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Effect of simulated soil salinity conditions and varieties of pigeon pea (*Cajanus Cajan* L.) on biochemical parameters

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Abstract
To ascertain the impact of soil salinity on pigeon pea biochemical parameters, a pot experiment was carried out. The experiment includes five salinity levels (Control, 40, 60, 80 and 100 meq l^{-1}) and four pigeon pea varieties (V_1: GJP-1, V_2: Vaishali, V_3: BDN-2, V_4: AGT-2) in Completely Randomized Design (Factorial) replicated three times. According to biochemical characteristics (proline content) variety V_4 (AGT-2) had the maximum proline accumulation (0.980 mole/g f. wt), which rose with increasing salt levels. Variety V_4 (AGT-2) outperformed the other examined varieties in terms of RWC, chlorophyll a, chlorophyll b, and total chlorophyll content in pigeon pea leaves at 45 DAS. These metrics also declined as the saline level rose. So it can be concluded that variety V_4 (AGT-2) is salt-tolerant due to the highest concentration of proline, RWC, chlorophyll a, chlorophyll b and total chlorophyll content were obtained in it.

Keywords: Pigeon pea, salinity levels, bio-chemical parameter, proline and chlorophyll

Introduction

Pigeon pea (*Cajanus Cajan* L.) belongs to the genus: Cajanus, subtribe-Cajaninae, tribe-Phaseoleae, order-Fabales, family-Fabaceae and sub-family Faboideae. The term 'pigeon pea' was coined in Barbados, where its seeds were considered an important pigeon feed.

Pigeon pea is adapted to the tropical and subtropical region and can be grown on marginal land and low fertilizer input, even under drought conditions. The growth habit is predominantly indeterminate but some genotypes show determinate growth. The branching pattern varies from erect to spreading. Pigeon pea is a predominantly photoperiod sensitive short-day plant and exhibit wide variation in days to flower among genotypes.

At present about 20% of the worlds cultivated land and approximately half of all irrigated land are affected by salinity. Therefore, salinity is one of the most significant abiotic factors limiting crop productivity. This is attributed to the fact that Na^+ competes with K^+ for binding sites essential for cellular function. The latter implication of these two macronutrients in salinity is thought to be one of the factors responsible for the reduction of the biomass and yield components of plants. A High concentration of salt in the root zone (rhizosphere) reduces soil water potential and the availability of water. As a result of this, a reduction of the water content leads to dehydration at the cellular level and osmotic stress is observed.

Resources and Research methods

A pot experiment was conducted during *Kharif* - 2019-20 at the Department of Agricultural Chemistry and Soil Science, College of Agriculture, JAU, Junagadh.

Chlorophyll content in leaves at 45 DAS

A method for the extraction of chlorophyll from leaf tissue without maceration (DMSO by Hiscox and Israelstam, 1979) [5] which described below:

Take 0.1 g of fresh plant sample from 4^{th} developed leaves into the test tube without maceration and then add 5 ml DMSO (Dimethyl sulfoxide) in the test tube bearing fresh leaf tissue. The reaction mixture takes 24 hours to extract chlorophyll. Then, colour intensity was measured at 645 nm for chlorophyll a and 663 nm for chlorophyll b on the spectrophotometer.

$$\text{Chlorophyll a (mg/gf. wt)} = \frac{12.2\ x\ (A\ 663) - 2.69\ x\ (A\ 645) x\ V}{10}$$

$$\text{Chlorophyll b (mg/gf. wt)} = \frac{22.9\ x\ (A\ 645) - 4.68\ x\ (A\ 663) x\ V}{10}$$

$$\text{Total Chlorophyll (mg/gf. wt)} = \frac{20.2\ x\ (A\ 645) + 8.02\ x\ (A\ 663)\ x\ V}{10}$$

Where, A= Absorbance at specific wavelength
V = Volume of DMSO in ml.

Proline content in leaves at 45 DAS

Proline content was determined by the free proline method developed by Bates *et al.* (1973) through the following procedure:

The leaf tissue was ground in 3% sulfosalicylic acid and filtered with Whatman no. 2 filter paper. Five ml of filterate was recorded with 5 ml of ninhydrin (1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml of 6 M orthophosphoric acid) and 5 ml of glacial acetic acid in a test tube for one hour at 96 °C on the water bath. The reaction mixture was than extracted with 5 ml toluene and colour intensity was measured at 520 nm on the spectrophotometer.

$$\text{Proline (}\mu\text{ molar/gf.wt)} = \frac{\text{OD} \times \text{Toluene (ml)} \times 5}{\text{Sample weight (g)} \times 115.5 \times \text{aliquot (ml)}}$$

