# EFFECT OF INDUCED DROUGHT ON DIFFERENT GROWTH AND BIOCHEMICAL ATTRIBUTES OF BLACK GRAM *(Vigna mungo L.)* AND GREEN GRAM *(Vigna radiata L.)*

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## ABSTRACT

Drought is one of the major abiotic stresses which adversely affect crop growth and yield. Drought induced changes are mainly related to altered metabolic functions, such as reduced synthesis of photosynthetic pigments, accumulation of osmoprotectants like proline in the cell, decline in the cell membrane stability and alterations in physiological parameters including plant height, leaf area and cell membrane stability. A study was conducted during January-April,2010 in the experimental field of Tezpur University, Assam with two major pulses black gram (*Vigna mungo* L., cv. PU19) and green gram (*Vigna radiata L.*, cv. Pratap) to see the biochemical as well as physiological changes resulting from exposure to various osmotic stress intensities and subsequent relief of the same. Accumulation of proline in leaves was found to be increased during stressed period and decreased in the subsequent recovery stages. Watering was done regularly up to 36 days until the plants were grown to an average height of 4 cm. Then watering was stopped and a condition of water deficit was maintained for 10 days, 15 days and 20 days in each type of plants and labeled as $T_1$, $T_2$ and $T_3$ respectively. Reduction in all the other parameters was observed during stressed period with substantial increase in recovery stage followed by net reduction in yield. Positive correlations of soil moisture with leaf chlorophyll, chlorophyll stability index, plant height, leaf area, cell membrane stability and yield were obtained while it is found to be negatively correlated with leaf Proline concentration. Black gram variety PU19 was found to be more resistant than green gram variety pratap against drought stress.

**Key Words :** Proline, Chlorophyll, Plant height, Leaf area, Cell membrane stability, Yields

## INTRODUCTION

Stress is defined as any biotic or abiotic factor of environment that affects plant's physiological and biochemical activity along with growth and development in such a way that plant perform below the average for a region. Drought is a meteorological term and can be commonly defined as the absence of precipitation for a significant period of time. It also results in a condition when water lost in the form of transpiration from the plant exceeds the availability of water in soil. Drought is a major abiotic stress; distributed widely across the world over 1.2 billion hector area in rain fed agricultural land[1]. It is a perennial problem in India which is evidenced by the very recent drought occurred during the year 2002 resulting in the reduction of GDP in agriculture by 3.1% along with a loss of agricultural income of around 29,000 crores. Drought or water deficit stress is more severe in terms of yield and economic gain particularly when it occurs at the reproductive phase of plants[2].

A complex response (in terms of physiological, biochemical and molecular level) is shown by the plants exposed to drought and depending on that plants show differential adaptation and tolerance mechanisms[3]. Drought stress effects on plants are generally evident in terms of reduced growth[4], loss of membrane stability and integrity[5], reduction in essential pigments like chlorophyll[6] etc. However, the degree of these responses

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varies from plant to plant, species to species. Accumulation of proline in plants is a well known tolerance mechanism to drought stress which acts as cellular osmotic adjustor and also protect and stabilizes essential cell components like protein, photosynthetic apparatus and detoxify Reactive Oxygen Species (ROS)[7] etc.

Pulse crops green gram *(Vigna radiata L.)* and black gram *(Vigna mungo L.)* are two most important protein sources that are grown in all over India as well as in Assam. In addition, it also plays an important role in sustaining soil fertility by fixing atmospheric nitrogen. Therefore in the present experiment we tried to evaluate the various physiological and biochemical changes taking place in selected pulse cultivars under drought stress.

## AIMS AND OBJECTIVES

1. To study the effect of drought stress on some important physiological and biochemical parameters in two popular black gram *(Vigna mungo L.)* and green gram *(Vigna radiata L.)* cultivars commonly grown in Assam.
2. To observe the changes in these parameters during drought and recovery period.
3. To study the overall effect of drought on yield components.

