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PHYSIO-BIOCHEMICAL ANALYSIS OF PIGEON PEA (*CAJANUS CAJAN L.*) GENOTYPES UNDER SALINITY STRESS CONDITION

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ABSTRACT

Environmental stresses limit agricultural productivity worldwide. Stresses have an effect on crop species. Among the many environmental stresses, salinity is inflicting immense modification in the plant growth and physiology and agricultural productivity. Pigeonpea (*Cajanus cajan*) is a vital grain legume of the Indian landmass, South-East Asia and geographic region. Over 85% of the globe pigeonpea is produced and consumed in India wherever it's a key crop for food and nutritionary security of the individuals. In the present study, pot culture experiment were conducted to estimate the impact on NaCl stressed pigeon pea (*Cajanus cajan L.*). The seeds of 10 pigeon pea genotypes were seeded in plastic pots. After 20 days of sowing, the plants were treated with 50mM concentration of sodium chloride. The plants samples (leaf) were collected for estimating pigment, Physiological and biochemical contents. The sodium chloride treatments have an effect on the chlorophyll-a, b, total pigment, protein content and different parameters. Similarly, the amino acid and proline content were additionally affected with increasing concentration of NaCl to a bigger extent when put next to manage in all the treatments. As per the result obtained in our study, AH-06-7, MANAK, ICP 5028, H-2001-25, SGBS-6 and ICP 14085 were observed as salinity tolerant varieties and remaining four i.e., PAU881, A. H-06-9, MAL 15 and ICPL 88039 were identified

as sensitive cultivars based on the physiological, biochemical and antioxidants enzymes response under salinity stress condition.

Keywords: Pigeon pea, Salt stress, Pigments, Proline, Antioxidants enzymes, MSI

INTRODUCTION

Global agriculture is going through a whole lot of challenges like producing 70% extra food for a further 2.3 billion people by 2050 **[8]**. The major pigeon pea producing area in the country lies between 20°N to 30°N covering the Indo – Gangetic plains (State of Punjab, Haryana, Uttar Pradesh, Madhya Pradesh, Rajasthan and Bihar) but in Uttar Pradesh, Meerut district lies between 28^0 57' to 28^0 02' North latitude and 77^0 40' to 77^0 45' East longitude in the Indo – Gangetic plains of India. It is bound on the north by Muzaffarnagar district, in the south by Bulandshahar district while Ghaziabad and Baghpat districts from the southern and western limits.

However, the productivity of pulses is not growing in parallel with the food requirement. The lesser productivity is because of numerous abiotic stresses, of which enhanced soil salinity is one of the important reasons. The negative impact of salinity is caused by Na+ and Cl- ions generating the serious conditions for plant growth **[13, 14]**. Pigeonpea or Red Gram (*Cajanus cajan* (L.) Millspaugh) is an important food legume for the tropical and subtropical regions of Indian subcontinent, South-East Asia and East Africa **[19]**. It plays important role in food and nutritional security because it is a rich source of proteins, minerals and vitamins. Pigeon pea is capable being in symbiosis with rhizobia and thereby enriches soil through nitrogen fixation. Symbiotic bacteria (*Bradyrhizobium*) colonizing root nodules of pigeonpea fix atmospheric nitrogen up to 40 kg/ha in a cropping season and its deep root system improves soil structure **[4]**. Salinity tolerance is a polygenic individual regulates by interaction of many alleles manage physiological parameters **[28]**. Those physiological parameters showed genetic variability for salinity tolerance. As a result there may be require to investigate physiological importance of specific developments in plant edition and productivity below saline conditions. To this point, research associated with salinity tolerance mechanisms have denoted that the deduction of plant growth and yield beneath salinity is typically attributed to collective effects of reduced osmotic capacity of soil solution, disruption in nutrient uptake and toxic effects of hazardous ions, cumulation of

toxic salts (mainly sodium and chloride ions) are the main causative factors for the physiological harm under salinity, disturbs metabolic processes and all Physio-biochemical tendencies **[24]**. These encompass the suppression of several crucial processes, in most cases beginning from restrained water uptake and assimilation of many vital nutrients i.e., K^+ ,Ca^{+2}, Mg^{+2} results in reduced turgidity and growth **[1, 31]**.

