

# Growth Responses and Changes in Protein and Nucleic Acids of *Cajanus cajan* L. under Normal, Excess and Deficient Water Supply

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**Abstract** – In order to compare growth responses and biochemical changes under different water regimes, *Cajanus cajan* plants were grown under normal conditions of day and night. Selected growth parameters were plant height, branch number, leaf number, leaf area and stem diameter. Water potential of leaves at different stages was also determined. Protein content, protease activity, RNA and DNA were estimated among biochemical changes to observe the variation by changing the water supply. These plants belonged to one of the three categories viz. those getting water supply (1) twice, (2) five times and (3) not even once during 120-day of growth. Lesser supply of water resulted marked fall in water potential values whereas excess supply was responsible for relatively higher values at various stages of leaf growth. Marked variations were noticed in height, branch number per plant, number of leaves, leaf area and stem diameter. Among growth responses, excess supply of water did not cause marked change while deficient availability of water drastically affected chosen parameters. Both excess and deficient water supply brought down the amount of protein, RNA and DNA in comparison to plants getting normal supply. Water stress has severely affected growth processes and metabolism of pigeon pea plants.

**Keywords** – Branches, DNA, Leaf Area, Leaf Number, Plant Height, Protease Activity, Protein, RNA, Water Supply.

## I. INTRODUCTION

All living cells contain water. It is the most abundant molecular species in actively growing cells. Leaf and petal cells contain as much as 80-90 percent water. However, deviation from the normal supply of water has adverse effect on all aspects of growth and metabolism [1]–[3]. Water stress and other abiotic stresses affect growth and various plant processes including photosynthesis, respiration and nitrogen metabolism [4]. Flooding is another kind of water stress that has also received adequate attention of various workers [5]. Major progress has been made to characterize stress – related promoters, transcription factors and signaling components [6]. But, seldom we come across studies where growth performance and biochemical changes have been compared by supplying different amount of water. Attempt was made, therefore, to find out how pigeon pea plants would perform under three distinct conditions of water supply viz. normal, excess (not flooding) and deficient. Growth parameters and changes in protein and nucleic acids were selected for comparison under these conditions.

## II. MATERIALS AND METHODS

### A. Plant material and growth conditions

Certified pigeon pea seeds (*Cajanus cajan* L. cv. UPAS-120) obtained from C.C.S. Haryana Agricultural University, Hisar, India were used for growing plants in the experimental beds within wire cage maintained in the Botanical garden of Botany Department, Kurukshetra University, Kurukshetra under natural condition of day and night. Nine experimental plots were prepared, each one measuring 2x2 m<sup>2</sup>. Each experimental plot was provided with 40 kg of dung manure, mixed properly to maintain uniformity. Selected crop required proper irrigation only twice for satisfactory performance. On the basis of irrigation, 3 plots each of three sets were named as follows:

- (1) Normal water (NW) – Water was provided twice to these experimental beds during 120-day of plant growth.
- (2) Above normal water (AbN) – Excess water was provided to these plants as irrigation was carried out 5 times during 120-day growth period.
- (3) Deficit water (DW) – Experimental plot did not receive irrigation during 120-day of plant growth.

### B. Physiological analyses

The leaf water potential was determined by pressure chamber technique [7]. Plant height, number of branches and leaves; and leaf area were determined as average of ten plants. Leaf area was measured using the formula of Stickler et al. [8] as given below:

$$A = L \times W \times 0.75$$

Where A = Leaf area; L = Leaf length; W = Leaf width and 0.75 = a constant.

### C. Biochemical analyses

Chlorophylls were extracted with 80% acetone and estimated by the method of Arnon [9]. Protein content was estimated following the method of Bradford [10] using coomassie brilliant blue G-250 dye. The procedure of protease extraction was a slight modification of that described by Yomo and Varner [11] and appeared elsewhere [12]. Protease activity was expressed as moles of tyrosine g<sup>-1</sup> fresh weight h<sup>-1</sup>. RNA and DNA were determined by a modified method of Ogur and Rosen [13]; the extraction process removed the interfering compounds and involved defatting [14]. Each biochemical analysis was based upon three replicates; and for each replicate three aliquots were taken for recording data.

### III. RESULTS

Results have been summarized in Table 1-3. Zero day stage in tables indicate 90-day old pigeon pea plants from when data were collected at 10-day intervals. Plants grown under varying amount of water exhibited different values of water potential. At any growth stage, water potential was highest in those plants getting above normal water followed by normal and deficit water supply. With the advancement of growth, water potential value decreased gradually in these plants under all the three water regimes. Marked reduction in water potential was noticed under deficit water (Table 1).