## MATERIAL AND METHODS

**Description of the experimental site**

The experiment was conducted during January-April, 2010 at experimental field of Tezpur University which is located at North bank plain zone of Assam ($26^0$14´ N and $92^0$50´ E) at Tezpur, India. The soil of the experimental site is characterized by silty textured soil having slightly acidic in reaction. The maximum and minimum average air temperature recorded during the experimental period ranges from 14.7$^0$C to 28.5$^0$C and the average rainfall recorded was 2.48 mm.

**Experimental design**

Soils were collected from farmers' field which is located outside Tezpur University campus. The collected soils were ground and mixed thoroughly with dry FYM @ of 10gm/kg of soil. The prepared soils were then put into 56 equal sized polythene bags with 1 kg of soil each. The polythene bags were arranged in Completely Randomized Design (CRD) with four treatments and seven replications. Seeds of pulse cultivars namely black gram (cv. PU19) and green gram (cv. Pratap) were collected from Regional Agricultural Research Station (RARS), Shillongoni, Nagaon, Assam, India. Before sowing, seeds were soaked in running tap water and then about 5-7 seeds were sown per bag on 28$^{th}$ of January, 2010. Watering was done regularly up to 36 days until the plants were grown to an average height of 4 cm. After that watering was stopped and a condition of water deficit was maintained for 10 days, 15 days and 20 days in each type of plants and labeled as $T_1$, $T_2$ and $T_3$ respectively. The remaining plants were allowed to grow in controlled condition (i.e. with regular watering) and labeled as C. A temporary rain shelter made up of bamboo and polythene sheet was prepared to avoid rainfall.

**Soil Analysis**

The collected soils were processed and various physico-chemical parameters like- bulk density (Db), soil pH, water holding capacity of soil and organic matter content were analyzed using standard methods at the beginning of the experiment. Soil moisture content was determined by gravimetric method at regular interval.

**Plant morpho-physiological analysis**

Plant physiological parameters such as plant height, leaf area and cell membrane stability were measured before starting of treatment, during treatment as well as in the recovery period of drought for each treatment including the control. Leaf area was recorded by using a laser leaf area meter (model CI-203, USA). Membrane stability index was determined by recording the electrical conductivity of leaf leachates in doubled distilled water at 40 and 100$^0$C[8].

$$\text{Membrane stability index} = 100 - \{(C_1/C_2) \times 100\}$$

$C_1$ = electrical conductivity at 40 ºC

$C_2$ = electrical conductivity at 100 ºC

**Plant biochemical analysis**

Chlorophyll-a, chlorophyll-b and total chlorophyll were determined. Chlorophyll stability

index was also calculated periodicaly[9.] using the following formula :

(CSI %) = (Total Chlorophyll under stress/Total Chlorophyll under control) × 100

Leaf proline content was determined at various treatment levels[10]. Yield and different yield attributing parameters such as number of pods per plant, total number of seeds per plant and finally the weight of seeds per plant was recarded on maturity. The obtained data were analyzed using the statistical package PASW statistics[18].

## RESULTS AND DISCUSSION

Soil moisture content was analyzed at various stages of the treatment viz. before the onset of treatments, during the stress period and at recovery stages and are presented in **Table 1** and **Table 2.**

**Effect of drought stress on plant growth parameters**

In the present study, plant growth parameters in terms of height and leaf area were observed and are presented in **Table 3** and **Table 4.** Drought stress has been found to decline the linear growth of shoots in both the cultivars as compared to those of untreated ones. Upon re-watering, both black gram and green gram plants started to recover in terms of height but did not reach the control level. This reduction in plant growth in terms of height is due to the loss of cell turgor which greatly suppresses cell expansion and cell growth thereby inhibiting the linear growth of shoot.

