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The Physiology of Salt Tolerance in Four Genotypes of Chickpea during Germination

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ABSTRACT

The physiology of salt tolerance in chickpeas (*Cicer arietinum*) is low. Therefore, after screening of a large number of genotypes, two tolerant (SG-11 & DHG-84-11) and two susceptible (Pusa-256 & Phule G-5) chickpea genotypes were germinated in sterilized germination boxes under different levels of salt stress (NaCl : $CaCl_2$: Na_2SO_4) viz., 0.0 (control), 4.0 and 8.0dSm^{-1} in order to investigate the physiological basis of salt tolerance. The experiment was carried out in completely randomised design in three replications under simulated conditions. It was terminated after 8 days and the germinated seeds were subjected to various analyses. At maximum salinity stress, there was comparatively more accumulation of sugar, protein, proline and phenol in tolerant genotypes along with higher amylase, peroxidase, catalase and lower protease activities. All the characters were positively and significantly correlated. Some of these indices might be useful for improving chickpea genotypes against salinity stress.

Keywords: Chickpea, Hydrolytic enzymes, Organic metabolites, Oxidative enzymes, Salt stress.

INTRODUCTION

Salinity is one of the major environmental stresses that affects crop productivity. Excessive irrigation and poor drainage facilities are the major contributing factors for soil salinity in agricultural lands and about one-third of world irrigated land is being affected by soil salinity (El-Saidi, 1997).

Chickpeas are produced in thirty-five countries but the major production areas are the Indian subcontinent, the Mediterranean region, Ethiopia and Mexico. Despite the release of one hundred fifty cultivars over the past sixty years, neither total production of chickpeas nor productivity per unit area have increased significantly. However, among the abiotic stresses, salinity is the most important yield reducer. Chickpeas are normally grown on receding soil moisture in the post rainy season, which would tend to increase salt concentration in the soil solution. The cost of soil reclamation is frequently so high that it is not possible to reclaim such soil for crop production. Exploitation of genetic variability in cultivated species offers the possibility of developing salt tolerant crops (Epstein *et al*., 1980). Thus, to permit crop growth on natural saline soils considerable enhancement of salinity tolerance could be required for the chickpea which is a relatively salt sensitive legume (Lauter and Munns, 1986). For conducting this experiment, a large number of chickpea genotypes were screened first for their relative tolerance against salinity stress on the basis of germination percentage and vigour index (Singh and Singh, 1999). SG-11 and DHG-84-11 were identified as tolerant genotypes, whereas Pusa-256 and Phule G-5 were susceptible. The present work was planned to understand the physiological basis of salt tolerance in chickpeas during germination. In this study the organic me-

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tabolites, namely, sugar, protein, proline and phenol along with the hydrolytic enzymes, namely amylase and protease and the oxidative enzymes, namely peroxidase and catalase were measured and analysed.

MATERIALS AND METHODS

Two salt tolerant genotypes (SG-11 and DHG-84-11) and two genotypes susceptible to salt (Pusa-256 & Phule G-5) were provided by Rajendra Agricultural University.

These seeds of four genotypes were surface sterilized with 0.1 per cent $HgCl_2$ solution for two minutes and then thoroughly washed with distilled water. One hundred seeds of each genotype were taken in three replications and the experiment was carried out in a completely randomised design (CRD). The salt solution was prepared by taking $NaCl:CaCl_2:Na_2SO_4$ in the ratio of 7:2:1 and the electrical conductivity of different salinity levels was adjusted by a direct reading conductivity meter. The seeds were germinated at different salinity levels, namely 0.0 (control), 4.0 and 8.0 dSm^{-1} in sterilized germination boxes lined with blotting papers and kept in a Biological Oxygen Demand (BOD) incubator at 25 + 2°C. The experiment was terminated after 8 days. Eight-day-old germinated seeds were subjected to following various analyses. Quantitative traits of total soluble sugar, soluble protein, free proline and total phenol were measured by the procedures described by Miller (1959), Lowry *et al*. (1951). Bates *et al*. (1973) and Farkas and Kiraly (1962), respectively. Activities of amylase, protease, peroxidase and catalase were assayed by the procedures described respectively by Mc Cready *et al*. (1950), Dubey and Rani (1990), Palmiano and Juliano (1973) and Kar and Mishra (1976).

With regard to sugar estimation, 2 ml of water extract was mixed with 2 ml of 2 NHCl and placed in a boiling water bath. The contents were neutralised with 5N NaOH. The total sugar was then determined using DNS reagent and the absorbance was recorded at 520 nm. A standard curve was prepared with D-glucose.