Table 1: *Cajanus cajan* showing changes in water potential (in bars  $\pm$  S.E.) of leaves at different stages of growth under different water regimes.

Stages	Normal Water (NW)	Above Normal Water (AbN)	Deficit Water (DW)
0-day	-7.80 $\pm$ 0.14	-6.80 $\pm$ 0.24	-16.00 $\pm$ 0.14
10-day	-9.00 $\pm$ 0.28	-6.60 $\pm$ 0.17	-15.30 $\pm$ 0.21
20-day	-10.90 $\pm$ 0.21	-8.10 $\pm$ 0.07	-17.60 $\pm$ 0.28
30-day	-10.50 $\pm$ 0.44	-8.60 $\pm$ 0.42	-17.70 $\pm$ 0.42
40-day	-10.70 $\pm$ 0.64	-8.40 $\pm$ 0.43	-17.80 $\pm$ 0.26
50-day	-13.20 $\pm$ 0.19	-10.30 $\pm$ 0.23	-18.20 $\pm$ 0.17
60-day	-14.90 $\pm$ 0.20	-12.60 $\pm$ 0.84	-22.60 $\pm$ 1.08

Table 2 shows changes in plant height, number of branching per plant, number of leaves per plant, leaf area and stem diameter. Supply of water has profound effect on various aspects of growth. As far as plant height is concerned, it is largest when plant has received above normal water than the other two conditions. Plants having no irrigation became stunted and showed marked reduction in height in comparison to plants having normal and above normal water supply. Maximum height of plants was observed at 10-day whereas 20 to 60-day stages registered a sharp decrease. Very little increase in height was noticed in plants between zero to 60-day getting no water. Plants with normal and above normal water supply between 40 to 60-day stage also showed negligible variation in longitudinal growth. Number of branches per plant showed increment upto 20-day while stem diameter increased upto 30-day followed by a decline irrespective of quantum of irrigation. It was interesting to note that leaf number per plant registered increment between zero to 30-day only followed by decrease in those plants receiving normal water supply. However, above normal water and deficit water were responsible for gradual decline in leaf number right from 10-day stage. It was equally interesting to observe that leaf area exhibited gradual rise during zero to 40 and 50-day stage when plants had normal and excess water supply respectively. Leaf area increment, however, continued even at 60-day in plants having deficit water. Zero to 10-day period was remarkable in showing sharp rise in leaf area in all plants irrespective of water status;

but significant difference could be seen in plants having water (normal or excess) or devoid of it.

#### A. Protein and protease activity

Amount of protein in pigeon pea leaves registered smaller increments between 0 to 40-day stages followed by a gradual decline – in plants having normal water supply. Plants getting either excess or no water supply exhibited slightly lesser quantity of protein in all stages. Forty day and later stages of leaves appear to be associated with the senescent stages. Protease activity showed gradual increment between 0 to 60-day stages in all plants irrespective of the irrigation water. In most of the cases the protease activity was in the order of plants getting deficit water > above normal water > normal water (Table 3).

#### B. RNA and DNA

The distribution pattern of RNA and DNA was almost similar, the amount of former was much greater than the latter (Table 3). Zero to 30-day stages are characterized by their gradual increments whereas later stages showed stepwise decline. In almost all the stages leaf samples showed maximum amount of RNA and DNA in plants getting normal irrigation and the least amount was found in plants having deficient water.

### IV. DISCUSSION

Water potential data of leaves revealed gradual decline with leaf expansion, growth and senescence irrespective of water supply. However, values were comparatively higher in plants getting above normal irrigation and lowest under deficit water. Drastic reduction in plant height, branching and number of leaves under deficient water supply can be considered as an adaptational response as opined by Turner and Begg [15]. Turgor of leaves tend to increase with leaf expansion in presence of water while reduction in turgor under deficient condition reduced leaf growth [16] – [17]. Increment in abscisic acid (ABA) may be significant during reduced leaf expansion, turgor and plant height [18].

Protein values indicate increment with leaf maturity and development during first thirty days followed by degradation. Increment in protein with leaf maturation has been noticed earlier also [19]. Breakdown of protein was slow during 30 to 40-day and faster thereafter. Much higher rate of protease activity at these stages prove this point. Water stress has caused increased proteolysis as reported in earlier studies [20].

Present investigation has clearly revealed that leaf development and expansion are associated with rise in nucleic acid level irrespective of water status of plants and the onset of senescence showed their gradual depletion. The loss of RNA and DNA was maximum in plants having least supply of water. Water stress can trigger sharp rise in RNase activity [21]-[22]. Significant decline in nucleic acids has been reported during leaf senescence [23]. Water deficiency is also responsible for rise in ABA concentration [24].