**Table 1: Soil moisture content (%) during the stress period and at recovery stages of black gram (ABT-At the beginning of treatment, DAT-Days after treatment)**

| Soil moisture content (%) | | | | | | |
|---|---|---|---|---|---|---|
| Treatment | | | | | | |
| | **ABT** | **3 DAT** | **10 DAT** | **15 DAT** | **20DAT** | **30DAT** |
| **C** | 91.31 | 90.17 | 87.67 | 86.19 | 90.23 | 89.06 |
| **$T_1$** | 91.02 | 49.60 | 24.01 | 56.78 | 66.06 | 81.32 |
| **$T_2$** | 89.70 | 50.23 | 22.77 | 13.14 | 40.82 | 69.08 |
| **$T_3$** | 92.43 | 52.19 | 20.34 | 12.81 | 6.79 | 46.28 |

**Table 2 : Soil moisture content (%) during the stress period and at recovery stages of green gram (ABT-At the beginning of treatment, DAT-Days after treatment)**

| Soil moisture content (%) | | | | | | |
|---|---|---|---|---|---|---|
| Treatment | | | | | | |
| | **ABT** | **3 DAT** | **10 DAT** | **15 DAT** | **20DAT** | **30DAT** |
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Hence it can be inferred that the slow decline in this growth parameter might be due to lack of adequate moisture in plant root zone **(Table 1 and Table2)** which can be evidenced from the positive correlation between soil moisture and height of the plant **(Table 7)**. Which is in confirmatery with the findings os eariler workers.[11, 12]

Various studies have indicated that water stress has an inhibiting effect on the leaf area of treated plants. Leaf area measurements taken over the experimental period has revealed that there is a considerable diminish in leaf area of the stressed plants than the plants grown under controlled moisture environment. However, re-watering had brought an increment in leaf size **(Table 4)** in all the treatments. The reduction of leaf area was more prominent in $T_3$ plants that faced longest period of water deficit (20 days).

**Table 3: Effect of drought stress on height of black gram and green gram (ABT- At the beginning of treatment, DAT-Days after treatment)**

| Days of treatment | Black gram | | | | Green gram | | | |
|---|---|---|---|---|---|---|---|---|
| | C | $T_1$ | $T_2$ | $T_3$ | C | $T_1$ | $T_2$ | $T_3$ |
| ABT | 7.52 | 7.55 | 9.15 | 7.65 | 8.00 | 5.25 | 9.40 | 9.10 |
| 3 DAT | 9.69 | 9.16 | 9.33 | 8.00 | 9.54 | 5.31 | 10.04 | 9.63 |
| 10 DAT | 12.58 | 9.25 | 10.05 | 9.12 | 11.27 | 6.35 | 10.40 | 10.00 |
| 15 DAT | 16.61 | 11.60 | 10.11 | 9.85 | 12.49 | 9.50 | 11.71 | 10.56 |
| 20 DAT | 18.60 | 13.81 | 13.86 | 11.35 | 18.18 | 12.22 | 13.95 | 11.13 |
| 30 DAT | 19.15 | 17.15 | 15.08 | 13.95 | 20.71 | 14.27 | 16.10 | 13.21 |

Reduction in leaf area was found to be in the order of $T_3>T_2>T_1$ for both the cultivars. Correlation study between soil moisture content and leaf area reveals the dependency of the later with the former parameter. Under water deficit condition, plants first show reduction in cell division resulting in reduced cell number and stop cell elongation inhibiting leaf expansion. This modification in leaf anatomy is one of the basic causes which lead to reduction in average leaf size under water limiting situation[13,14]

**Effect of drought stress on cell membrane stability**

**Fig. 1** and **Fig. 2** shows a considerable decrease in the membrane stability in the plants grown under drought stress condition as compared to the control plants for both the cultivars. Plants kept under 20 days of water deficit ($T_3$) had the lowest membrane stability value compared to the other plants including the plants grown under control condition. The decrease in cell membrane stability was found to be more in green gram. But when re-watering was done, the stressed plants of both the cultivars started to regain membrane stability. This decrease in cell membrane stability is due to increase in the permeability of the cell membrane which increases the leakage of electrolyte from the cell across the Membrane[15]. Membrane stability index was found to be positively correlated with soil moisture content for both black gram and green gram cultivars **(Table 7)**.