Protein was quantified by taking 100 mg of dried samples of plant material homogenised in 80 per cent ethanol and centrifuged. The residue was washed three to four times and was treated with trichloroacetic acid (TCA). The residue was defatted two to three times. The protein content was measured using Folin-Ciocalteau phenol reagent. The absorbance was read at 650 nm. A standard curved was prepared with bovine serum albumin (BSA).

As regards proline estimation, the extraction of proline was carried out by homogenising dried material in aqueous sulphosalicylic acid. The reaction mixture was extracted with toluene. The upper layer was pipetted out and its absorbance was read at 520 nm. A standard curve was prepared with L-proline.

For measuring phenols, a water extract of the sample prepared for sugar estimation was used. A water extract was taken in a test tube and Folin-Ciocalteau phenol reagent was added followed by Na_2CO_3 solution. Any precipitate, if formed, was removed by centrifugation. The absorbance was read at 650 nm. A standard curve was prepared with pyrogallol.

With regard to amylase activity, the reaction mixture was prepared by adding a citrate buffer and starch solution to an aliquot of the enzyme extract in a test tube. The tube was then incubated for five minutes at 30°C and sugar contents were determined using DNS reagent. One unit of amylase activity was defined as the amount of enzyme that liberated 10 µg glucose under the conditions of assay.

As regards protease, the procedure hinges upon the determination of amino-acids released during the hydrolysis of BSA by the proteolytic enzymes. The BSA solution was added to the enzyme extract and incubated for two hours. The reaction was stopped using trichloracetic acid and the absorbance was recorded at 570 nm. One unit of protease activity was defined as the amount of

enzyme, which released 1 µg of amino-acids from BSA under the conditions of assay.

For peroxidase, the reaction mixture contained the enzyme extract and pyrogallol solution in phosphate buffer. The reaction was initiated by adding 1 per cent H_2O_2 solution. After two minutes H_2SO_4 was added to stop the reaction. Purpurogallin, the product formed by the enzymatic oxidation of pyrogallol was extracted in n-butanol. Its absorbance was read at 420 nm. One unit of peroxidase activity was defined as the change in absorbance of 0.01 under the conditions of assay.

With regard to catalase activity, a reaction mixture was prepared by taking enzyme extract and 1 per cent solution of H_2O_2. The reaction was stopped by the addition of H_2SO_4 and titrated against 0.005 N solution of $KMNO_4$ to a faint purple colour, which persisted for at least fifteen seconds. One unit of catalase activity was defined as the amount of enzyme that catalysed the breakdown of 1 µmol of H_2O_2 under the conditions of assay.

RESULTS AND DISCUSSION

Total soluble sugars declined in all genotypes with the increasing of salinity stress (Table 1). Significant differences were observed between the genotypes of tolerant and susceptible groups for total sugar. The reduction in the sugar quantity of under salt stress condition may be attributed to suppressed amylase activity (Murumkar and Chavan, 1987) which causes reduced hydrolysis of reserve polysaccharides from cotyledons. A salt induced reduction in the

Table 1. The effect of salinity (NaCl:CaCl2:Na_2SO_4) on organic metabolites in four chickpea lines.

Genotypes	Salinity (dSm^{-1})	Sugar (mg g^{-1} dry wt.)	Protein (mg g^{-1} dry wt.)	Proline (mg g^{-1} dry wt.)	Phenol (mg g^{-1} dry wt.)
SG-11 (T)	0.0	58.33	155.00	0.71	5.06
	4.0	44.16	138.78	0.88	6.06
		(-24.29)	(-10.48)	(+23.94)	(+19.76)
	8.0	34.16	125.00	1.10	7.16
		(-41.43)	(-19.35)	(+54.92)	(+41.50)
DHG-84-11 (T)	0.00	59.16	155.00	0.75	5.36
	4.00	48.33	128.75	0.88	6.33
		(-18.30)	(-16.93)	(+17.33)	(+18.09)
	8.0	35.83	118.75	1.16	7.73
		(-39.43)	(-23.38)	(+54.66)	(+44.21)
Pusa-256 (S)	0.0	67.50	152.50	0.81	6.46
	4.0	44.16	122.75	0.88	5.36
		(-34.57)	(-19.50)	(+8.64)	(-17.02)
	8.0	26.66	105.00	0.98	4.73
		(-60.50)	(-31.14)	(+20.98)	(-26.78)
Phule G-5 (S)	0.0	64.16	156.25	0.85	6.13
	4.0	39.16	119.78	0.93	4.86
		(-38.96)	(-23.35)	(+9.41)	(-20.71)
	8.0	21.66	102.80	1.01	4.10
		(-66.24)	(-34.20)	(+18.82)	(-33.11)

	S.Em.			C.D. at 5%		
	Stress (S)	Genotypes (G)	S x G	Stress (S)	Genotypes (G)	S x G
Sugar	0.870	1.010	1.750	2.550	2.950	5.110
Protein	0.910	1.060	1.840	2.680	3.100	5.370
Proline	0.007	0.009	0.015	0.023	0.026	0.046
Phenol	0.060	0.070	0.120	0.180	0.200	0.360

T = Tolerant group S = Susceptible group

Figures in parentheses indicate per cent increase (+) or decrease (-) over control.

