

## Physiological and biochemical responses of salt-tolerant and salt-sensitive pigeonpea genotypes to salinity

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### Abstract

The present study was carried out to evaluate the physiological and biochemical changes induced by salt stress in ten cultivars of *Cajanus cajan* L. under laboratory conditions and to screen the salinity tolerant and sensitive cultivars based on the results obtained. The productivity of crops is not increasing in parallel with the food demand. The lower productivity is attributable to various abiotic stresses, of which increased soil salinity is one of the foremost causes. The negative effect of salinity is caused by Na<sup>+</sup> and Cl<sup>-</sup> ions producing the critical conditions for plant survival. The obvious outcome of salinity includes membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and improper metabolic functions, including photosynthesis which ultimately leads to plant demise. In order to overcome this problem genotypes that are resistant to salinity need to be identified for further use in crop improvement programmes. Crops vary significantly in their threshold limits of salt tolerance. A well-focused approach combining the molecular, physiological, biochemical and metabolic aspects of salt tolerance is essential to alleviate the drastic effects of salinity and develop salt-tolerant crop varieties. Ten genotypes of pigeonpea were selected for their comparative analysis under salinity stress. These were ICP939, ICP1126, ICP1279, ICP2698, ICP8793, ICP11059, ICP13011, ICP14722, ICP14900, ICP11946) collected from NBPGR, New Delhi. According to the results obtained in our study, Eight among the ten *Cajanus cajan* L. cultivars namely EC ICP 939, ICP2698, ICP 1126, ICP1279, ICP11059, ICP13011, ICP14722, ICP14900 were identified as salinity tolerant cultivars and two among them namely ICP 8793 and ICP11946 were identified as salinity sensitive cultivars based on their biochemical response towards salinity.

**Keywords:** genotypes, salinity, photosynthesis, membrane damage, abiotic stress

### Introduction

World agriculture is facing a lot of challenges like producing 70% more food for an additional 2.3 billion people by 2050. However, the productivity of crops is not increasing in parallel with the food demand. The lower productivity is attributable to various abiotic stresses, of which increased soil salinity is one of the foremost causes. The negative effect of salinity is caused by Na<sup>+</sup> and Cl<sup>-</sup> ions producing the critical conditions for plant survival. The obvious outcome of salinity includes membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and improper metabolic functions, including photosynthesis which ultimately leads to plant demise. Crops vary significantly in their threshold limits of salt tolerance. A well-focused approach combining the molecular, physiological, biochemical and metabolic aspects of salt tolerance is essential to alleviate the drastic effects of salinity and develop salt-tolerant crop varieties. The exploitation of genetic differences of available germplasm has the greatest significance, because it helps to identify the genotypes performing well even under saline conditions. Screening of crops for tolerance can strengthen the breeding programs by identifying genotypes with high salt tolerance and yield potential. This strategy involves comparative investigation of various morphological, physiological, biochemical, enzymatic and ionic responses, together with the study of differential expression pattern of genes/proteins concerned with salt tolerance at different developmental stages under salt stress in salt-sensitive and salt-tolerant cultivars. Soil salinization is one of the serious forms of soil degradations, which can arise

from natural causes and human-mediated activity, such as irrigation in arid and semi-arid regions. Approximately 20% of the irrigated lands in the world are presumably affected by soil salinization<sup>[1]</sup>. Pigeonpea [*Cajanus cajan* (L.) Millsp.] is the sixth most important grain legume of tropics and subtropics. Because of its multiple uses, it plays an important role in subsistence agriculture. It is an important pulse crop that performs well in poor soils and regions where moisture availability is unreliable or inadequate (Kimani, 2001)<sup>[8]</sup>. In India, pigeonpea is mainly grown in the regions lying between 140N and 290N latitudes with mean annual rainfall ranging between 600 and 1500 mm.

