



## Research Article

# IMPACT OF WATER DEFICIT STRESS ON BIOCHEMICAL CHARACTERISTICS OF PIGEONPEA CULTIVAR

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**Abstract-** A field experiment was conducted using split plot design with the four irrigation level and the biochemical traits like chlorophyll content, carotenoid, protein, free amino acid, proline and glycine betaine were determined. The biochemical parameters like chlorophyll content, carotenoids content and protein significantly decreased under the water stress condition. Significantly highest accumulation of other biochemical parameters such as glycine-betaine, proline and amino acid increased under the stress condition compared to irrigation condition. Among all the six genotypes, GT-102 showed higher seed yield which was followed by C-11 due to accumulation of glycine- betaine and proline was higher under the water stress condition. Thus it enabled the genotype to thrive better in the water stress condition and produce higher yield under water deficient.

**Keywords-** Pigeonpea, Cultivar, Water Stress, Fresh weight, Biochemical

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## Introduction

Pigeonpea (*Cajanus cajan*(L.) Millsp.) is one of the major grain legume (pulse) crops of the tropics and subtropics. The Indian subcontinent, accounts for about 90% of the global production. Drought is one of the major constraint which decreases the productivity of pigeonpea. These variations for maturity have direct relevance on the survival and fitness of the crop in different agro-ecological niches (Choudhary[1]). Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savoure [2]). Occurrence of quaternary ammonium compound glycine betaine (GB) in response to oxidative stress like salinity is a common phenomenon. Drought stress has profound effects on the GB accumulation in *Cajanus cajan*. Accordingly, the present investigation was carried out to find out the effect of water stress on the biochemical characteristics of the pigeonpea cultivars and its yield with four irrigation level.

## Materials and Methods

The study was carried out on "Effect of water stress on, biochemical character and yield of pigeonpea (*Cajanus cajan* L. Millsp.) under *rabi* season", 2013-2014 at College Farm, N. M. College of Agriculture, Navsari Agricultural University, Navsari. The weather during the growing season was normal and favourable for crop growth. The seed of six varieties of pigeon pea i.e. GT-102, Bharboot local, GNP – 304, GT-1, AGT-2, C-11 were studied under four different irrigation levels (I<sub>1</sub>- all irrigation given at 25, 50 and 75 DAS, I<sub>2</sub>- two irrigation given 25 and 50 DAS, I<sub>3</sub>- one irrigation given 25 DAS and I<sub>4</sub>- Rainfed).

## Chlorophyll content

The chlorophyll pigments viz., chlorophyll 'a', chlorophyll 'b' and total chlorophyll content in leaf were determined by dimethyl sulfoxide method (DMSO) of Hiscox

and Israelstam [3]. Top fully expanded leaf was brought in polyethylene bags kept in ice box from the field and was cut into small pieces. Known weight of leaves (100 mg) was kept in test tubes containing 7.0 ml of dimethyl sulfoxide (DMSO). The test tube incubated at 65°C for 30 minutes. Leaf residue was removed by decanting the solution and final volume was made to 10 ml with DMSO. The absorbance of the extract was measured at 645, 652 and 663 nm by spectrophotometer (Model No: UV-1800, Shimadzu, UV spectrophotometer) and a blank was run using DMSO. The chlorophyll content is calculated using the formula:

$$\text{Chlorophyll 'a'} = \frac{12.7 (A663) - 2.69 (A645) \times V}{1000 \times w \times a} \quad (\text{mg/g fr. wt.})$$
$$\text{Chlorophyll 'b'} = \frac{22.9 (A645) - 4.68 (A663) \times V}{1000 \times w \times a} \quad (\text{mg/g fr. wt.})$$

Total Chlorophyll = Chlorophyll 'a' + Chlorophyll 'b' (mg/g fr. wt.)