Where, OD = from conversion table
Mol. wt. of proline =115.5
Make up volume in ml. = 5

Relative water content in leaves at 45 DAS

Relative water content was determined by rapid estimates of relative water content method developed by Richard and Gail (1974), which described below:

A composite sample of fresh leaves was taken and the fresh weight is determined, followed by flotation on water for up to 4 hr. The turgid weight is then recorded, and the leaf tissue is subsequently oven-dried to a constant weight at about 85^0 C RWC is calculated by

$$\text{RWC (\%)} = \frac{\text{(Fresh weight} - \text{Dry weight)}}{\text{(Turgid weight} - \text{Dry weight)}}\ x\ 100$$

Fig 1: Experiment view at the stage of germination

Fig 2: Overall view of an experiment

Results

Effect of Salinity and Variety on bio-chemical parameters

Individual as well as the combined effect of salinity levels and varieties on biochemical parameters like chlorophyll a, chlorophyll b, total chlorophyll content, proline content and RWC at 45 DAS.

Different levels of salinity and varieties significantly affected biochemical parameters (Table 1). The proline content increased with increasing levels of salt concentration, thus the highest value of proline content was obtained in level S_5 (100 meq l^{-1}). The highest values of Relative water content, chlorophyll a, chlorophyll b and total chlorophyll were recorded in level S_1 (Control).

Significantly the highest proline content at 45 DAS was observed with variety V_4 (AGT-2). The maximum value of RWC, chlorophyll a, chlorophyll b and total chlorophyll were attained in V_4 (AGT-2). (Table 1)

The interaction effect of salinity levels and varieties significantly affected proline content, chlorophyll a, chlorophyll b and total chlorophyll (Table 2, 3, 4 and 5).

Table 1: Effect of salinity levels and varieties on biochemical parameters in leaves of pigeon pea at 45 DAS

Treatments	Proline (µmole/g f. wt)	RWC (%)	Chlorophyll a (mg/g f. wt)	Chlorophyll b (mg/g f. wt)	Total chlorophyll (mg/g f. wt)
Salt concentration(Salinity) (S)					
S_1: Control	0.75	54.36	16.32	10.32	26.89
S_2: 40 meq l^{-1}	0.86	49.44	15.30	9.30	24.85
S_3: 60 meq l^{-1}	0.87	43.13	14.67	8.67	23.57
S_4: 80 meq l^{-1}	0.92	42.38	13.66	7.66	21.55
S_5: 100 meq l^{-1}	0.98	40.69	13.11	7.11	20.46
S.E.M. ±	0.02	1.23	0.16	0.11	0.33
C.D. (P=0.05)	0.06	3.51	0.47	0.33	0.95
Variety (V)					
V_1: GJP-1	0.80	42.41	13.37	7.37	20.97
V_2: Vaishali	0.87	42.59	14.40	8.40	23.04
V_3:BDN-2	0.85	45.48	14.74	8.74	23.72
V_4: AGT-2	0.98	53.52	15.94	9.94	26.12
S.E.M. ±	0.02	1.1	0.15	0.10	0.30
C.D. (P=0.05)	0.05	3.14	0.42	0.29	0.85

S x V Interaction					
S.E.M. ±	0.04	2.46	0.33	0.23	0.66
C.D. (P=0.05)	0.11	NS	0.94	0.65	1.90
C.V. %	7.74	9.25	3.89	4.59	4.91

Table 2: Interaction effect of salinity levels and varieties on proline (µmole/g f. wt) content in leaves of pigeon pea at 45 DAS

	S_1: Control	S_2: 40 meq l^{-1}	S_3: 60 meq l^{-1}	S_4: 80 meq l^{-1}	S_5: 100 meq l^{-1}	Mean
V_1: GJP-1	0.71	0.79	0.80	0.81	0.87	0.80
V_2: Vaishali	0.74	0.87	0.87	0.90	0.98	0.87
V_3: BDN-2	0.79	0.84	0.86	0.87	0.88	0.85
V_4: AGT-2	0.74	0.94	0.94	1.09	1.18	0.98
Mean	0.75	0.86	0.87	0.92	0.98	
S.E.M. ±	0.04		C.D. (p = 0.05)		0.11	

Table 3: Interaction effect of salinity levels and varieties on chlorophyll a (mg/g f. wt) content in leaves of pigeon pea at 45 DAS