Germination of seeds, one of the most critical phases of plant life, is greatly influenced by salinity (Misra and Dwivedi, 2004)<sup>[10]</sup>. Higher level of salt stress inhibits the germination of seeds while lower level of salinity 3 induces a state of dormancy (Khan and Weber 2008). The chlorophyll content of tissues decreased with an increase in salinity. Zhao *et al.* (2007)<sup>[32]</sup> reported that salinity reduced leaf chlorophyll in the oat plant. The decrease in Chl content under salt stress is a commonly reported phenomenon and in various studies the Chl concentration were used as a sensitive indicator of the cellular metabolic state (Chutipaijit *et al.* 2011)<sup>[4]</sup>. Chlorophyll fluorescence has been used to evaluate the integrity of photosystem II upon exposure to stress (Shabala, 2002)<sup>[18]</sup>. The in vivo effects of salinity on chlorophyll fluorescence have been described for several crop species (Smillie and Nott, 1982; Sayed, 2003)<sup>[21, 17]</sup>. Fluorescence parameters have been used to screen for salinity tolerance in barley, wheat and corn (Monneveux *et al.*, 1990; Belkhdja *et*

al., 1994) [12, 2]. Under saline environments, the plant lipid metabolism is interrupted as a result of oxidative damage to membrane lipids by active oxygen species and lipid peroxidation (Hernandez and Almansa, 2002; Misra and Gupta, 2006) [24, 11]. Salinity proved to have a significant impact on dry and fresh weight, root volume and stem diameter. Agricultural production losses due to water-logging and salinity in India are estimated to be around USD 28.5 million (ICRISAT Report, 2011) [6]. In Tamil Nadu, the increasing threat of salinity requires an in-depth study for salinity tolerance. In order to overcome this problem genotypes that are resistant to salinity need to be identified for further use in crop improvement programmes. The present study was carried out to evaluate the physiological and biochemical changes induced by salt stress in ten cultivars of *Cajanus cajan* L. under laboratory conditions and to screen the salinity tolerant and sensitive cultivars based on the results obtained.

**Materials and Methods**

The present study was carried out to understand the tolerance mechanism to salinity stress in pigeonpea [*Cajanus cajan* (L.) Millsp.] Genotypes. Ten genotypes of pigeonpea were selected for their comparative analysis under salinity stress. These were ICP939, ICP1126, ICP1279, ICP2698, ICP8793, ICP11059, ICP13011, ICP14722, ICP14900, ICP11946) collected from NBPGR, New Delhi. The seedlings were grown in pots with garden soil (pH 7.9 and electrical conductivity, EC 0.4dSm<sup>-1</sup>), temperature 28±1°C and this soil have some macro nutrients such as N(44), P(11.0), K(78) and micro nutrients such as Fe(5.8), Mn(9.0) no Zn content and Cu(0.4). This soil have no calcium carbonate. The seeds were surface sterilized with mercuric chloride, washed with distilled water and soaked in distilled water for 24 hours. After sprouting the seeds were sowed in the plastic pots.

**Plant Growth Condition and Salinity Treatments**

Pigeon pea seedlings were grown under controlled climatic condition temperature of 24-30°C and stress for 14 days. The plants were watered regularly. The seedlings were grown in pure water (Ph 7.20 and electrical conductivity, EC 0.13dSm<sup>-1</sup>) bicarbonate (1.00mEq/L), Chloride (0.60mEq/L), Calcium (1.00mEq/L), Magnesium (0.40mEq/L). After 4-7 days, uniform seedlings with fully developed trifoliolate leaves were transplanted into each of the plastic pots and arranged into three levels of salt stress. The plants were classified into three types of salt treatments in different varieties of cultivars.

- Control - Seeds treated in water regularly.
- Test - Seeds treated in 100 mM NaCl,

Treatments were triplicated 3 times and arranged into a randomized complete Block Design. At the end of 15<sup>th</sup> day, the growth parameters and photo synthetic pigments and stress parameters were measured in the cultivar seedlings.

