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Morpho-physiological and Yield responses of Black gram (*Vignamungo* L.) and Green gram (*Vigna radiata* L.) genotypes under Drought at different Growth stages

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Abstract

This study was carried out to evaluate the response pattern of black gram (Vignamungo L.*) and green gram (Vigna radiate* L.*) genotypes under water drought stress imposed at vegetative, early reproductive and pod filling stages on the basis of morpho-physiological traits and yield. Four commonly grown genotypes- T9, KU 301 (black gram) and Pratap, SG 21-5 (green gram) were arranged in randomized block design with three replications. Drought stress was found to have significant inhibitory impact on all the studied traits. Positive correlation of seed yield was obtained with relative leaf water content, plant height, leaf number, leaf area and shoot: root biomass. Early reproductive stage was proved to be the most critical for drought stress as it greatly reduced seed yield (T9-31.28%, KU 301- 48.52%, Pratap-37.12%, SG 21-5- 56.98%). Among the studied genotypes, T9 and Pratap were identified as drought tolerant with higher values of DTI, RP, MP and HI.*

Key words: Drought, Early reproductive, Pod filling, Relative leaf water content, Vegetative, Yield.

Introduction

Drought can be defined as the absence of adequate moisture necessary for a plant to grow normally to complete its life cycle[1]. It is considered as one of the main a biotic stresses that limit crop production worldwide. Plants exhibit physiological, biochemical and molecular responses at both the cellular and whole plant levels when exposed to drought. Plants have differential adaptation potential towards drought[2]. However, the response of crops to drought varies with degree and duration of stress, variety, growth stage of the crop and soil type. Significant reduction in relative water content (a useful indicator of plant water balance) was observed in drought stressed plants of wheat[3]. Drought caused impaired mitosis, cell elongation and expansion resulted in reduced growth and yield traits[4]. Water deficits reduce the number of leaves per plant, individual leaf size and leaf longevity by decreasing the soil's water potential[5]. Under drought, greater allocation of biomass to root is associated with the benefits in terms of water uptake capacity to meet the demand of water in plant body[6].

Black gram (*Vignamungo* L.) and green gram (*Vigna radiata* L.) are two important short duration grain legumes, highly rich in protein and play an important role in sustaining soil fertility by fixing atmospheric nitrogen. Besides its widespread culinary use, they hold a significant cultural and religious place in Assamese culture. In Assam, these two crops are subjected to frequent drought due to selection of marginal lands to grow these crops and prevailing insufficient irrigation facility with erratic rainfall pattern. Though many reports are available about the responses of plant under water stress but very few experimental studies have been done in this particular region focused mostly on these two crops. In this study we aimed to test the sensitivity of black gram and green gram genotypes by comparing relative leaf water content, morphological characteristics and yield indexes of the genotypes (two each of black gram and green gram) commonly grown in Assam, India exposed to water deficit at three different growth stages. We also examined the most critical growth stage and the genotypic variability of drought tolerance among them.

Materials and Methods

Experimental Site: The experiment was conducted during September-November, 2012 at the experimental field of Tezpur University campus located at north bank plain zone of Assam (26º14´ N and 92º50´ E) at Tezpur, India. The maximum and minimum average temperature recorded during the experimental period ranges from 22.74 to 22.96 ^{o}C and the average rainfall recorded was 0.14 mm. The experimental site is characterized by silt loam textured soil being slightly acidic in nature.

Experimental Design: The field was ploughed with the help of a tractor. Fertilizers were applied at 15: 35:10 kg NPK ha^{-1} according to the package of practice. A temporary rain shed was constructed in the field with PVC (polyvinyl chloride) film (of about 0.15 mm thickness and 85% of transmittance) to avoid rainfall. The experiment was led in randomized block design with three replications under stress and non-stress conditions. Genotypes taken for the experiment wereT9, KU-301(black gram) and Pratap, SG21-5 SG 21-5 (green gram). Seeds were collected from Regional Agricultural Research Station (RARS), Shillongoni, Nagaon (Assam), India and were sown in the field on 5th of September, 2012 maintaining there quisite gap of 10 and 30 cm between plants and rows respectively.