**Table 4: Effect of drought stress on leaf area of black gram and green gram (ABT- At the beginning of treatment, DAT-Days after treatment)**

| Days of treatment | Black gram | | | | Green gram | | | |
|---|---|---|---|---|---|---|---|---|
| | C | $T_1$ | $T_2$ | $T_3$ | C | $T_1$ | $T_2$ | $T_3$ |
| ABT | 1.33 | 2.26 | 2.24 | 1.01 | 1.29 | 0.89 | 2.8 | 0.89 |
| 3DAT | 4.66 | 4.93 | 3.41 | 2.37 | 2.12 | 1.94 | 2.93 | 1.50 |
| 10DAT | 5.45 | 5.23 | 3.44 | 2.78 | 4.96 | 2.14 | 3.14 | 1.97 |
| 15DAT | 7.89 | 7.16 | 4.03 | 3.43 | 6.37 | 3.09 | 3.73 | 2.12 |
| 20DAT | 7.89 | 7.53 | 4.92 | 4.30 | 9.87 | 6.22 | 5.76 | 2.39 |
| 30DAT | 10.56 | 9.34 | 6.73 | 5.34 | 13.25 | 9.14 | 7.71 | 6.19 |

**Effect of drought stress on biochemical parameters**

Plants subjected to drought stress shows a rapid loss of their leaf chlorophyll content with increasing intensity of stress[16]. In the present study, chlorophyll-a and chlorophyll-b content of both black gram and green gram plants showed a decreasing trend with the increasing duration of drought (data not presented) which proved that these photosynthetic pigments are sensitive to water deficit condition[17]. But at recovery stage the level of chlorophyll-a and b started to

increase in all treatments.

Total chlorophyll content was estimated for all the treatments of both black gram and green gram cultivars and presented in **Fig. 3** and **Fig. 4**. The rate of decrease was rapid during prolonged drought condition ($T_3$). On recovery, the level of