**Biochemical parameters analyzed**

The photosynthetic pigments (Litchenthaler, 1987) [9], various biochemical parameters such as soluble sugars (Jayaraman, 1981) [26] total soluble proteins (Lowry et al., 1951) [29], soluble starch (Hodge and Hofreiter, 1962), free amino acids Moore, 1968) [30], proline (Bates et al., 1973) [1], electrolyte leakage (Sutinen et al., 1992), ascorbate (Mukherjee and Choudhini, 1983), TBARS Heath and Packer,

1968) the activities of catalase (Chance and Maely, 1955) [23], peroxidase(Chance and Maely, 1955) [23], nitrate reductase (Jaworski, 1971) [25] and polyphenol oxidase(Chance and Maely, 1955) [23] were measured both in salinity treated and control *Cajanus cajan* L.cultivars. All measurements were made on samples in triplicates and data was analyzed statistically using Students't test. The chlorophyll fluorescence was measured using pulse modulated OS-30P Chlorophyll Fluorometer (Winn Avenue Hudson, NH 03051 USA) with RS 232 port. All measurements were made on samples in ten replicates.



Fig 1: Cultivars of *cajanus cajan* L.

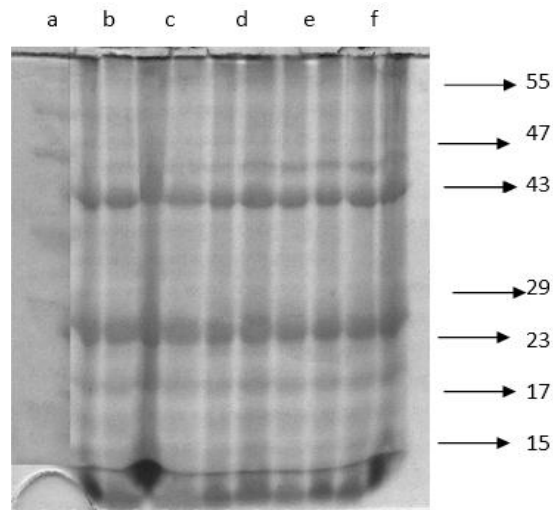


Fig 2

Electrophoretic profile of thylakoid membrane proteins in *Cajanus cajan* L.Cultivars. Gel lanes were loaded with equal amount of protein (100µg). a. Standard marker protein, Cultivars-- b. ICP939, c. ICP1126, d. ICP1279, e. ICP2698, f. ICP8793,

a g h i j k

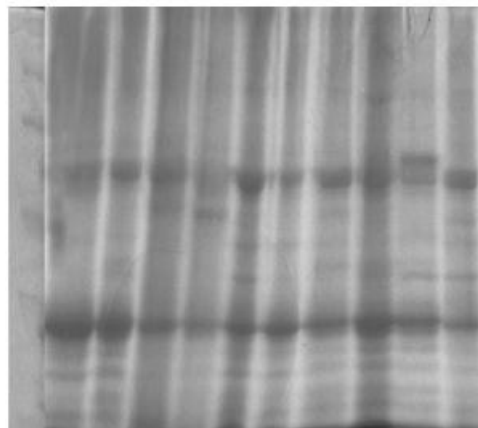


Fig 3

a. Standard marker protein Cultivars-- g. ICP11059, h. ICP13011, i. ICP14722, j. ICP14900, k. ICP11946

**Table 1:** Salinity (100 mM NaCl) induced changes in photosynthetic pigment composition of 10 cultivars of *Cajanus cajan L.* values represent mean  $\pm$  SD (n=9). The values in parentheses indicated percentage difference.