Where,

A645 = Absorbance of the extract at 645 nm

A663 = Absorbance of the extract at 663 nm

a = Path length of Cuvette (1 cm)

V = Final volume of the chlorophyll extract (10 ml)

w = Fresh weight of the sample (0.10 g)

## Proline

Free proline content was estimated by following the method of Bates *et al.* [4]. A known weight (0.5 g) of fresh leaf sample was macerated in a mortar using 10 ml of 3 percent sulphosalicylic acid. The extract was filtered and 2.0 ml of the filtrate

was used for proline estimation. To this 2.0 ml of filtrate, 2.0 ml of acid ninhydrin reagent (2.5 g of ninhydrin dissolved in 40 ml of 6.0 M orthophosphoric acid and 60 ml of glacial acetic acid), 2.0 ml of glacial acetic acid were added and placed in boiling water bath for one hour. Following this, test tubes containing the samples were transferred to an ice bath for cooling. The contents of each test tube were transferred to a separatory funnel and 6.0 ml of toluene was added, shaken thoroughly and allowed for few minutes for separation of two layers. The lower layer was discarded and the upper toluene layer containing the colour complex was taken into a test tube. The optical density was read at 520 nm using spectrophotometer (Model No: UV-1800, Shimadzu, UV spectrophotometer) and the proline content was calculated as follows. where, OD = Optical density at 520 nm V = Total volume of the extract in ml d = Fresh weight / dry weight ratio f = Fresh weight taken for proline estimation (mg) 2 = Volume of extract taken for proline estimation.

$$\text{Proline content } (\mu\text{g} / \text{gdryweight}) = 36.2311 \times \text{OD} \times V \times d / 2 \times f$$

### Glycine betaine content

Estimation of glycine betaine was done by following the method of Grieve and Grattam [5]. Leaf tissues (2.0g) were finely- ground using liquid nitrogen in mortar-pestle. A finely ground plant sample 0.5g of each genotype was mechanically shaken with 20 ml of de-ionized H<sub>2</sub>O for 24 hours at 25°C. The samples were then filtered. The extracts were diluted 1:1 with 2N H<sub>2</sub>SO<sub>4</sub>. Aliquots (0.50 ml) were measured into 2.0 ml eppendorf tubes and cooled in ice-water for 1 hr. Cold KI- I<sub>2</sub> reagent (0.20 ml) was added to each tube and reactants gently stirred on a vortex mixer. The tubes were stored at 0-4°C for 16 hrs and then centrifuged at 10000 rpm for 15 mins at 0°C. The supernatants were carefully aspirated. The per-iodide crystals were dissolved in 9.0 ml of 1, 2- dichloroethane. Vigorous vortex mixing was frequently required to effect complete solubilisation in the developing solvent. After 2-2.5 hrs, the absorbance was measured at 365 nm on a Spectrophotometer (UV- 1800). Reference standard of GB (50-200 microgram/ml) were prepared in 1N H<sub>2</sub>SO<sub>4</sub>. Standard curve were prepared and the GB content of sample calculated.

$$\text{Glycine betaine} = \text{Sample O.D.} \times \text{Graph factor} \times \text{Dilution factor}$$

### Protein

Protein concentration of each extract of seed protein was estimated by method of Lowry *et al.*, [6]. Protein extract (25 µl) were taken in test tube and volume was made upto 1 ml with millipore water. A tube with 1 ml of water served as blank. Five ml of solution C was mixed by vortexing and kept for 10 min. Then 0.5 ml of solution D (Folin & Ciocalteu reagent) was mixed with vortex and kept it room temperature for 30 min. Absorbance was read at 660 nm. A standard curve was prepared with bovine serum albumin in the range of 10-80 µg.

### Amino acid

The amino acid in the free pool of plant cells or protein hydrolysate can be determined by using ninhydrin (Lee and Takahashi, [7]). Supernatant (0.4 ml) was taken from the ethanol extract prepared for total soluble sugar content and 5 ml of ninhydrin reagent (5:12:2; 1% ninhydrin in 0.5 M citrate buffer pH 5.5: Glycerol: 0.5M Citrate buffer pH 5.5) was added followed by vigorous shaking. The tubes were placed in boiling water bath for 12 minutes and brought to room temperature under running water. The absorbance was read at 570 nm against blank prepared by adding 0.4 ml 80% ethanol in place of extract. Standard curve was prepared by glycine in the range of 0-80 µg concentration.

$$\text{Total free amino acids (mg/g)} = \text{Sample O.D.} \times \text{Standard O.D.} \times \text{Dilution Factor}$$

### Result

The experimental results of the water stress on the biochemical parameters are resulted below of the six different pigeon genotypes. The accumulation of the chlorophyll, glycine betaine, carotenoid, proline, protein and amino acids were recorded at different days after sowing of the pigeon pea genotypes.