Four treatments were given: T_1– irrigation throughout the growing period (control), T_2– withdrawal of irrigation for 15 days at vegetative stage (25 days after sowing), T_3– withdrawal of irrigation for 15 days at early reproductive stage (35 days after sowing), T_4 – withdrawal of irrigation for 15 days at pod filling stage (45 days after sowing).

Soil Analysis: Gravimetric method was employed to measure the moisture content of soil at weekly interval throughout the crop growing period. For each treatment irrigation was withheld until the plots reached a stress level of 30 % of plant available water. It took almost 10 days to reach this stress level and was maintained for 15 days. After this period, regular watering was done in all the plots. All measurements were taken at an interval of 7 days up to the end of stress period.

Plant Analysis: Plant height (cm) was measured using a meter ruler by averting the distance from soil level to the top of each plant. Total number of leaves (three fully expanded leaflets) was recorded for both control and stressed plants. After separating the plants into shoot and root, they were oven dried at 80^0C for 72 hours and weighed to determine the dry weight of shoot and root biomass. Using these values, shoot: root ratio was calculated. Leaf area was recorded non- destructively by using a laser leaf area meter (model CI-203, USA). RWC was calculated according to Lin and Ehleringer[7]. After taking the leaf fresh weight it was submerged in distilled water for 12 hours and turgid weight was recorded and finally dried it at 70ºC for 48 h to obtain the dry weight.

To study the overall effect of drought on yield components, harvesting was done when 75% of the pods mature indicating full darkish pod and brittle on slight pressure. Various yield and yield attributing parameters like number of pod per plant, seeds per pod and finally the weight of seeds per plant were recorded. From these data we obtained the following yield indexes

Mean productivity (MP): $(\text{Yield}_{\text{control}} + \text{Yield}_{\text{drought}})/2$.
Rate productivity (RP): $\text{Yield}_{\text{drought}}/ \text{Yield}_{\text{control.}}$
Drought tolerance index (DTI): $(\text{Yield}_{\text{drought}} \times \text{Yield}_{\text{control}})/ \text{Mean yield}_{\text{control.}}$
Harvest index: (Economic yield/ Biological yield) × 100.

Statistical analysis: Mean values were taken from the measurements of three replicates and the "Standard Error" of the means was calculated. Two-way ANOVA was applied to determine the significance of the results among genotypes, different treatments and the interaction effect between genotype and treatments. Duncan's multiple range tests (DMRT) were performed at p = 0.05. Correlation study was also done to find out the linear relationship of soil water potential (Ψ_S) seed yield with the studied parameters. All the statistical analyses analysis were done using SPSS for Windows (version 16.0).

Results and Discussion

Effect of drought stress on Relative leaf water content (RLWC): Significant decrease in relative leaf water content (RWC) was observed in all the treatments (Table -1). This deviation in RWC may be attributed to differential ability of the genotypes to absorb water from soil and or the ability to control transpiration loss of water through stomata. It may also be due to variations among the tested genotypes to accumulate and adjust osmolytes to maintain tissue turgor and hence physiological activities[8]. In our experiment, T9 and Pratap gave better yield than KU 301 and SG 21-5. They also maintained higher percentage of RWC in all the treatments which indicates their better tolerance capacity against the applied drought. Correlation study between RWC and seed yield gave a highly significant positive correlation (Table -4). The interaction effect of genotype and treatment was also found to be significant for both the crops ($p \leq 0.05$).

Effect of drought stress on plant height: Water stress during vegetative stage was most detrimental in terms of height (Figure-1). Mean values of the data indicated that this impact was more prominent in KU 301 and SG 21-5 for all the treatments. This reduced plant height under water deficit was the outcome of reduced cell turgor which decreased the rate of cell division and cell expansion as the process of cell growth and development[9]. In species like *A. esculentus*, it was observed that the decline in cell enlargement and more leaf senescence was associated with reduced plant height during drought[10]. Water stress did not affect plant height significantly during pod filling stage since the vegetative growth of the plant almost ceases at this period. The interaction effect between genotype and treatment was statistically significant for both black gram and green gram ($p \leq 0.05$). A positive correlation was obtained between plant height and seed yield (Table -4).