Cultivars	Chlorophyll a (mg.g fw <sup>-1</sup> )		Chlorophyll b (mg.g fw <sup>-1</sup> )		Total Chlorophyll(mg.g fw <sup>-1</sup> )		Carotenoids (mg.g fw <sup>-1</sup> )	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
ICP939,	1.32 $\pm$ 0.02	1.29 $\pm$ 0.01 (-2)	0.66 $\pm$ 0.01	0.65 $\pm$ 0.01 (-1)*	1.98 $\pm$ 0.02	1.91 $\pm$ 0.01 (-3)#	0.57 $\pm$ 0.01	0.56 $\pm$ 0.01 (-2)*
ICP1126,	1.33 $\pm$ 0.02	1.32 $\pm$ 0.02 (-1)*	0.62 $\pm$ 0.01	0.61 $\pm$ 0.01 (-1)*	1.95 $\pm$ 0.02	1.93 $\pm$ 0.03 (-1)	0.61 $\pm$ 0.01	0.59 $\pm$ 0.01 (-3)
ICP1279,	1.28 $\pm$ 0.13	1.30 $\pm$ 0.02 (2)#	0.61 $\pm$ 0.01	0.60 $\pm$ 0.01 (-2)#	1.89 $\pm$ 0.01	1.90 $\pm$ 0.02 (1)*	0.59 $\pm$ 0.02	0.57 $\pm$ 0.01 (-3)#
ICP2698,	1.26 $\pm$ 0.01	1.30 $\pm$ 0.01 (3)	0.68 $\pm$ 0.01	0.65 $\pm$ 0.01 (-4)	1.94 $\pm$ 0.01	1.95 $\pm$ 0.02 (1)#	0.63 $\pm$ 0.01	0.61 $\pm$ 0.01 (-2)#
ICP11059,	1.25 $\pm$ 0.01	1.29 $\pm$ 0.02 (2)	0.61 $\pm$ 0.01	0.60 $\pm$ 0.01 (-1)#	1.86 $\pm$ 0.01	1.88 $\pm$ 0.02 (1)#	0.64 $\pm$ 0.02	0.63 $\pm$ 0.02 (-1)*
ICP13011,	1.31 $\pm$ 0.02	1.33 $\pm$ 0.02 (2)*	0.67 $\pm$ 0.01	0.66 $\pm$ 0.01 (-2)#	1.98 $\pm$ 0.02	1.99 $\pm$ 0.02 (0.3)*	0.64 $\pm$ 0.02	0.63 $\pm$ 0.01 (-1)*
ICP14722,	1.27 $\pm$ 0.01	1.32 $\pm$ 0.01 (4)	0.61 $\pm$ 0.01	0.60 $\pm$ 0.008 (-2)#	1.88 $\pm$ 0.02	1.92 $\pm$ 0.02 (1)	0.63 $\pm$ 0.01	0.62 $\pm$ 0.01 (-2)#
ICP14900	1.25 $\pm$ 0.01	1.3 $\pm$ 0.02 (4)	0.62 $\pm$ 0.01	0.61 $\pm$ 0.01 (-1)*	1.87 $\pm$ 0.02	1.91 $\pm$ 0.03 (2)	0.57 $\pm$ 0.01	0.56 $\pm$ 0.01 (-2)*
ICP11946	1.31 $\pm$ 0.02	1.14 $\pm$ 0.03 (-13)	0.65 $\pm$ 0.01	0.50 $\pm$ 0.03 (-23)	1.96 $\pm$ 0.03	1.64 $\pm$ 0.05 (-16)	0.57 $\pm$ 0.01	0.44 $\pm$ 0.03 (-22)
ICP11946	1.32 $\pm$ 0.02	1.14 $\pm$ 0.04 (-13)	0.61 $\pm$ 0.01	0.49 $\pm$ 0.03 (-19)	1.94 $\pm$ 0.02	1.64 $\pm$ 0.03 (-15)	0.62 $\pm$ 0.02	0.49 $\pm$ 0.02 (-10)

\* Not Significant, # P $\leq$ 0.05-P=0.01, the remaining values represents P $\leq$ 0.01

**Table 2:** Salinity (100 mM NaCl) induced changes in biochemical characteristics of 10 cultivars of *Cajanus cajan L.* The values represent mean  $\pm$  SD, (n=9). The value in the parenthesis indicates percentage difference.