### Chlorophyll [Table-1]

There were significant results observed in the chlorophyll content among the six pigeon pea genotypes in the given irrigation conditions. At 50 DAS it was observed that the mean chlorophyll content was found maximum in the genotype GT-1 (3.03 mg g<sup>-1</sup>FW) while the minimum mean chlorophyll content observed was in the genotype Bharboot local (2.90 mg g<sup>-1</sup> FW). The irrigation in which the chlorophyll content was found highest was I<sub>0</sub> condition (3.73 mg g<sup>-1</sup>FW) while the minimum was recorded in the irrigation condition I<sub>3</sub> (2.53 mg g<sup>-1</sup>FW). At 75 DAS it was found that I × V interaction remained significant. It was noted that the irrigation I<sub>0</sub> was at par with the irrigation I<sub>1</sub> while in the interaction the treatment I<sub>0</sub>G<sub>2</sub> remained at par with I<sub>0</sub>G<sub>4</sub>. At 75 DAS it was observed that the chlorophyll content decreased as the stress increased. It was resulted that the mean chlorophyll content was found maximum in the genotype AGT-2 (1.90 mg g<sup>-1</sup>FW) while the minimum chlorophyll content was found in the genotype C-11 (1.88 mg g<sup>-1</sup>FW). The mean chlorophyll content in the irrigation was recorded highest in the irrigation level I<sub>0</sub> (1.95 mg g<sup>-1</sup>FW) and the least chlorophyll content was found in the irrigation level I<sub>3</sub> (1.86 mg g<sup>-1</sup>FW). The irrigation was found to be significant while the genotype were found to be non- significant. The interaction of the genotypes and the irrigation was found to be significant. Statistically the irrigation I<sub>0</sub> remained at par with irrigation I<sub>1</sub> while the treatment combination I<sub>0</sub>G<sub>4</sub> and I<sub>0</sub>G<sub>5</sub> remained at par with the treatment I<sub>0</sub>G<sub>1</sub>.

### Carotenoid content [Table-1]

The six genotypes of pigeon pea found significant differences in carotenoids content. The variety GT-1(2.30 mg<sup>-1</sup>FW) showed highest carotenoids content. The minimum carotenoids content was found in the variety C-11(1.90 mg<sup>-1</sup> FW). The carotenoid content significantly declined due to water stress at 50 DAS (I<sub>2</sub> and I<sub>3</sub>). The variety GT-1 recorded highest carotenoid content due to water stress at 50 DAS which was followed by GT-102. Among different irrigation intervals, under I<sub>0</sub> (2.17 mg<sup>-1</sup> FW) showed significantly highest carotenoid content as compared to other water stress conditions which was statistically at par with I<sub>1</sub>(2.17 mg<sup>-1</sup>FW) treatment. The lowest value of carotenoids was observed under I<sub>3</sub> (1.98 mg<sup>-1</sup> FW) treatment at 50 DAS. The interaction of these varieties with the water stress levels on carotenoids was found to be non- significant. The carotenoids content again reduced at 75 DAS. The highest carotenoids content was observed in the variety GT-1(1.98 mg<sup>-1</sup>FW) and the minimum carotenoids content was found in the variety C-11 (1.40 mg<sup>-1</sup>FW). The lowest carotenoids content was noted in the irrigation level I<sub>3</sub> (1.40 mg<sup>-1</sup>FW) while the maximum of the carotenoids content was observed in I<sub>0</sub> (1.68 mg<sup>-1</sup>FW). The interaction effect was found to be non significant for carotenoids content.

