	0.160	0.148	0.124

S.E.M. ± 0.0029; C.D. at 5% P (N.S.)

(e) Interaction V × D

S. No.	Variety	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	120 Hrs.
1.	RS4-807	0.168	0.204	0.218	0.232	0.272
2.	RS4-865	0.120	0.144	0.172	0.184	0.216

S.E.M. ± 0.0028; C.D. at 5% P (N.S.)

(f) Interaction "Seedling Age × Salinity Level"

Seedling Age	Control	2EC	4EC	6EC	8EC
24 Hrs.	0.187	0.222	0.237	0.257	0.312
48 Hrs.	0.162	0.182	0.207	0.227	0.262
72 Hrs.	0.147	0.172	0.187	0.202	0.242
96 Hrs.	0.127	0.157	0.182	0.182	0.212
120 Hrs.	0.097	0.136	0.162	0.172	0.192

S.E.M. ± 0.024; C.D. at 5% P (N.S.)

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