Cultivars	Soluble sugars (mg.g fw <sup>-1</sup> )		Soluble starch (mg.g fw <sup>-1</sup> )		Free amino acids (mg.g fw <sup>-1</sup> )		Soluble proteins (mg.g fw <sup>-1</sup> )	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
ICP939,	10.5 $\pm$ 0.6	25.9 $\pm$ 0.67 (26)	16.7 $\pm$ 0.17	17.9 $\pm$ 0.2 (7)	18.9 $\pm$ 0.24	22.8 $\pm$ 0.4 (10)	28.6 $\pm$ 0.42	32.9 $\pm$ 0.19 (15)
ICP1126,	19.7 $\pm$ 0.59	25.3 $\pm$ 0.6 (29)	15.9 $\pm$ 0.14	17.7 $\pm$ 0.28 (11)	18.3 $\pm$ 0.2	22.8 $\pm$ 0.34 (25)	25.8 $\pm$ 0.41	32.2 $\pm$ 0.23 (25)
ICP1279,	19.5 $\pm$ 0.22	23.9 $\pm$ 0.2 (-23)	15.5 $\pm$ 0.27	16.8 $\pm$ 0.13 (8)	19.1 $\pm$ 0.1	23.2 $\pm$ 0.16 (22)	25.5 $\pm$ 0.60	32.7 $\pm$ 0.24 (28)
ICP2698,	19.9 $\pm$ 0.28	24.8 $\pm$ 0.38 (25)	16.0 $\pm$ 0.1	15.6 $\pm$ 0.07 (-3)	18.9 $\pm$ 0.08	22.8 $\pm$ 0.12 (10)	26.8 $\pm$ 0.34	32.5 $\pm$ 0.3 (21)
ICP11059,	10.6 $\pm$ 0.10	24.0 $\pm$ 0.61 (16)	16.2 $\pm$ 0.13	17.6 $\pm$ 0.15 (8)	18.8 $\pm$ 0.15	22.6 $\pm$ 0.21 (10)	28.1 $\pm$ 0.41	32.5 $\pm$ 1.21 (15)
ICP13011,	19.9 $\pm$ 0.64	25.4 $\pm$ 0.65 (28)	16.6 $\pm$ 0.23	17.9 $\pm$ 0.22 (7)	19.2 $\pm$ 0.14	22.3 $\pm$ 0.26 (21)	28.6 $\pm$ 0.46	32.9 $\pm$ 0.47 (15)

ICP14722,	10.7±0.14	24.0±0.51 (15)	15.9±0.2	17.7±0.3 (11)	18.8±0.41	22.8±0.37 (21)	26.3±0.73	32.3±0.34 (23)
ICP14900	19.9±0.38	24.7±0.54 (24)	15.4±0.3	16.4±0.18 (7)	18.7±0.3	22.9±0.37 (22)	28.0±0.41	32.5±1.16 (16)
ICP11946	19.9±0.42	16.1±0.88 (-19)	15.9±0.2	14.3±0.33 (-10)	18.8±0.41	17.3±0.47 (-8)	26.3±0.73	24.6±1.16 (-7)
ICP11946	19.4±0.31	15.4±1.09 (-21)	15.4±0.25	13.3±0.27 (-14)	18.7±0.3	17.3±0.42 (-8)	28.0±0.41	26.4±0.45 (-6)

\* Not significant, # P≤0.05-P=0.01, the remaining values represents P≤0.01.

**Table 3:** Salinity (100 mM NaCl) induced changes in proline, electrolyte Leakage and TBARS of 10 cultivars of *Cajanus cajan L.* The values represent mean ± SD, n=9. The value in the parentheses indicated percentage difference.