### Proline content [Table-2]

Under irrigated conditions at 50 DAS the mean proline content was 1.71(mg g<sup>-1</sup> FW) at I<sub>0</sub> level, 1.78 (mg g<sup>-1</sup>FW) at I<sub>1</sub> irrigation, 3.59 (mg g<sup>-1</sup>FW) at I<sub>2</sub> irrigation and 3.63 (mg g<sup>-1</sup> FW) at I<sub>3</sub> irrigation. The maximum of the proline content was observed in the irrigation level I<sub>3</sub> 3.63 (mg g<sup>-1</sup>FW) at 50 DAS which was followed by the irrigation level I<sub>2</sub> (3.59 mg g<sup>-1</sup>FW), while the minimum proline content was observed in the irrigation level I<sub>0</sub> 1.71 (mg g<sup>-1</sup>FW).The genotype GT-1 proved the best at 50 DAS with the highest proline accumulation 2.88 (mg g<sup>-1</sup>FW) which was followed by the genotype C-11 2.87 (mg g<sup>-1</sup>FW). The interaction of the irrigation and the genotype was found significant at 50 DAS. The maximum proline accumulation was found in I<sub>3</sub>G<sub>4</sub> while the minimum was found in the I<sub>0</sub>G<sub>3</sub>. Under the irrigation condition at 75 DAS the mean proline content was observed different at different irrigation levels. At 75 DAS the irrigation condition I<sub>0</sub> was found with least proline accumulation 2.70 (mg g<sup>-1</sup> FW) which resulted better while the maximum proline accumulation was observed at the irrigation level I<sub>3</sub> 5.63 (mg g<sup>-1</sup>FW). The genotype AGT-2 and C-11 showed the highest proline accumulation 5.16 (mg g<sup>-1</sup>FW). The interaction of irrigation and the variety was found significant with maximum proline accumulation in the I<sub>3</sub>G<sub>5</sub> and I<sub>3</sub>G<sub>6</sub> while the minimum proline accumulation was observed in I<sub>0</sub>G<sub>3</sub>.

### Glycine betaine [Table-2]

The accumulation of the glycine betaine increased with the increase in the stress.

**Table-1** Effect of irrigation intervals on Chlorophyll content (mg g<sup>-1</sup>FW) and carotenoid content (mg<sup>-1</sup>FW) at 50, and 75 DAS of six pigeonpea varieties

Genotypes	Chlorophyll content (mg g <sup>-1</sup> FW)										Carotenoid (mg <sup>-1</sup> FW)									
	50 DAS					75 DAS					50 DAS					75 DAS				
	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	Mean	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	Mean	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	Mean	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	Mean
GT-102	2.37	2.87	2.90	3.67	<b>2.95</b>	1.84	1.87	1.88	1.96	<b>1.89</b>	2.33	2.22	2.11	2.05	<b>2.18</b>	1.83	1.72	1.61	1.55	<b>1.68</b>
Bharboot local	2.23	2.63	2.80	3.93	<b>2.90</b>	1.87	1.88	1.86	1.95	<b>1.89</b>	2.03	2.03	1.93	1.95	<b>1.98</b>	1.53	1.53	1.43	1.45	<b>1.49</b>
GNP-304	2.60	2.77	2.67	3.83	<b>2.97</b>	1.85	1.87	1.87	1.95	<b>1.89</b>	2.10	2.17	1.97	1.87	<b>2.03</b>	1.60	1.67	1.47	1.37	<b>1.53</b>
GT-1	2.63	2.83	2.80	3.87	<b>3.03</b>	1.84	1.87	1.88	1.97	<b>1.89</b>	2.44	2.51	2.13	2.11	<b>2.30</b>	2.01	1.94	1.63	1.61	<b>1.80</b>
AGT-2	2.60	2.83	2.80	3.60	<b>2.96</b>	1.87	1.87	1.87	1.97	<b>1.90</b>	2.19	2.19	2.09	2.01	<b>2.12</b>	1.69	1.69	1.59	1.48	<b>1.62</b>
C-11	2.77	2.63	2.90	3.50	<b>2.95</b>	1.86	1.88	1.89	1.90	<b>1.88</b>	1.94	1.93	1.83	1.90	<b>1.90</b>	1.44	1.43	1.40	1.33	<b>1.40</b>
<b>Mean</b>	<b>2.53</b>	<b>2.76</b>	<b>2.81</b>	<b>3.73</b>	2.96	<b>1.86</b>	<b>1.87</b>	<b>1.88</b>	<b>1.95</b>	4.72	<b>2.17</b>	<b>2.17</b>	<b>2.01</b>	<b>1.98</b>	2.09	<b>1.68</b>	<b>1.66</b>	<b>1.52</b>	<b>1.47</b>	4.72
	I	G	I x G			I	G	I x G			I	G	I x G			I	G	I x G		
<b>S.E.m±</b>	0.03	0.03	0.06			0.03	0.03	0.07			0.02	0.03	0.06			0.02	0.02	0.05		
<b>C.D.@ 5 %</b>	0.09	NS	0.18			0.10	0.10	0.19			0.08	0.08	NS			0.07	0.07	NS		
<b>C.V. %</b>	3.87	3.63				2.62	2.47				4.95	4.59				5.69	5.38			