Table 2: *Cajanus cajan* showing changes in plant height (cm  $\pm$  S.E.), number (No.) of branching per plant, number of leaves per plant, leaf area (cm<sup>2</sup>  $\pm$  S.E.) and stem diameter (cm  $\pm$  S.E.) at different stages of growth.

Parameters	0-day			10-day			20-day			30-day		
	Above normal	Normal	Deficit	Above normal	Normal	Deficit	Above normal	Normal	Deficit	Above normal	Normal	Deficit
Plant height	166.93 $\pm$ 6.24	147.50 $\pm$ 2.17	66.86 $\pm$ 3.37	167.80 $\pm$ 9.42	148.84 $\pm$ 4.18	68.11 $\pm$ 2.23	158.42 $\pm$ 4.32	138.36 $\pm$ 4.22	70.56 $\pm$ 1.30	142.17 $\pm$ 2.32	132.48 $\pm$ 2.08	70.12 $\pm$ 2.21
No. of branching per plant	42.30 $\pm$ 1.65	37.66 $\pm$ 1.60	14.00 $\pm$ 2.16	43.60 $\pm$ 0.54	45.66 $\pm$ 0.98	22.00 $\pm$ 1.24	48.30 $\pm$ 1.65	51.00 $\pm$ 0.47	23.30 $\pm$ 0.27	46.83 $\pm$ 0.82	48.30 $\pm$ 0.36	24.83 $\pm$ 0.49
No. of leaves per plant	321.66 $\pm$ 21.10	206.60 $\pm$ 9.15	75.30 $\pm$ 4.01	309.00 $\pm$ 27.61	265.10 $\pm$ 12.35	60.33 $\pm$ 4.54	305.00 $\pm$ 2.16	273.30 $\pm$ 2.88	54.12 $\pm$ 1.18	292.00 $\pm$ 1.89	274.01 $\pm$ 2.81	48.30 $\pm$ 0.54
Leaf area	3.90 $\pm$ 0.24	3.42 $\pm$ 0.14	1.90 $\pm$ 0.01	11.50 $\pm$ 0.01	9.20 $\pm$ 0.78	6.70 $\pm$ 0.32	15.70 $\pm$ 0.92	14.80 $\pm$ 0.78	10.40 $\pm$ 0.60	17.90 $\pm$ 1.02	18.20 $\pm$ 0.92	14.10 $\pm$ 0.42
Stem diameter	1.16 $\pm$ 0.02	1.07 $\pm$ 0.03	0.54 $\pm$ 0.01	1.20 $\pm$ 0.02	1.11 $\pm$ 0.03	0.71 $\pm$ 0.01	1.18 $\pm$ 0.04	1.12 $\pm$ 0.02	0.69 $\pm$ 0.01	1.21 $\pm$ 0.01	1.12 $\pm$ 0.01	0.83 $\pm$ 0.00
Parameters	40-day			50-day			60-day					
	Above normal	Normal	Deficit	Above normal	Normal	Deficit	Above normal	Normal	Deficit			
Plant height	130.20 $\pm$ 2.04	129.12 $\pm$ 1.15	69.83 $\pm$ 0.90	129.48 $\pm$ 1.08	128.90 $\pm$ 1.25	69.23 $\pm$ 1.08	129.16 $\pm$ 4.57	128.70 $\pm$ 6.21	69.23 $\pm$ 1.79			
No. of branching per plant	37.60 $\pm$ 0.49	43.32 $\pm$ 0.72	21.38 $\pm$ 1.18	32.51 $\pm$ 0.23	36.83 $\pm$ 0.36	17.83 $\pm$ 0.36	30.80 $\pm$ 0.38	32.66 $\pm$ 0.27	17.60 $\pm$ 0.32			
No. of leaves per plant	245.40 $\pm$ 1.90	231.10 $\pm$ 1.19	42.66 $\pm$ 0.27	192.60 $\pm$ 1.18	188.00 $\pm$ 0.72	40.30 $\pm$ 1.18	143.00 $\pm$ 7.36	173.70 $\pm$ 1.20	32.72 $\pm$ 0.32			
Leaf area	23.20 $\pm$ 1.20	211.50 $\pm$ 1.00	16.70 $\pm$ 0.92	23.40 $\pm$ 2.10	21.20 $\pm$ 1.48	18.10 $\pm$ 1.08	21.30 $\pm$ 0.87	18.80 $\pm$ 0.36	18.30 $\pm$ 0.42			
Stem diameter	1.16 $\pm$ 0.00	1.10 $\pm$ 0.00	0.78 $\pm$ 0.00	1.14 $\pm$ 0.01	1.07 $\pm$ 0.01	0.69 $\pm$ 0.00	1.13 $\pm$ 0.00	1.07 $\pm$ 0.01	0.69 $\pm$ 0.00			

Table 3: *C. cajan* L. cv. UPAS – 120 showing changes in protein (mg/100 mg dry weight  $\pm$  S.E.) protease activity (n moles of tyrosine g<sup>-1</sup> fr. wt. hr<sup>-1</sup>  $\pm$  S.E.) RNA and DNA ( $\mu$ g g<sup>-1</sup> dry wt.  $\pm$  S.E.) during development and senescence of leaves under three water regimes.