Cultivars	Proline (mg.g fw <sup>-1</sup> )		Electrolyte Leakage (%)		TBARS(melondialdehyde) (mmoles g fw <sup>-1</sup> )	
	Control	Treatment	Control	Treatment	Control	Treatment
ICP939,	55±0.66	54.5±0.5 (-1)*	33.0±0.75	32.6±0.82 (-1)*	3.79±0.06	3.78±0.05 (<-1)*
ICP1126,	54.1±0.78	54.1±0.83 (<1)*	30.7±0.83	30.7±0.6 (<1)*	3.79±0.05	3.72±0.08 (-2)#
ICP1279,	52.4±0.34	51.7±0.6 (-1)#	29.6±0.42	29.4±0.55 (-1)*	3.91±0.1	3.84±0.01 (-2)*
ICP2698,	50.7±0.5	50.3±0.6 (-1)*	29.6±0.85	29.5±0.75 (<-1)*	3.58±0.07	3.67±0.13 (2)*
ICP11059,	53.8±0.5	52.8±0.43 (-2)	32.3±0.64	32.2±0.71 (<-1)*	4.06±0.07	4.11±0.08 (1)*
ICP13011,	54.9±0.72	54.4±0.51 (-1)*	32.6±0.74	32.6±0.53 (<-1)*	3.79±0.05	3.77±0.04 (<-1)*
ICP14722,	54.1±0.86	54.2±0.87 (<1)*	30.6±0.74	30.8±0.66 (<1)*	3.75±0.09	3.75±0.06 (<1)*
ICP14900	53.6±0.52	52.7±0.36 (-2)*	30.7±0.83	30.7±0.56 (<-1)*	3.89±0.12	3.86±0.1 (-1)
ICP11946	54.1±0.86	55.9±1.30 (3)	30.6±0.74	35.2±0.83 (15)	3.75±0.09	4.32±0.2 (15)
ICP11946	53.6±0.52	55.5±1.3 (3)	30.7±0.83	37.8±1.2 (23)	3.89±0.12	4.61±0.26 (19)

\* Not significant, # P≤0.05-P=0.01, the remaining values represents P≤0.01.

**Table 4:** Salinity (100 mM NaCl) induced changes in antioxidants of 10 cultivars of *Cajanus cajan L.* The values represent mean ± SD, n=9. The value in the parentheses indicated percentage difference.

Cultivars	Ascorbic acid (mg.g fw <sup>-1</sup> )		Catalase (Units g fw <sup>-1</sup> )		Peroxidase (Units g fw <sup>-1</sup> )		Polyphenol oxidase (Units g fw <sup>-1</sup> )	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
ICP939,	4.18±0.03	4.5±0.05 (6)	9.5±0.09	9.0±0.1 (-5)	4.13±0.08	4.94±0.02 (10)	0.54±0.02	0.63±0.01 (16)
ICP1126,	4.19±0.06	4.57±0.06 (9)	9.8±0.07	8.86±0.09 (-10)	4.21±0.05	4.86±0.04 (15)	0.56±0.01	0.66±0.02 (17)
ICP1279,	4.11±0.05	4.62±0.03 (12)	9.52±0.05	8.71±0.05 (-8)	4.24±0.1	5.08±0.07 (10)	0.50±0.01	0.64±0.02 (27)
ICP2698,	4.26±0.06	4.91±0.02 (15)	9.82±0.05	9.0±0.08 (-8)	3.96±0.04	4.69±0.08 (18)	0.53±0.02	0.63±0.02 (19)
ICP11059,	4.27±0.02	4.92±0.07 (15)	8.9±0.07	8.16±0.15 (-8)	4.19±0.05	4.80±0.02 (15)	0.58±0.01	0.69±0.02 (19)
ICP13011,	4.10±0.06	4.57±0.06 (9)	9.8±0.01	8.83±0.12 (-10)	4.13±0.08	4.94±0.02 (10)	0.56±0.01	0.66±0.02 (18)
ICP14722,	4.11±0.05	4.62±0.03 (12)	9.49±0.01	8.59±0.01 (-10)	4.23±0.03	4.86±0.03 (15)	0.50±0.01	0.64±0.02 (27)
ICP14900	4.18±0.08	4.57±0.07 (9)	9.47±0.1	8.691±0.07 (-9)	3.94±0.07	4.67±0.1 (18)	0.5±0.02	0.64±0.01 (28)
ICP11946	4.29±0.16	4.54±0.13 (6)	9.49±0.1	10.2±0.3 (7)	4.23±0.03	3.56±0.05 (-16)	0.50±0.01	0.46±0.03 (-8)
ICP11946	4.18±0.08	4.57±0.07 (9)	9.47±0.1	9.8±0.07 (4)	3.94±0.07	3.39±0.12 (-14)	0.50±0.02	0.44±0.04 (-12)

\* Not significant, # P≤0.05-P=0.01, the remaining values represents P≤0.01.