**Table-2** Effect of irrigation intervals on Proline (mg g<sup>-1</sup> FW) and Glycine betaine (µg g<sup>-1</sup> DW) at 50, and 75 DAS of six pigeonpea varieties

Genotypes	Proline (mg g <sup>-1</sup> FW)										Glycine betaine (µg g <sup>-1</sup> DW)									
	50 DAS					75 DAS					50 DAS					75 DAS				
	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	Mean	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	Mean	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	Mean	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	Mean
GT-102	1.91	1.90	3.78	3.72	<b>2.83</b>	2.78	5.55	5.88	5.97	<b>5.04</b>	0.857	0.873	1.873	1.887	<b>1.373</b>	0.873	1.873	2.627	2.657	<b>2.008</b>
Bharboot local	1.46	1.48	3.37	3.56	<b>2.47</b>	2.37	4.88	4.87	4.96	<b>4.27</b>	0.823	0.843	1.760	1.773	<b>1.300</b>	0.843	1.760	2.657	2.627	<b>1.972</b>
GNP-304	1.21	1.75	2.84	2.88	<b>2.17</b>	2.44	3.02	3.90	4.95	<b>3.58</b>	0.827	0.813	1.760	1.807	<b>1.302</b>	0.813	1.760	2.663	2.690	<b>1.982</b>
GT-1	1.96	1.87	3.83	3.87	<b>2.88</b>	2.83	5.90	5.84	5.95	<b>5.13</b>	0.847	0.830	1.863	1.880	<b>1.355</b>	0.830	1.863	2.653	2.630	<b>1.994</b>
AGT-2	1.88	1.82	3.87	3.85	<b>2.86</b>	2.88	5.87	5.91	5.98	<b>5.16</b>	0.847	0.860	1.870	1.837	<b>1.353</b>	0.860	1.870	2.657	2.740	<b>2.032</b>
C-11	1.86	1.85	3.87	3.88	<b>2.87</b>	2.87	5.85	5.94	5.98	<b>5.16</b>	0.860	0.850	1.860	1.860	<b>1.358</b>	0.850	1.860	2.667	2.700	<b>2.019</b>
<b>Mean</b>	<b>1.71</b>	<b>1.78</b>	<b>3.59</b>	<b>3.63</b>	2.68	<b>1.86</b>	<b>1.87</b>	<b>1.88</b>	<b>1.95</b>	4.72	<b>0.843</b>	<b>0.845</b>	<b>1.831</b>	<b>1.841</b>	1.340	<b>0.845</b>	<b>1.831</b>	<b>2.654</b>	<b>2.674</b>	2.001
	I	G	I x G			I	G	I x G			I	G	I x G			I	G	I x G		
<b>S.E.m±</b>	0.01	0.02	0.04			0.03	0.03	0.07			0.0059	0.006	0.0126			0.006	0.005	0.0106		
<b>C.D.@ 5 %</b>	0.05	0.05	0.10			0.10	0.10	0.19			0.0204	0.018	0.036			0.022	0.015	0.0303		
<b>C.V. %</b>	2.36	2.31				2.62	2.47				1.86	1.63				1.37	0.92			