Therefore, decrease uptake of Na^{+2} or Cl^- and their subsequently transport and compartmentalization, upkeep of ion homeostasis and stimulation of antioxidant systems are taken into consideration the biochemical and physiological basis of salinity tolerance **[20, 22, 23, 29, 7]**. Most of the researchers have reported that variability within the salinity tolerance mechanisms of the rice cultivars **[15, 16]** indicates the potential of various traits to cope with salinity stress condition. The present study was carried out to evaluate the physio-biochemical changes induced by salinity stress in ten pigeon pea genotypes and screened the salinity sensitive and tolerant genotypes based on the present observation.

MATERIAL AND METHODS

The germplasm of 10 pigeon pea genotypes were collected from Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut, and experiment was conducted at our college i.e., in Keral Verma Subharti College of Science, Department of Biotechnology, Swami Vivekanand Subharti University, Meerut. Sterilization process of seed of pigeon pea was done by 0.2% $HgCl_2$ Solution for 6 minute with continuous shaking and then thoroughly washed with distilled water. All the sterilized seed were sown (5 seed per pots) in the plastic pots and all the pots were irrigated with tap water up to one week from the day after sowing. After one week of sowing pots were irrigated with tap water as control or with 50 mM concentration of saline solution (NaCl) which was given time to time as per the experiment design.

Salinity tolerant and sensitive genotypes were identified based on the following parameters -

Chlorophyll Content (mg g^{-1} fresh wt.): Chlorophyll analysis (mg/g fresh weight) was determined by following the method of researcher **[2]**. Ethanol 80 %, 5 ml of was taken in test tubes and immediately weighed (0.1 g) fresh leaf samples were added, immersed in ethanol and tubes were capped. Extract was kept in water bath at 80 ºC for 10 min. Extract was cooled in dark room at room temperature. The optical density was

measured by exposing to lower light by using a UV Spectrophotometer.

Assessing carotenoid content in plant leaves with reflectance spectroscopy by method of Gitelson **[9]**.

Total Soluble Sugar Content (mg/g): Total soluble sugar was determined based on the method given by Yemm and Willis **[32]**.

Starch Content determination (mg.g fw^{-1}): Starch was estimated by the following anthrone reagent method described by Mc. Cready **[18]**.

Free amin acid (mg.g fw^{-1}): A novel colorimetric determination of free amino acids content by method of Chen **[5]**.

Soluble Protiens (mg.g fw^{-1}): Extraction yield of soluble protein by method of Choi **[6]**.

Proline Content (mg.g fw^{-1}): Proline (mg/g fresh weight) amounts were determined by following Bates **[3]**. Fresh sample weight of 0.1 g of leaves was added in 5 ml of 3 % sulfosalycylic acid in test tubes, ground, and then allowed to settle. Then, 2 ml from supernatant was mixed with 2 ml each of glacial acetic acid and ninhydrin reagent and was boiled for 1 hr in water bath at 100 ºC. After an hr, the reaction was cooled in ice bath and finally 4 ml of toluene were added, vortexed and the absorbance of the supernatant was read at 520 nm on the UV Spectrophotometer.

Membrane Stability Index: Leaf membrane stability index (MSI) was determined by recording the electrical conductivity of leaf lichgates in double distilled water 40^0C and 100^0C. Leaf samples (0.1 g) were cut into discs of uniform size and taken in test tubes containing 10 ml of double distilled water in two sets of the entire sample. One set was kept at 40^0C for 30 minutes and another set at 100^0 C in boiling water bath for 15 minutes and their respective electrical conductivities as C_1 and C_2 were measured by conductivity meter using protocol described by saadalla **[25]**.