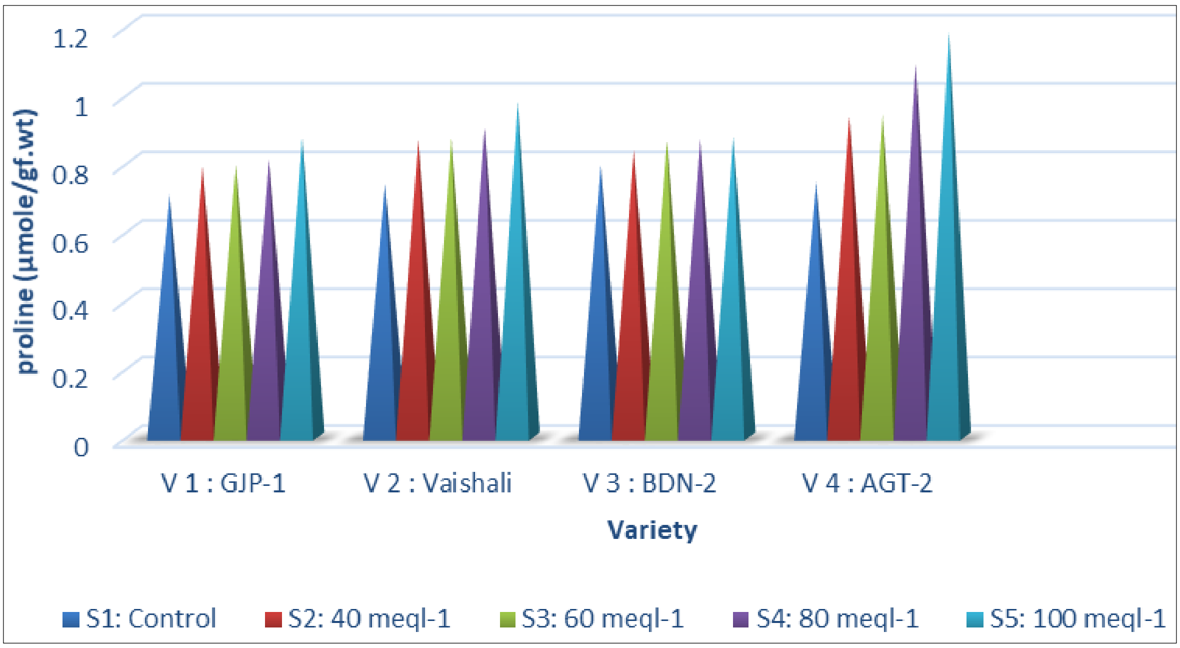
	S_1: Control	S_2: 40 meq l^{-1}	S_3: 60 meq l^{-1}	S_4: 80 meq l^{-1}	S_5: 100 meq l^{-1}	Mean
V_1: GJP-1	15.09	14.05	13.69	12.04	11.96	13.37
V_2: Vaishali	16.03	15.08	14.81	13.07	13.01	14.40
V_3: BDN-2	16.01	15.08	14.89	14.69	13.04	14.74
V_4: AGT-2	18.17	17.00	15.27	14.82	14.43	15.94
Mean	16.32	15.30	14.67	13.66	13.11	
S.E.M. ±	0.328		C.D. (p = 0.05)		0.937	

Table 4: Interaction effect of salinity levels and varieties on chlorophyll b (mg/g f.wt) content in leaves of pigeon pea at 45 DAS

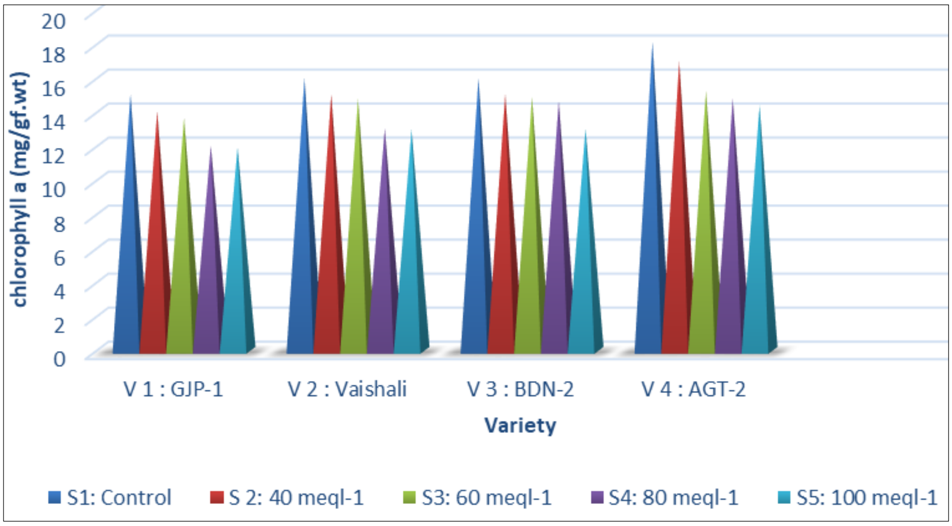
	S_1: Control	S_2: 40 meq l^{-1}	S_3: 60 meq l^{-1}	S_4: 80 meq l^{-1}	S_5: 100 meq l^{-1}	Mean
V_1: GJP-1	9.09	8.05	7.69	6.04	5.96	7.37
V_2: Vaishali	10.03	9.08	8.81	7.07	7.01	8.40
V_3: BDN-2	10.01	9.08	8.89	8.69	7.04	8.74
V_4: AGT-2	12.17	11.03	9.27	8.82	8.43	9.94
Mean	10.32	9.30	8.67	7.66	7.11	
S.E.M. ±	0.23		C.D. (p = 0.05)		0.65	