The maximum accumulation of the glycine betaine was found in the I<sub>3</sub> stress condition at both 50 and 75 DAS and the minimum was observed in the I<sub>0</sub> condition. The pigeon pea genotypes at 50 DAS ranged from 1.300 ( $\mu\text{g g}^{-1}$  DW) to 1.373 ( $\mu\text{g g}^{-1}$  DW). Statistically it was observed that the genotype GT-102 1.373 ( $\mu\text{g g}^{-1}$  DW) remained at par with GT-1 1.355 ( $\mu\text{g g}^{-1}$  DW). The maximum glycine betaine content at 50 DAS was observed in the genotype GT-1 1.355 ( $\mu\text{g g}^{-1}$  DW), while the least glycine betaine content was observed in the genotype Bharboot local 1.300 ( $\mu\text{g g}^{-1}$  DW). The accumulation of glycine betaine was found maximum in I<sub>3</sub> 1.841 ( $\mu\text{g g}^{-1}$  DW) while the minimum glycine betaine content was observed in I<sub>0</sub> (0.843  $\mu\text{g g}^{-1}$  DW). It was resulted that the treatment I<sub>3</sub> remained at par with the I<sub>2</sub>. Also the interaction of the irrigation and the genotype was found to be significant. Here the interaction I<sub>3</sub>G<sub>2</sub> was at par with the I<sub>1</sub>G<sub>6</sub>. The glycine betaine content at 75 DAS also increased as the stress condition increased. The maximum glycine betaine content was observed in the genotype AGT-2 2.032 ( $\mu\text{g g}^{-1}$  DW) while the minimum of the glycine betaine content was observed in the genotype Bharboot local 1.972 ( $\mu\text{g g}^{-1}$  DW). Statistically the significant result was observed in the genotypes. The genotype AGT-2 2.032 ( $\mu\text{g g}^{-1}$  DW) remained at par with the genotype C-11 with 2.019 ( $\mu\text{g g}^{-1}$  DW) content. The glycine betaine content was found maximum in the irrigation level I<sub>3</sub> 2.674 ( $\mu\text{g g}^{-1}$  DW), while the minimum of the glycine betaine content was observed in the irrigation I<sub>0</sub> 0.845 ( $\mu\text{g g}^{-1}$  DW). The irrigation condition given was found to be significant where I<sub>3</sub> was found at par with I<sub>2</sub> condition. The results also showed that the significant results were obtained in the interaction of the irrigation and the genotypes. The irrigation and genotype interaction I<sub>3</sub>G<sub>5</sub> 2.740 ( $\mu\text{g g}^{-1}$  DW) was at par with I<sub>2</sub>G<sub>6</sub> 2.667 ( $\mu\text{g g}^{-1}$  DW).

### Protein content [Table-3]

Under I<sub>3</sub> irrigation condition the protein content significantly decreased over all the given irrigated condition. Under irrigated condition, the mean protein content in was I<sub>0</sub> 0.018 (mg g<sup>-1</sup> FW), I<sub>1</sub> 0.016 (mg g<sup>-1</sup> FW), I<sub>2</sub> 0.016 (mg g<sup>-1</sup> FW) and I<sub>3</sub> 0.015 (mg g<sup>-1</sup> FW) which shows that I<sub>0</sub> irrigation had the maximum protein content and I<sub>3</sub> had the minimum protein content. The maximum protein content was recorded in GT-102 0.017 (mg g<sup>-1</sup> FW) and also the genotype C-11 0.017 (mg g<sup>-1</sup> FW). The minimum protein content was observed in the genotype Bharboot local, GNP-304, GT-1 and AGT-2 0.016 (mg g<sup>-1</sup> FW) respectively. The irrigation was found to be significant, where I<sub>0</sub> remained significantly at par with I<sub>1</sub>. And the genotypes were also found to be significant where genotype GT-102 remained significantly at par with C-11. Similarly the irrigation genotype interaction was also found significant where the interaction I<sub>0</sub>G<sub>1</sub> was significantly at par with I<sub>0</sub>G<sub>2</sub>, I<sub>0</sub>G<sub>4</sub> and I<sub>0</sub>G<sub>6</sub>.

### Free Amino acid [Table-3]

The amino acid content was found to be significantly increased with the increasing stress. The mean amino acid content found maximum with the treatment I<sub>3</sub> 2.864 (mg g<sup>-1</sup> FW), while the minimum amino acid content was noted in the irrigation level I<sub>0</sub> 2.576 (mg g<sup>-1</sup> FW). The genotype which stood better in the given water stress condition was C-11 2.718 (mg g<sup>-1</sup> FW). The minimum amino acid content was observed in the genotype GT-1 2.708 (mg g<sup>-1</sup> FW). The irrigation I<sub>3</sub> remained at par with the irrigation I<sub>2</sub>. And also the interaction of genotype and the irrigation was observed significant. It was observed that the interaction I<sub>3</sub>G<sub>1</sub> 2.890 (mg g<sup>-1</sup> FW) was at par with I<sub>3</sub>G<sub>2</sub> 2.857 (mg g<sup>-1</sup> FW).