Stage	Protein	Protease Activity	RNA	DNA
<b>0-day</b>				
NW	8.42 $\pm$ 0.11	1621 $\pm$ 108	0.382 $\pm$ 0.001	0.084 $\pm$ 0.001
AbN	7.20 $\pm$ 0.30	2469 $\pm$ 172	0.286 $\pm$ 0.001	0.058 $\pm$ 0.001
DW	8.01 $\pm$ 0.21	2147 $\pm$ 150	0.303 $\pm$ 0.003	0.058 $\pm$ 0.003
<b>10-day</b>				
NW	10.84 $\pm$ 0.09	1982 $\pm$ 120	0.440 $\pm$ 0.003	0.098 $\pm$ 0.001
AbN	10.98 $\pm$ 0.16	2480 $\pm$ 108	0.334 $\pm$ 0.001	0.082 $\pm$ 0.003
DW	10.08 $\pm$ 0.11	2672 $\pm$ 142	0.359 $\pm$ 0.005	0.060 $\pm$ 0.001
<b>20-day</b>				
NW	12.32 $\pm$ 0.11	2168 $\pm$ 108	0.472 $\pm$ 0.001	0.121 $\pm$ 0.003
AbN	12.08 $\pm$ 0.08	2502 $\pm$ 120	0.456 $\pm$ 0.003	0.092 $\pm$ 0.003
DW	12.30 $\pm$ 0.24	2847 $\pm$ 146	0.380 $\pm$ 0.003	0.076 $\pm$ 0.001
<b>30-day</b>				
NW	12.64 $\pm$ 0.20	2408 $\pm$ 112	0.498 $\pm$ 0.003	0.136 $\pm$ 0.001
AbN	13.02 $\pm$ 0.30	2842 $\pm$ 126	0.508 $\pm$ 0.001	0.092 $\pm$ 0.001
DW	12.80 $\pm$ 0.11	3196 $\pm$ 152	0.420 $\pm$ 0.001	0.096 $\pm$ 0.003
<b>40-day</b>				
NW	12.84 $\pm$ 0.11	2420 $\pm$ 132	0.518 $\pm$ 0.003	0.097 $\pm$ 0.005
AbN	10.24 $\pm$ 0.03	2972 $\pm$ 150	0.438 $\pm$ 0.001	0.084 $\pm$ 0.003
DW	10.32 $\pm$ 0.32	3246 $\pm$ 138	0.372 $\pm$ 0.001	0.066 $\pm$ 0.003
<b>50-day</b>				
NW	9.90 $\pm$ 0.10	3692 $\pm$ 172	0.308 $\pm$ 0.001	0.072 $\pm$ 0.001
AbN	8.02 $\pm$ 0.12	3872 $\pm$ 184	0.282 $\pm$ 0.005	0.050 $\pm$ 0.003
DW	9.98 $\pm$ 0.20	3698 $\pm$ 120	0.238 $\pm$ 0.005	0.046 $\pm$ 0.001
<b>60-day</b>				
NW	9.24 $\pm$ 0.11	3846 $\pm$ 172	0.178 $\pm$ 0.001	0.050 $\pm$ 0.003

<b>AbN</b>	8.01 ± 0.09	3846 ± 128	0.144 ± 0.003	0.032 ± 0.001
<b>DW</b>	7.62 ± 0.21	4296 ± 148	0.126 ± 0.003	0.027 ± 0.001

NW – Plants having normal water, AbN – Above normal water, DW – Deficit water

## V. CONCLUSION

This study has revealed how water availability affects plant growth and metabolism in pigeon pea. Non-availability of water during 120-day growth period severely affected height, number of branches, number of leaves, leaf area and stem diameter. Moreover, metabolic processes have been slowed down resulting decline in protein, RNA and DNA. Although selected growth parameters do not show reduction when plants received above normal water; amounts of protein, RNA and DNA were lower than those of normal plants.

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 Biochemistry of senescence in leaves and reproductive organs of pigeon peas (ICAR financed, 1985-1988).  
 Regulation of chlorophyll and carotenoid degradation and influence of amino acids during leaf senescence (UGC financed, 2004-2007).  
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