Table 5: Interaction effect of salinity levels and varieties on total chlorophyll (mg/g f.wt) content in leaves of pigeon pea at 45 DAS

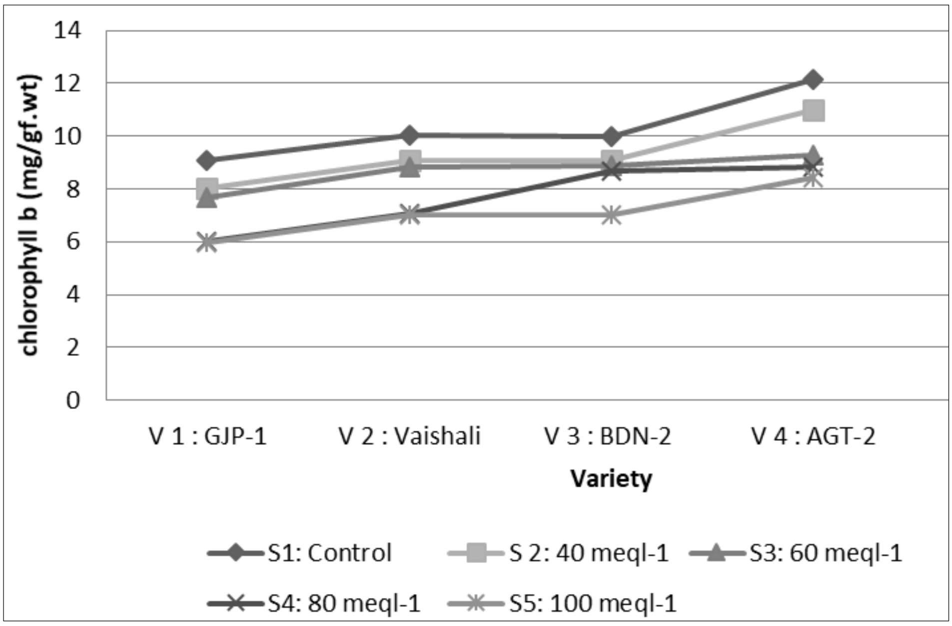
	S_1: Control	S_2: 40 meq l^{-1}	S_3: 60 meq l^{-1}	S_4: 80 meq l^{-1}	S_5: 100 meq l^{-1}	Mean
V_1: GJP-1	24.42	22.34	21.62	18.32	18.15	20.97
V_2: Vaishali	26.29	24.40	23.86	20.38	20.27	23.04
V_3: BDN-2	26.26	24.40	24.02	23.62	20.32	23.72
V_4: AGT-2	30.58	28.25	24.78	23.88	23.09	26.12
Mean	26.89	24.85	23.57	21.55	20.46	
S.E.M. ±	0.665		C.D. (p = 0.05)		1.9	