TBARS (melondialdehyde) (mmoles g fw^{-1}): MDA content was measured according to the modified method of Heath and Packer **[12]**. 1g fresh tissue was taken and ground in 10 ml of 0.5% TBA in 20% TCA. The mixture was incubated at 95º C in water bath for 30 min and quickly cooled in an ice bath. The homogenate was centrifuged and absorbance of the supernatant was read at 532 nm.

Ascorbic acid content (mg.g fw^{-1}): The extraction and estimation of ascorbic acid was done by method of Oser **[21]**. For indophenol method a 3.4moles/litre solution of 2,6- dichlorophenolindophenol sodium

solution was prepared. 5ml of the above solution was then diluted to 50ml with deionised water warmed, filtered into an amber-coloured bottle and then standardized with 0.8mg/100ml ascorbic acid dissolved in metaphosphoric acetic acid.

Catalase (Units g fw^{-1}): **Catalase (EC 1.11.1.6) activity was determined polarographically by the method of Sgherri [26]**.

Peroxidase (Units g fw^{-1}): Peroxidase (GPOD, EC 1.11.1.7) activity was determined following the method of Zheng **[34]**.

Polyphenol oxidase (Units g fw^{-1}): Extraction and partial characterization of polyphenol oxidase determined. by the method of Wuyts **[30]**.

RESULTS AND DISCUSSION

According to the consequences received in our investigation, the pigeon pea cultivars were classified into highly tolerant, tolerant, moderate, sensitive and highly sensitive cultivars under saline treatment condition **[10]**. In the salinity treated cultivars i.e, AH-06-7, MANAK, ICP 5028, H-2001-25, SGBS 6, ICP 14085, plant pigments like chlorophyll a, chlorophyll b, total chlorophyll and carotenoids (**Table 1**) were showed minimum percentage of decrease in tolerant cultivars as compared to sensitive. The biochemical variable i.e., soluble sugar, soluble starch, soluble protein and free amino acid of all pigeon pea cultivars (**Table 2**) showed an increase under salinity stress condition, when compared to the control plant. The different antioxidants ascorbic acid, catalase and peroxidase **(Table 4)** showed an increase under salinity stress condition, when compared to the control plant. The Stress parameter such as proline, membrane stability index (MSI) and melondialdehyde (**Table 3**) have showed minimum significant variation. These observation showing the tolerance of above mentioned cultivars towards salinity treatment condition. In the experiment other pigeon pea cultivars i.e., PAU881, AH-06-9, MAL 15 and ICPL 88039 the plant pigments like chlorophyll a, chlorophyll b total chlorophyll and carotenoids were showed maximum percentage of decrease in the mentioned cultivars as compared to tolerant. The different antioxidant ascorbic acid, catalase and peroxidase (**Table 4**) showed a significantly increase under salinity stress condition, when compared to the control plant. The stress parameters such as proline, membrane stability index (MSI) and melondialdehyde have showed significant increase under salinity stress condition. The observation of four cultivars showing the

sensitivity towards salinity treatment condition.

Salinity can have an effect on increase in a number of approaches; the primary segment of the growth reaction is due to the osmotic impact of the salt inside the soil solution and produces a set of results equal to the ones of water strain due to drought. Thereafter, there may be an additional effect on growth of the plant, if immoderate amounts of salt enter the plant they'll sooner or later rise to toxic tiers inside the older transpiring leaves, inflicting untimely or premature senescence. This can lessen the amount of assimilate that the plant can produce, and a reduction in this assimilate transported to the mounting tissue may additionally in addition restrict growth. Some researcher have observed that the plant pigment (chlorophyll content) decreases under salinity stress condition **[33, 9]**. Chloroplast ultra structure was changed under salinity stress condition **[11, 12]**. Photosynthetic rate is also decreased under saline condition **[27]**. Most of the researchers were observed that under salinity stress condition proline content accumulation in different plants like pigeon pea **[17]**.