Table 2. The effect of salinity ($NaCl:CaCl_2:Na^2SO^4$) on hydrolytic and oxidative enzymes in four chickpea lines.

Genotypes	Salinity (dSm^{-1})	Amylase (Units g^{-1} fresh wt.)	Protease (Units g^{-1}fresh wt.)	Peroxidase (Units g^{-1} fresh wt.)	Catalase (Units g^{-1} fresh wt.)
SG-11 (T)	0.0	145.33	88.66	146.66	16.67
	4.0	97.33	121.91	166.66	18.16
		(-33.02)	(+37.50)	(+13.63)	(+8.93)
	8.0	74.66	152.91	186.66	20.13
		(-48.62)	(+72.50)	(+27.27)	(+20.75)
DHG-84-11(T)	0.00	160.00	95.31	126.66	23.10
	4.00	113.33	135.21	146.66	24.58
		(-29.16)	(+41.86)	(+15.79)	(+6.40)
	8.0	80.00	167.03	173.33	27.39
		(-50.00)	(+76.10)	(+36.84)	(+48.57)
Pusa-256 (S)	0.0	141.33	104.18	166.66	12.20
	4.0	66.66	158.66	146.66	11.05
		(-52.83)	(+52.20)	(-12.00)	(-9.42)
	8.0	37.33	193.25	126.66	9.73
		(-73.58)	(+85.50)	(-24.00)	(-20.24)
Phule G-5 (S)	0.0	165.33	95.31	173.33	15.01
	4.0	77.33	148.77	153.33	12.53
		(-53.22)	(+56.10)	(-11.53)	(-16.52)
	8.0	42.66	180.23	133.33	10.55
		(-74.19)	(+89.10)	(-23.07)	(-29.71)

	S. Em.			C. D. at 5%		
	Stress (S)	Genotypes (G)	S x G	Stress (S)	Genotypes (G)	S x G
Amylase	1.61	1.85	3.22	4.69	5.42	9.39
Protease	1.91	2.21	3.83	5.60	6.47	11.20
Peroxidase	3.33	3.84	6.66	9.72	11.23	19.45
Catalase	0.09	0.10	0.18	0.26	0.30	0.53

T = Tolerant group S = Susceptible group

Figures in parentheses indicate per cent increase (+) or decrease (-) over control.

amount of sugar has also been reported by Singh and Singh (1995). At 8.0 dSm^{-1}, comparatively more accumulation of sugar in SG-11 (34.16) and DHG-84-11 (35.83) is on account of the higher activity of amylase in tolerant genotypes (Table 2). A greater accumulation of sugar lowers the osmotic potential of cells and reduces loss of turgidity in tolerant genotypes. The other possible role of sugar may be as a readily available energy source.

Salt stress causes a marked change in protein metabolism of plant tissues (Levitt, 1980). The protein content decreased in all genotypes (Table 1), particularly, in susceptible genotypes. A salt induced decrease of protein content has been reported by Singh and Singh (1995). In the present study, increased protein hydrolysis seems to be the possible reason for a decrease in the level of protein under salt stress. The susceptible genotypes showed higher protease activity with increased salt stress (Table 2).

The proline content showed an increasing trend in all genotypes of chickpea under salt stress (Table 1), especially in tolerant genotypes in keeping with the result of Singh and Singh (1995) and Durgaprasad *et al*. (1996). Proline acts as an intracellular osmotic solute for maintenance of osmotic balance between cytoplasm and vacuole (Flowers *et al*., 1977). In addition to its role as an osmolyte and a reservoir of carbon and nitrogen, proline has been shown to protect plants against free radical induced damage (Matysik *et al*., 2002) Slow utilization of proline for protein synthesis and stimulation of glutamate conversion to proline during stress

Table 3. Correlation coefficient 'r' of biochemical characters with germination percentage at 8.0 dSm-1.