**Table 5:** Salinity (100 mM NaCl) induced changes in the relative levels of chlorophyll fluorescence emitted as minimal fluorescence ( $F_0$ ), Variable fluorescence ( $F_v$ ) and the ratio of variable to maximum fluorescence ( $F_v/F_m$ ) of 10 cultivars of *Cajanus cajan L.* under laboratory conditions. The values represent mean  $\pm$  SD of 10 replicates.

Cultivars	$F_0$		$F_m$		$F_v/F_m$		$F_v$	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
ICP939,	55.4 $\pm$ 1.84	68.4 $\pm$ 3.95	337.8 $\pm$ 4.02	336.5 $\pm$ 6.88	0.836 $\pm$ 0.0059	0.797 $\pm$ 0.0100	282.4 $\pm$ 4.62	268.1 $\pm$ 5.74
ICP1126,	58.3 $\pm$ 1.89	71.3 $\pm$ 3.68	343.2 $\pm$ 4.71	343.3 $\pm$ 7.21	0.830 $\pm$ 0.0058	0.792 $\pm$ 0.0084	284.9 $\pm$ 4.99	272 $\pm$ 5.52
ICP1279,	64.1 $\pm$ 2.69	75.6 $\pm$ 6.64	354.3 $\pm$ 5.93	362.7 $\pm$ 5.52	0.819 $\pm$ 0.0079	0.791 $\pm$ 0.0188	290.2 $\pm$ 6.32	287.1 $\pm$ 8.96
ICP2698,	50.1 $\pm$ 1.91	71.1 $\pm$ 4.04	353.7 $\pm$ 5.93	351 $\pm$ 6.63	0.858 $\pm$ 0.0067	0.797 $\pm$ 0.0098	303.6 $\pm$ 6.95	279.9 $\pm$ 5.47
ICP11059,	56.3 $\pm$ 1.64	66.8 $\pm$ 4.16	342.6 $\pm$ 5.78	331.6 $\pm$ 12.1	0.836 $\pm$ 0.0060	0.798 $\pm$ 0.0139	286.3 $\pm$ 6.51	264.8 $\pm$ 12.4
ICP13011,	52.5 $\pm$ 1.96	62.9 $\pm$ 4.51	358 $\pm$ 6.39	346.1 $\pm$ 10.5	0.853 $\pm$ 0.0069	0.818 $\pm$ 0.0136	305.5 $\pm$ 7.47	283.2 $\pm$ 10.9
ICP14722,	57 $\pm$ 2.54	67.6 $\pm$ 4.88	370.1 $\pm$ 6.74	358.4 $\pm$ 9.49	0.846 $\pm$ 0.0075	0.811 $\pm$ 0.0142	313.1 $\pm$ 7.34	290.8 $\pm$ 10.3
ICP14900,	60.8 $\pm$ 2.74	75.2 $\pm$ 5.79	358.8 $\pm$ 7.48	340.8 $\pm$ 8.65	0.830 $\pm$ 0.0090	0.779 $\pm$ 0.0187	298 $\pm$ 8.89	265.6 $\pm$ 10.7
ICP11946	53 $\pm$ 2.40	55.2 $\pm$ 4.96	272.5 $\pm$ 5.66	163.4 $\pm$ 16.4	0.805 $\pm$ 0.0101	0.661 $\pm$ 0.0288	219.5 $\pm$ 6.38	108.2 $\pm$ 13.9
ICP11946	53.5 $\pm$ 1.72	59.9 $\pm$ 5.24	272 $\pm$ 4.37	180.9 $\pm$ 15.8	0.803 $\pm$ 0.0069	0.668 $\pm$ 0.0247	218.5 $\pm$ 4.55	121 $\pm$ 13.0