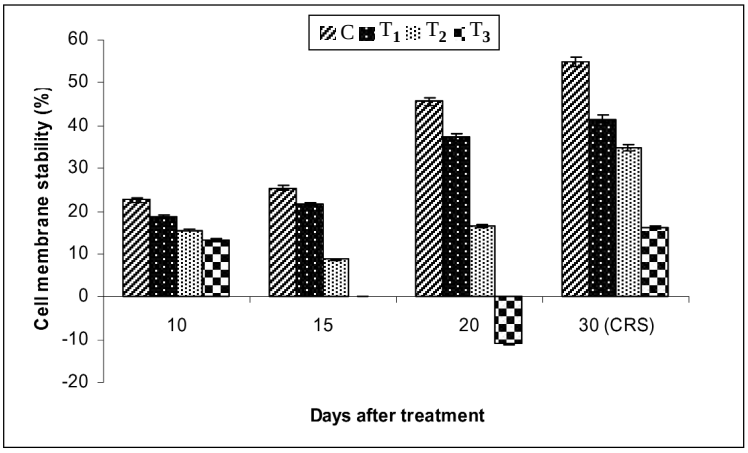


**Fig. 1:** Effect of drought stress on cell membrane stability of black gram (CRS-Complete Recovery Stage)

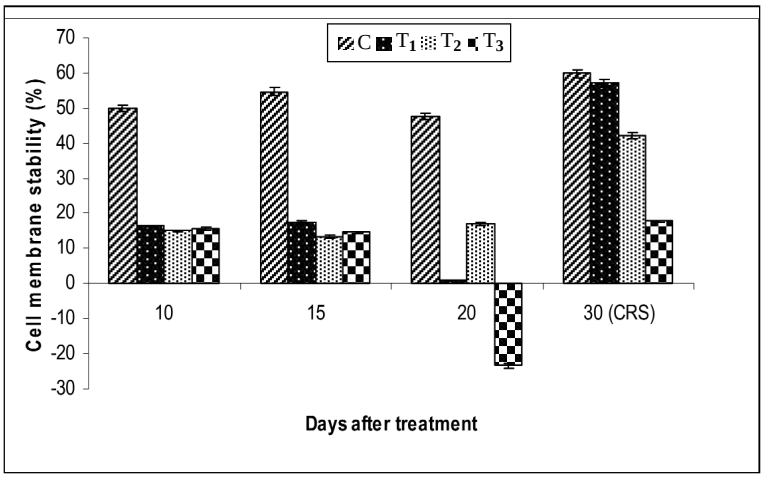


**Fig. 2 :** Effect of drought stress on cell membrane stability of green gram (CRS-Complete Recovery Stage)

total chlorophyll started to increase but could not reach the total chlorophyll content of the control plants. This reduction in leaf chlorophyll content under drought stress might be due to the cause of excessive swelling of chloroplast membranes, distortion of the lamellae vesiculation and the appearance of lipid droplets[18]. This degradation is considered as one of the consequences of drought stress which has resulted from sustained photo-inhibition and photo breeding[19]. In the present study, it was also observed that the rate of decrease of chlorophyll during drought is higher in green gram compared to black gram and the black gram cultivar also maintained a relatively higher ratio of chlorophyll a/b ($T_1$) .

Analysis of chlorophyll stability index also revealed the similar trend of change during the stress and recovery periods. In the present study, a continuous decrease in chlorophyll stability index was observed during the drought period and it was found to increase in both the pulse cultivars with the subsequent recovery (Table 5). Chlorophyll Stability Index (CSI) is an indicator of the stress tolerance capacity of plants and it indicates how

well chlorophyll can perform under stress[20]. A higher CSI helps plants to withstand stress through better availability of chlorophyll by maintaining more dry matter production, and higher productivity. The drop down of CSI in green gram was faster than that of black gram. The correlation between Chlorophyll Stability Index (CSI) and soil moisture content was found to be positively significant **(Table 7)** in both black gram and green gram for all the treatments.

Leaf proline content of both black gram and green gram cultivars were increased during the stressed period and were found to decline during the recovery period **(Fig. 5** and **Fig. 6)**. Proline content is used as an indicator of drought tolerance capacity of plant tissue as