Table 1: Table indicate the changes in of Photosynthetic pigments of 10 pigeonpea cultivars (*Cajanus cajan L.*) under salinity (50 mM) stress condition. The value in parentheses indicated % differences

Cultivars	Chlorophyll *a* (mg.g fw^{-1})		Chlorophyll *b* (mg.g fw^{-1})		Total Chlorophyll(mg.g fw^{-1})		Carotenoids (mg.g fw^{-1})	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
AH-06-7	1.48±0.03	1.28±0.02 (-2)	0.69±0.01	0.55±0.02 (-1)*	1.85±0.03	1.74±0.02 (-3)#	0.55±0.02	0.53±0.02 (-2)*
MANAK	1.46±0.02	1.28±0.04 (3)	0.68±0.01	0.55±0.02 (-4)	1.85±0.02	1.73±0.01 (1)#	0.61±0.02	0.58±0.02 (-2)#
ICP 5028	1.38±0.03	1.27±0.02 (-1)*	0.66±0.01	0.51±0.02 (-1)*	1.83±0.03	1.68±0.02 (-1)	0.58±0.02	0.57±0.02 (-3)
H-2001-25	1.38±0.12	1.26±0.03 (2)#	0.66±0.01	0.51±0.02 (-2)#	1.83±0.02	1.68±0.03 (1)*	0.56±0.04	0.55±0.02 (-3)#
SGBS 6	1.33±0.02	1.22±0.03 (2)	0.63±0.01	0.48±0.02 (-1)#	1.77±0.03	1.64±0.04 (1)#	0.62±0.03	0.61±0.02 (-1)*
ICP 14085	1.33±0.01	1.21±0.04 (2)*	0.63±0.01	0.48±0.02 (-2)#	1.77±0.01	1.64±0.05 (0.3)*	0.62±0.01	0.61±0.02 (-1)*
PAU 881	1.31±0.02	1.18±0.02 (4)	0.61±0.01	0.47±0.07 (-2)#	1.76±0.03	1.63±0.03 (1)	0.61±0.02	0.60±0.02 (-2)#
AH-09-9	1.31±0.02	1.18±0.02 (4)	0.61±0.01	0.47±0.02 (-1)*	1.75±0.01	1.63±0.02 (2)	0.55±0.02	0.54±0.02 (-2)*
MAL 15	1.30±0.03	1.16±0.02 (-13)	0.60±0.01	0.45±0.02 (-23)	1.72±0.03	1.60±0.02 (-16)	0.55±0.02	0.42±0.02 (-22)
ICPL 88039	1.30±0.04	1.16±0.03 (-13)	0.60±0.01	0.45±0.04 (-19)	1.72±0.04	1.60±0.01 (-15)	0.60±0.02	0.46±0.03 (-10)

*** Not Significant, # P≤0.05-P=0.01, the remaining values represents P≤0.01**

Table 2: Table indicate the changes in biochemical characteristics of 10 pigeonpea cultivars (*Cajanus cajan L.*) under salinity (50 mM) stress condition. The value in parentheses indicated % differences