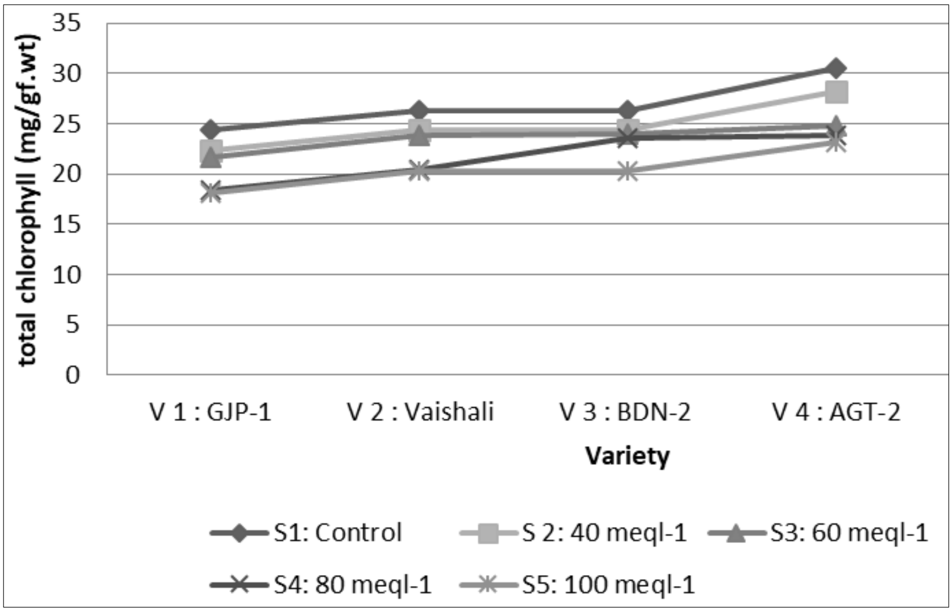
Graph 1: Effect of salinity levels and varieties on proline content in leaves of pigeon pea at 45 DAS



Graph 2: Effect of salinity levels and varieties on chlorophyll a content in leaves of pigeon pea at 45 DAS



Graph 3: Effect of salinity levels and varieties on chlorophyll b content in leaves of pigeon pea at 45 DAS



Graph 4: Effect of salinity levels and varieties on total chlorophyll content in leaves of pigeon pea at 45 DAS

Discussion

Reduction in proline with increasing salinity concentration was also reported by Turan *et al.* (2007) [9]. Proline accumulation in salt-stressed plants is a primary defense response to maintain the osmotic pressure in a cell. Turan *et al.* (2009) [9] reported that the present study shows that the salt treatments induced an increase in proline concentration. Higher proline was noticed at higher salinity levels coupled with higher Na^+ constitute the osmoregulation substances, which favours water uptake from saline medium, thereby enabling the plants to maintain their physiological activity. Proline is a bio-indicator of stress conditions, particularly regarding salinity or drought stresses reported by Kholova *et al.* (2010) [7]. Faster activation and high levels of proline accumulation are probable reasons for Flip 97-43c (T1) tolerance during stress conditions.

The negative effect on plant water relations was induced by an rise in soluble salts that decelerate the uptake of water and nutrients causing osmotic effects and toxicity (Yang *et al.*, 2009 [11] and Jiang *et al.*, 2014) [6].

Chlorophyll a concentrations were reduced with increasing salinity levels, the reason behind the reduction in chlorophyll concentration is probably due to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions reported by Ali *et al.*, (2005) [1]. The reduction could be due to overall mineral deficiency, leading to the inhibited synthesis of chlorophyll coupled to rapid chlorophyll degradation (Amuthavalli and Sivasankaramoorthy, 2012) [2]. Similarly, study done by revealed that chlorophyll a contents decreased with increasing salt stress. The differential response observed in tolerant and susceptible genotypes in terms of chlorophyll content could be due to the presence of mechanisms, which prevented chlorophyll degradation in tolerant genotypes than in susceptible ones reported by Chakraborty *et al.* (2012) [4]. Also reported that chlorophyll a reduced with increasing salt stress. The reduction could be due to overall mineral deficiency, leading to the inhibited synthesis of chlorophyll coupled to rapid chlorophyll a degradation (Amuthavalli and Sivasankaramoorthy, 2012) [2]. Tayyab and Ahmed (2016) showed chlorophyll a (Chl a) to be more sensitive to salt stress compared to chlorophyll b (Chl b) in pigeon pea.

The differential response determined in tolerant and susceptible genotypes in terms of chlorophyll content could be due to presence of mechanisms that prevented chlorophyll degradation in tolerant genotypes than susceptible ones reported by Chakraborty *et al.* (2012) [4]. Literature available pointed out more chlorophyll and carotenoid contents in wheat under control conditions than compared salt-stressed plants reported by Sairam *et al.* (2005) [12].

Conclusion

Salinity is a complex situation and a single factor cannot indicate the salt tolerance of the crop. It was observed that the relative water content, chlorophyll a, chlorophyll b and total chlorophyll decreased with increasing salinity levels of irrigation water, while proline increased with increasing salinity levels. A high concentration of proline may provide better performance under saline conditions, which was found highest in AGT-2 variety. AGT-2 also had highest relative water content, chlorophyll a, chlorophyll b and total chlorophyll. Thus, pigeon pea variety AGT-2 showed more salt tolerance compared to other varieties.

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