**Table-3** Effect of irrigation intervals on Protein and Free Amino acid (mg g<sup>-1</sup> FW) at 50, and 75 DAS of six pigeonpea varieties

Genotypes	Protein					Free Amino acid (mg g <sup>-1</sup> FW)				
	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	Mean	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	Mean
GT-102	0.016	0.018	0.015	0.018	0.017	2.570	2.667	2.740	2.890	2.717
Bharboot local	0.016	0.016	0.016	0.018	0.016	2.550	2.677	2.757	2.857	2.710
GNP-304	0.016	0.016	0.016	0.017	0.016	2.580	2.653	2.757	2.860	2.713
GT-1	0.015	0.016	0.015	0.018	0.016	2.633	2.653	2.710	2.837	2.708
AGT-2	0.015	0.017	0.016	0.017	0.016	2.560	2.667	2.767	2.863	2.714
C-11	0.015	0.018	0.017	0.016	0.017	2.563	2.680	2.753	2.877	2.718
Mean	0.015	0.016	0.016	0.018	0.016	2.576	2.666	2.747	2.864	2.713
	I	G	I x G			I	G	I x G		
S.E.m <sub>+</sub>	0.0001	0.0001	0.0002			0.007	0.0077	0.0153		
C.D.@ 5%	0.0003	0.0003	0.0006			0.0242	NS	0.0437		
C.V. %	1.7	1.65				1.08	0.98			

### Yield per plant (g/plant) [Table-4]

The maximum seed yield per plant was observed in the genotype GT-102 with the yield (46g/plant) and the minimum was observed in the genotype GNP-304 (33.20 g/plant). The irrigation in which the seed yield found highest was I<sub>0</sub> with the mean seed yield (40.16 g/plant) and the lowest yield in the irrigation I<sub>3</sub> with the mean seed yield of (36.03 g/plant). Statistically the result of the irrigation, genotype and the treatments combination was found to be significant. In the irrigation, the I<sub>0</sub> irrigation remained at par with I<sub>1</sub> irrigation. Also the genotype GT-102 was found at par with C-11 and the interaction I<sub>0</sub>G<sub>1</sub> was found at par with I<sub>1</sub>G<sub>1</sub>.

**Table-4** Effect of irrigation intervals on Seed yield (g/plant) of six pigeon pea varieties

Genotypes	Seed yield (g/plant)				
	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	Mean
GT-102	47.40	45.91	45.68	45.01	<b>46.000</b>
Bharboot local	36.68	34.89	34.86	34.57	<b>35.249</b>
GNP-304	35.17	34.35	33.60	30.04	<b>33.290</b>
GT-1	38.36	37.96	37.25	36.10	<b>37.417</b>
AGT-2	40.44	38.66	34.39	28.59	<b>35.521</b>
C-11	42.59	43.09	41.97	41.95	<b>42.399</b>
Mean	<b>40.106</b>	<b>39.144</b>	<b>37.959</b>	<b>36.043</b>	38.313
	I	G	I x G		
S.E.m <sub>+</sub>	0.79	0.65	1.29		
C.D.@ 5%	2.72	1.84	3.69		
C.V. %	8.70	5.83			

### Discussion

There was a significant result observed in the chlorophyll content and carotenoid content among the six pigeon pea genotypes in the given irrigation condition. The data of the chlorophyll content and carotenoids was recorded at 50 DAS and 75 DAS, which reduced significantly in the stress condition. The results were homogenous to the findings of Mafakheri *et al* [8]. The proline content significantly increased with the increase in the stress as compared to the irrigation conditions. The results were found homologous with Hoekstra and Buitinik [9]. It was indicated significant differences in glycine betaine content amongst the six pigeon pea genotypes. The data also indicated that glycine betaine content significantly increased due to stress. The glycine betaine content was recorded at 50 DAS and 75 DAS. The accumulation of the glycine betaine increased with the increase in the stress. The results are homologous to the findings of Jaleel *et al* [10] Drought stress has profound effects on the GB accumulation in *Cajanus cajan*. The protein content decreased with the increase in the stress as compared to the irrigation conditions. The results were similar as reported by Jaleel *et al* [10]. Free amino acid accumulation is more important account for most of the changes in osmotic potential. The accumulations of free amino acids under stress at all the growth stages indicate the possibility of their involvement in osmotic adjustment.

### Conflict of Interest: None declared

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