Cultivars	Soluble sugars(mg.g fw^{-1})		Soluble starch(mg.g fw^{-1})		Free amino acids(mg.g fw^{-1})		Soluble proteins (mg.g fw^{-1})	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
AH-06-7	19.8±0.6	24.5±0.62 (26)	16.7±0.15	16.9±0.22 (7)	18.5±0.24	21.8±0.41 (10)	28.6±0.42	31.9±0.19 (15)
MANAK	19.8±0.59	24.3±0.61 (29)	15.9±0.15	16.7±0.21 (11)	18.3±0.22	21.8±0.32 (25)	25.8±0.41	31.2±0.24 (25)
ICP 5028	19.5±0.22	23.9±0.22 (-23)	15.5±0.25	16.5±0.12 (8)	18.1±0.11	21.2±0.14 (22)	25.5±0.60	30.7±0.26 (28)
H-2001-25	19.5±0.28	23.8±0.39 (25)	15.2±0.14	16.2±0.05 (-3)	17.9±0.09	20.8±0.14 (10)	24.8±0.34	30.5±0.32 (21)
SGBS 6	18.2±0.10	23.0±0.63 (16)	14.5±0.14	15.6±0.17 (8)	17.2±0.16	20.3±0.22 (10)	24.4±0.41	30.±1.22 (15)
ICP 14085	18.2±0.64	22.8±0.64 (28)	14.5±0.24	15.6±0.25 (7)	17.2±0.14	20.3±0.25 (21)	24.4±0.46	28.9±0.48 (15)
PAU 881	18.0±0.14	22.7±0.52 (15)	14.0±0.23	15.4±0.31 (11)	16.8±0.42	19.8±0.36 (21)	24.3±0.73	28.3±0.32 (23)
AH-09-9	18.0±0.38	22.7±0.53 (24)	13.8±0.32	15.4±0.19 (7)	16.7±0.31	19.7±0.36 (22)	24.0±0.41	27.5±1.18 (16)
MAL 15	17.2±0.42	22.1±0.85 (-19)	13.4±0.21	15.3±0.35 (-10)	16.5±0.44	19.3±0.45 (-8)	23.3±0.73	26.6±1.18 (-7)
ICPL 88039	17.2±0.31	22.1±1.08 (-21)	13.4±0.22	15.3±0.26 (-14)	16.5±0.32	19.3±0.42 (-8)	23.0±0.41	26.4±0.42 (-6)

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

Table 3: Table indicate the changes in proline, electrolyte Leakage and TBARof 10 pigeonpea cultivars (*Cajanus cajan L.*) under salinity (50 mM) stress condition. The value in parentheses indicated % differences

Cultivars	Proline (mg.g fw^{-1})		Electrolyte Leakage (%)		TBARS(melondialdehyde) (mmoles g fw^{-1})	
	Control	Treatment	Control	Treatment	Control	Treatment
AH-06-7	54±0.68	55.1±0.52 (-1)*	27.6±0.72	30.6±0.81 (-1)*	3.30±0.06	3.15±0.04 (<-1)*
MANAK	54.1±0.74	55.1±0.79 (<1)*	27.7±0.81	30.7±0.60 (<1)*	3.26±0.05	3.05±0.07 (-2)#
ICP 5028	54.3±0.35	55.7±0.62 (-1)#	28.6±0.43	31.4±0.54 (-1)*	3.14±0.12	3.00±0.02 (-2)*
H-2001-25	54.3±0.40	55.8±0.63 (-1)*	28.8±0.82	31.4±0.73 (<-1)*	2.90±0.07	2.50±0.16 (2)*
SGBS 6	54.8±0.40	56.6±0.45 (-2)	32.3±0.65	33.2±0.72 (<-1)*	2.02±0.08	1.80±0.07 (1)*
ICP 14085	54.9±0.61	56.6±0.53 (-1)*	32.6±0.75	33.6±0.54 (<-1)*	1.90±0.06	1.73±0.08 (<-1)*
PAU 881	55.1±0.72	56.8±0.86 (<1)*	32.8±0.74	33.8±0.65 (<1)*	1.33±0.10	1.18±0.04 (<1)*
AH-09-9	55.6±0.53	56.8±0.35 (-2)*	32.8±0.80	33.8±0.55 (<-1)*	1.28±0.13	1.15±0.1 (-1)
MAL 15	55.8±0.84	56.9±1.32 (3)	33.6±0.75	34.2±0.84 (13)	0.50±0.08	0.35±0.2 (13)
ICPL 88039	55.8±0.50	56.9±1.30 (3)	33.7±0.82	34.8±1.22 (19)	0.15±0.13	0.05±0.27 (17)