## Results and Discussion

According to the results obtained in our study, the cultivars are classified into Salinity tolerant and sensitive cultivars. In the Salinity treated cultivars EC ICP 939, ICP2698, ICP 1126, ICP1279, ICP11059, ICP13011, ICP14722, ICP14900 the pigments Chl *a*, Chl *b*, total chlorophyll, carotenoids (Table.1)  $F_v/F_m$  (Table.5) and the biochemical parameters such as soluble sugars, free amino acids, soluble proteins, soluble starch (Table.2) and the antioxidants ascorbic acid, peroxidase, polyphenol oxidase (Table.4) have all shown an increase when compared to control plants whereas the free radical scavenging enzyme catalase was found to be decreased in salinity treated cultivars. The stress parameters, proline, electrolyte leakage and TBARS (Table.3) did not show any significant variation. These results showing the tolerance of these cultivars towards salinity treatment.

In the cultivars ICP 8793 and ICP11946, the pigments Chl *a*, Chl *b*, total chlorophyll, carotenoids,  $F_v/F_m$  and the biochemical parameters such as soluble sugar, free amino acids, soluble protein, soluble starch and the antioxidants ascorbic acid, peroxidase, polyphenol oxidase have all shown a decrease and the free radical scavenging enzyme catalase was found to be increased in salinity treated cultivars. The stress parameters, proline, electrolyte leakage and TBARS have shown significant increase. Results observed in these two cultivars showing their sensitivity towards salinity treatment. Salinity can affect growth in a number of ways; the first phase of the growth response is due to the osmotic effect of the salt in the soil solution and produces a suite of effects identical to those of water stress caused by drought. Later, there may be an additional effect on growth, if excessive amounts of salt enter the plant they will eventually rise to toxic levels in the older transpiring leaves, causing premature senescence. This will reduce the amount of assimilate that the plant can produce, and a reduction in this assimilate transported to the growing tissue may further limit growth. There was an earlier report saying that the total chlorophylls content decreases under NaCl salinity stress (Sadale, 2007) [15] in salt stressed Sorghum and maize plants. Salinity stress causes changes in chloroplast ultrastructure (Keiper *et al.*, 1998). There is also decrease in rate of photosynthesis under saline conditions (Sixto *et al.*, 2005) [20] in different species and clones of genus Populus. The depressive effect of salt stress on chlorophyll biosynthesis may be due to the formation of proteolytic enzymes such as chlorophyllase which is responsible for the chlorophyll degradation and

damaging the photosynthetic apparatus.

Change in chlorophyll contents due to salinity is the most obvious biochemical response (Sherif, 2012) [19]. Many authors have reported the increase in proline accumulation under salt stress in different plants such as pigeon pea (Waheed *et al.*, 2006) [22] the decrease in  $F_v/F_m$  in salinity treated plants might be due to damage produced by salinity stress. This is due to the impairment of photosynthetic system.

Plate 1&2 demonstrated the typical electrophoretogram of thylakoid membrane polypeptide profiles of *Cajanus cajan L. Cultivars*. Several polypeptides are present in similar amounts in both control and Salinity treated plants. The level of 43, 47 and 23-kDa polypeptides was found to be reduced in *Cajanus cajan L. Cultivars* ICP 8793 and ICP11946 when compared to the cultivars EC ICP 939, ICP2698, ICP 1126, ICP1279, ICP11059, ICP13011, ICP14722, ICP14900. This might be due to the increased rate of damage of these proteins when compared to their rate of repair (Barber, 1995). Several studies have been undertaken in the recent past to analyze stress proteins which are altered in response to salinity stress in high yielding rice (*Oryza sativa L.*) cultivars. Hence we have made an attempt to identify the salinity tolerant and sensitive cultivars of *Cajanus cajan L.* obtained from NBPGR, biochemically. The response of biochemical adaptation to the salinity stress has been studied for genetic improvement of commercial varieties.

## Conclusion

Eight among the ten *Cajanus cajan L.* cultivars namely EC ICP 939, ICP2698, ICP 1126, ICP1279, ICP11059, ICP13011, ICP14722, ICP14900 were identified as salinity tolerant cultivars and two among them namely ICP 8793 and ICP11946 were identified as salinity sensitive cultivars based on their biochemical response towards salinity.

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