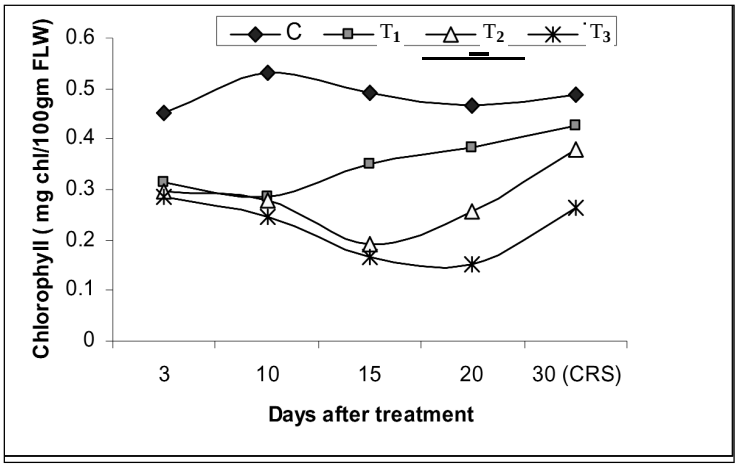


**Fig. 3 : Effect of drought stress on total chlorophyll content of black gram**
*(CRS- complete recovery stage)*

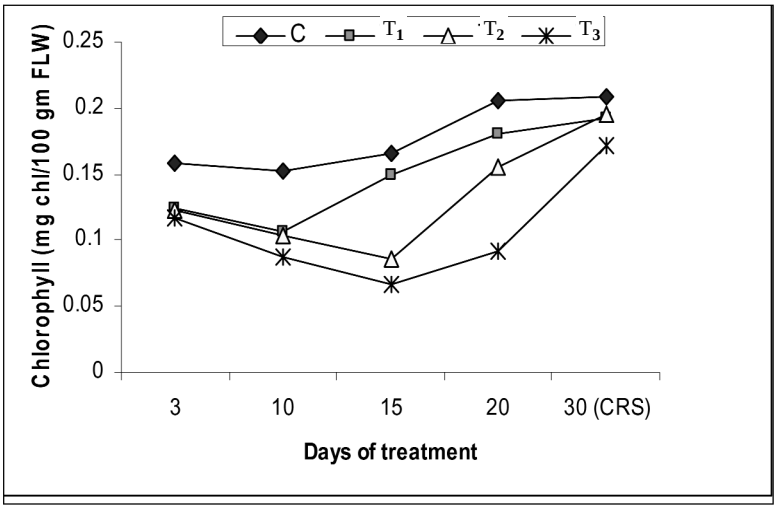


**Fig. 4 : Effect of drought stress on total chlorophyll content of green gram**
*(CRS- complete recovery stage)*

proline accumulation helps in maintaining a better osmotic balance in plant cells suffering from water deficit[21]. In the present study, more accumulation of proline was reported in leaf tissue with the increase in duration of drought stress ($T_3$). The rate of proline accumulation during drought was found to be more in green gram than black gram. This increased accumulation of proline might be due to the decreased activity of proline dehydrogenase, a catabolic

enzyme of proline[22]. The correlation between leaf proline content and soil moisture content was found to be negatively correlated **(Table 7)** for both the pulses.

**Effect of drought stress on yield**

The yield of both black gram and green gram were calculated using different yield attributing parameters such as number of pod per plant, total number of seed per plant, total seed weight per plant and the results are presented in **Table 6**.

**Table 5 : Effect of drought stress on Chlorophyll stability index (%) of black gram and green gram (DAT-Days after treatment)**

| | Black gram | | | Green gram | | |
|---|---|---|---|---|---|---|
| **DAT** | **$T_1$** | **$T_2$** | **$T_3$** | **$T_1$** | **$T_2$** | **$T_3$** |
| **3** | 69.91 | 65.92 | 63.49 | 79.11 | 77.84 | 74.05 |
| **10** | 54.15 | 52.45 | 46.60 | 69.28 | 67.97 | 56.86 |
| **15** | 71.07 | 36.86 | 35.43 | 90.30 | 52.12 | 40.06 |
| **20** | 81.62 | 52.13 | 35.47 | 88.29 | 75.60 | 44.87 |
| **30** | 87.7 | 77.45 | 53.89 | 92.78 | 93.75 | 82.21 |

The yields of both the pulses (black gram and green gram) were found to decrease at pre-anthesis drought stress treatment ($T_3$) compared to other treatments ($T_1$ and $T_2$). Which coincides with the results obtained by earlier workers in wheat[23]. Water stress significantly affected crop yield of both the cultivars in the order of $T_1 < T_2 < T_3$. Highest crop yield was obtained from $T_1$ and lowest value of crop yield was obtained from $T_3$. The higher crop yield obtained from $T_1$ might be due to the more adequate moisture at crop root zone during active growth stage which helps in better utilization of nutrients. The lower crop yield obtained from $T_3$ was because of withdrawal of water for a longer period resulting in a lack of moisture in active crop root zone particularly at the critical growth stages i.e. at pre-anthesis causing poor nutrient utilization and hindrance in flowering[24,25]. Most of the studies revel that drought stress greatly reduces the grain yield, which is dependent on the level of defoliation due to water stress during early reproduc-