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

Table 4: Table indicate the changes in antioxidants of 10 pigeonpea cultivars (*Cajanus cajan L.*) under salinity (50 mM) stress condition. The value in parentheses indicated % differences

Cultivars	Ascorbic acid (mg.g fw^{-1})		Catalase (Units g fw^{-1})		Peroxidase (Units g fw^{-1})		Polyphenol oxidase (Units g fw^{-1})	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
AH-06-7	4.18±0.04	5.5±0.04 (6)	2.50±0.08	3.20±0.12 (-5)	0.65±0.04	1.84±0.03 (10)	0.66±0.02	0.73±0.01 (16)
MANAK	4.19±0.05	5.57±0.05 (9)	2.58±0.09	3.26±0.09 (-10)	0.65±0.03	1.86±0.04 (15)	0.66±0.01	0.76±0.02 (17)
ICP 5028	4.21±0.02	5.62±0.04 (12)	3.82±0.06	3.71±0.04 (-8)	0.84±0.20	2.08±0.06 (10)	0.60±0.01	0.74±0.02 (27)
H-2001-25	4.21±0.01	5.63±0.03 (15)	3.82±0.07	3.80±0.07 (-8)	0.86±0.05	2.09±0.02 (18)	0.63±0.02	0.73±0.02 (19)
SGBS 6	4.27±0.02	5.92±0.06 (15)	4.28±0.05	4.46±0.12 (-8)	1.19±0.04	2.80±0.02 (15)	0.68±0.01	0.79±0.02 (19)
ICP 14085	4.28±0.01	5.92±0.05 (9)	4.52±0.02	4.53±0.13 (-10)	1.33±0.05	2.94±0.02 (10)	0.66±0.01	0.76±0.02 (18)
PAU 881	5.31±0.04	6.62±0.02 (12)	4.59±0.02	4.59±0.02 (-10)	1.53±0.07	3.16±0.03 (15)	0.60±0.01	0.54±0.02 (27)
AH-09-9	5.32±0.07	6.64±0.01 (9)	4.67±0.20	5.12±0.03 (-9)	1.64±0.08	3.17±0.1 (18)	0.60±0.02	0.54±0.01 (28)
MAL 15	5.39±0.13	6.74±0.13 (6)	4.88±0.20	5.24±0.20 (7)	1.83±0.02	3.56±0.05 (-16)	0.60±0.01	0.56±0.03 (-8)
ICPL 88039	5.40±0.03	6.77±0.06 (9)	4.88±0.20	5.86±0.40 (4)	1.94±0.09	3.89±0.12 (-14)	0.60±0.02	0.54±0.04 (-12)