Sl. No.	Character	Correlation coefficient 'r'
1.	Sugar	0.883
2.	Protein	0.987
3.	Proline	0.935
4.	Phenols	0.968
5.	Protease	0.974
6.	Amylase	0.946
7.	Peroxidase	0.872
8.	Catalase	0.972

Table value of (r) of 10 degree of freedom and at 5% probability level = 0.576 and at 10% probability level = 0.708.

may be the possible reason for proline accumulation (Stewart, 1972).

The tolerant genotypes showed a higher level of total phenols (Table 1), whereas a significant reduction was observed in susceptible genotypes which is the same as the results of Dostanova *et al*. (1979) and Latha *et al*. (1989). Phenols constitute a part of cellular solutes and provide a reducing environment to the system (Das *et al*., 1990). Levitt (1980) reported that salt stress exerts its effect through membrane peroxidation which indicates that oxygen free radicals are formed during stress. Thus, phenol accumulation in tolerant genotypes could be a cellular adaptative mechanism for scavenging the free radicals of oxygen and preventing subcellular damage during stress. The accumulation of phenols may be due to the complex or a set operation of three factors including decreased conversion of phenols to quinones by lower activity of polyphenol oxidase, high oxidation of carbohydrate via the pentose phosphate Shiikimate pathway and low utilization of phenols for lignin formation (Latha *et al*., 1989).

Salinity stress induces reactive oxygen species (ROS) production and leads to oxidative damages. These toxic oxygen species may react with macromolecules, the proteins and lipid components of membranes causing damage through lipid peroxidation resulting in increased permeability of the membrance. The antioxidant defence system of the plant comprises a variety of antioxidant molecules and enzymes (Arora *et al*., 2002). Among the antioxidative enzymes, activity of peroxidase and catalase were recorded under salt stress conditions. The activity of peroxidase increased in tolerant genotypes which is similar to the results of Gossett *et al*., 1993 and decreased in susceptible genotypes (Table 2). At 8 dSm^{-1}, the peroxidase activity in SG-11 (186.66) and DHG-84-11 (173.33) were higher compared with that in Pusa-256 (126.66) and Phule G-5 (133.33). The higher activity of peroxidase in salt tolerant genotypes may protect the cell membrane in a comparatively better way from lipid peroxidation due to H_2O_2 which is a toxic metabolite produced by plants in various ways (Chaudhuri and Choudhuri, 1993).

Catalase, another H_2O_2 scavenging enzyme, behaved differently in both the groups with an increase in salinity. In contrast to the susceptible lines, the catalase activity was increased in salt tolerant chickpeas under a high level of salinity (Table 2). This result is comparable to those of Hernandez *et al*. (1993). The higher catalase activity indicates that the salt tolerant lines had an increased ability to decompose H_2O_2 (Gossett *et al*., 1994).

A highly positive and significant correlation was observed between germination percentage and all the characters (Table 3). The peroxidase activity (0.872) and the protein content (0.987) were the lowest and the highest, respectively. It is possible that, some of these indices might be useful for improving chickpea genotypes to withstand salinity stress.

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فیزیولوژی تحمل به شوری به هنگام جوانه‌زنی در چهار ژنوتیپ نخود سفید (*Cicer arietinum*)

چکیده

ا. ک. سینگ

اطلاعات اندکی راجع به فیزیولوژی تحمل به شوری در محصول نخود وجود دارد. به همین منظور، پس از غربال مقدار زیادی ژنوتیپ نخود، دو ژنوتیپ متحمل (SG-11 و DHG-84-11) و دو ژنوتیپ حساس (Pusa-256 و Phule G-5) در جعبه‌های استریل جوانه‌زنی تحت سطوح مختلف تنش شوری (کلرور سدیم، کلرید کلسیم و سولفات سدیم) با غلظت‌های صفر (شاهد)، ۴ و ۸ دسی زیمنس بر متر جهت یافتن اساس فیزیولوژیکی تحمل به شوری بررسی شدند. آزمایش در قالب طرح بلوک تصادفی در سه تکرار در شرایط القاء شوری انجام گرفت و بذور جوانه‌زده پس از هشت روز مورد ارزیابی مختلف قرار گرفتند. در تیمار حداکثر تنش شوری، مقادیر نسبتاً بالائی قند، پروتئین، پرولین و فنول در ژنوتیپ‌های متحمل تجمع یافت که همراه با فعالیت زیادتر آمیلوز، پراکسیداز، کاتالاز و فعالیت کمتر پروتئاز بود. همه صفات مورد بررسی همبستگی مثبت و معنی‌دار نشان دادند. برخی از این صفات ممکن است برای اصلاح ژنوتیپ‌های نخود در مقابل تنش شوری مفید باشند.