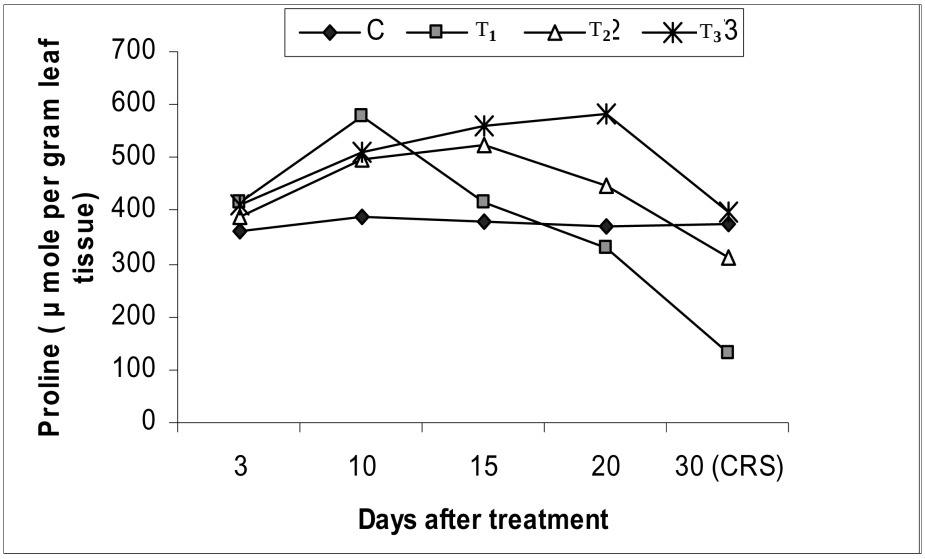


**Fig. 5 :** Effect of drought stress on proline content of black gram
*(CRS- complete recovery stage)*

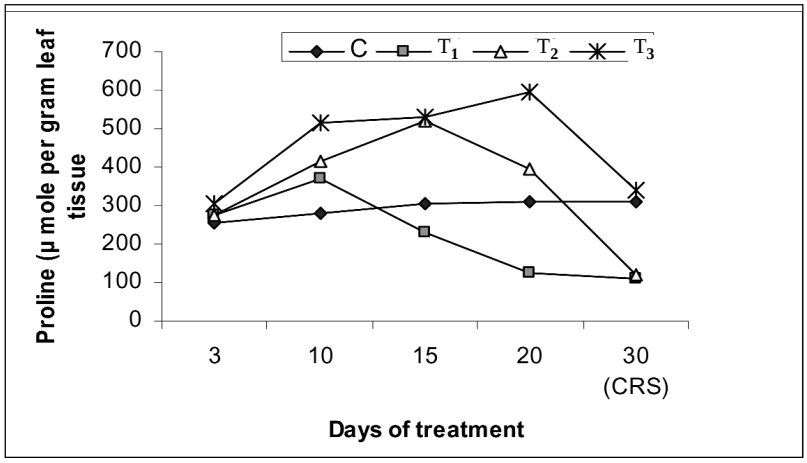


**Fig. 6 :** Effect of drought stress on proline content of green gram (CRS- complete recovery stage)

tive growth[26,27].

## CONCLUSION

In the present experiment, it has been observed that both the pulse cultivars were significantly affected by drought stress in terms of plant growth, leading to reduced productivity and yield. These losses were found to be more in the plants that were subjected to highest degree of drought (20 days of water deficit) which coincides one of the important crop growth stage that is pre-anthesis. The black gram cultivar maintained a higher level of yield compared to green gram in all the treatments which revels that black gram variety PU19 is more resistant than green

**Table 6 : Effect of drought stress on Yield of black gram and green gram**

| Yield attributing parameters | Black gram | | | | Green gram | | | |
|---|---|---|---|---|---|---|---|---|
| | C | $T_1$ | $T_2$ | $T_3$ | C | $T_1$ | $T_2$ | $T_3$ |
| No of pods per plant | 7 | 5 | 4 | 3 | 6 | 3 | 4 | 2 |
| Total number of seeds per plant | 203 | 105 | 96 | 42 | 156 | 100 | 45 | 18 |
| Total seed weight per plant (gm) | 9.09 | 7.35 | 2.88 | 1.68 | 12.5 | 6 | 2.7 | 0.72 |

**Table 7 : Correlation co-efficient of different parameters**

| | TC | | CSI | | PRO | | MSI | | HT | | LA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_1$ | GG | BG | GG | BG | GG | BG | GG | BG | GG | BG | GG | BG |
| MC | .953* | -.528 | .938* | .990** | -.968** | -.965** | .503 | .825 | .855 | .890* | .854 | .874 |
| $T_2$ | | | | | | | | | | | | |
| MC | .907* | .906* | .969** | .953* | -.969** | -992** | .781 | .839 | .608 | .638 | .546 | .691 |
| $T_3$ | | | | | | | | | | | | |
| MC | .763 | .909* | .939* | .964** | -.996** | -.983** | .599 | .786 | .163 | .026 | .361 | -.040 |

* Correlation significant at 0.05% probability level

** Correlation significant at 0.01% probability level

(MC- Moisture Content, TC - Total Chlorophyll, HT - Plant Height, LA - Leaf Area, CSI - Chlorophyll Stability Index, MSI - Membrane Stability Index, PRO- Proline Content)

gram variety Pratap against the induced drought stress.

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