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

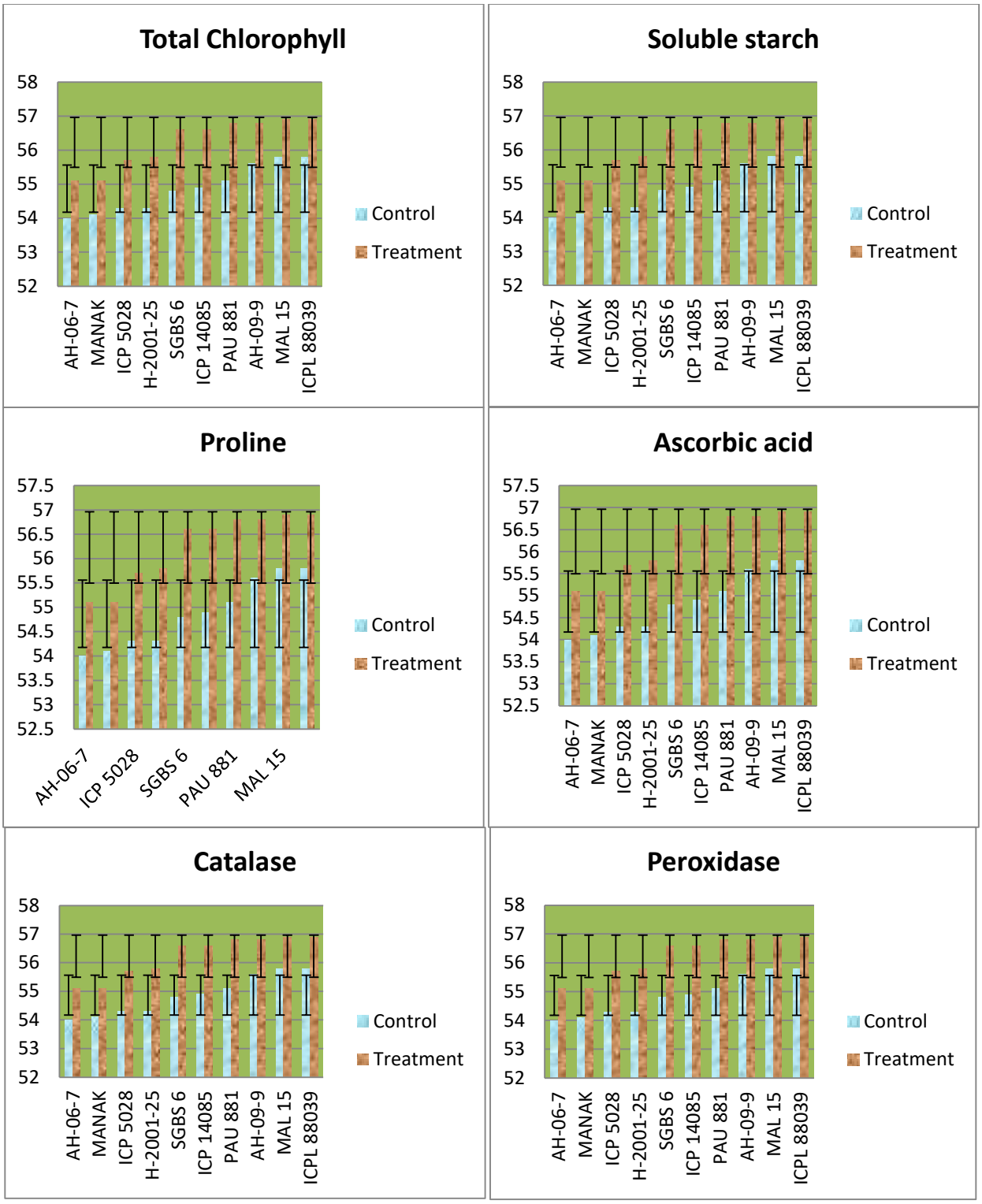

Figure 1: Tolerant and sensitive genotypes in controlled and stressed conditions – (a) Chlorophyll content (b) Soluble starch content (c) Proline content (d) Ascorbic acid (e) CAT activity (f) Peroxidase activity

CONCLUSION

On the basis of above mentioned findings following useful conclusions, both having fundamental and applied values, may be drawn. Overall growth, biochemical process and yield components of Pigeon pea crop were adversely affected under saline condition due to 50mM concentration of saline solution during crop growth and particularly grain development. Tolerant and sensitive genotypes among all were identified in controlled and stressed conditions in all growth parameters like Proline content, Electrolyte Leakage, TBARS, Chlorophyll content, MDA content, H_2O_2 content, Ascorbic acid, SOD activity, CAT activity and Peroxidase activity. In the present observation, six among the ten pigeon pea cultivars namely , AH-06-7, MANAK, ICP 5028, H-2001-25, SGBS 6 and ICP 14085 were observed as salinity tolerant varieties and remaining four i.e., PAU881, AH-06-9, MAL 15 and ICPL 88039 were identified as sensitive cultivars based on the physiological and biochemical response under salinity stress condition.

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