Effect of drought stress on leaf number: Significant reduction in leaf number of black gram and green gram plants were observed when subjected to stress for 15 days (Figure-2). Genotypes T9 and Pratap maintained higher leaf number in both the conditions than KU 301 and SG 21-5. Plants stressed at vegetative stage (T_2) recorded highest reduction in leaf number (T9-30.18%, KU 301-31.14%, Pratap-33.89%, SG 21-5-39.62%). Maximum abscission of leaf was observed in plants experiencing drought during pod filling stage (T_4) compared to other stages. The recorded lesser leaf number under water stress was resulted from the reduction and termination of new leaf production with increased leaf abscission. This higher leaf abscission may be linked with water stress induced production of more ethylene[11]. Irrespective of treatments and genotypes, the lowest number of leaves at pod filling was due to triggering of natural senescence process. Results of ANOVA showed significant ($p \leq 0.05$) interaction between genotype and treatment for green gram while it was non-significant for black gram. Leaf number was found to be positively correlated with seed yield (Table-4).

Effect of drought stress on leaf area: Total leaf areas of stressed plants were significantly lower than the control plants (Figure -3). Greater reduction of leaf area was observed in KU 301 and SG 21-5 in all the treatments. This modification in leaf area is one of the basic causes which lead to reduction in average leaf size under water limiting situation[12]. Irrespective of genotypes, plants stressed during vegetative stage (T_2) showed highest reduction of leaf area. This decrease can be attributed to suppression of leaf expansion through reduced cell division owing to loss of cell turgor[13]. The resulting smaller leaf area transpires less water and hence this can be considered a first line of defense against drought[14]. In the present experiment, although water stress was applied for same duration at all the stages, T_2 plants showed faster recovery with higher yield than T_3 and T_4 plants. This can be correlated to the findings of other researchers who suggested that both cell division and cell expansion were able to recover fully when stress occurred at early phases of leaf development[15]. From the analysis of variance (ANOVA), it was found that the interaction effect of genotypes and treatments was significant for black gram but it was non-significant for green gram. A positive correlation was obtained between leaf area and seed yield of all the studied genotypes (Table-4).

Effect of drought stress on shoot and root biomass: Results indicate that water restriction had a significant inhibitory impact on shoot and root biomass of all the studied genotypes (data not presented). Lowest reduction in biomass was recorded in those plants subjected to stress during pod filling stage. More reduction in shoot biomass was observed in KU 301 and SG 21-5 than other two genotypes. Results also showed a general increase in root biomass of all the genotypes irrespective of treatments. Highest increment in root biomass was recorded in T_2 plants followed by T_3 and T_4. Shoot: root ratio was significantly reduced by water stress while compared to non-stressed plants. Among the studied genotypes, the highest and lowest reduction of shoot: root ratio was observed in T_2 and T_4 respectively (Table-1). This higher reduction of shoot biomass during vegetative stage was due to water stress induced reduction of plant height and leaf senescence. Observed reduction in shoot: root ratio in all the treatments was the consequence of modulation of root length and density to maximize water uptake from the soil to guarantee the survival and growth under water stress condition[16]. Hence higher increment of root biomass in genotypes T9 and Pratap can be correlated to their tolerance capacity to water deficit condition to produce greater yield. Shoot: root biomass ratio was found to be positively correlated with seed yield (table-4). The interaction effect of treatment and genotype was found to be non-significant for green gram ($p \leq 0.05$).

Table-1
Relative leaf water content (RLWC) and shoot: root biomass ratio of black gram and green gram genotypes under control and stress condition (mean± standard error, C- control, D- drought, G- genotype, T- treatment)

Genotypes		Relative leaf water content (%)			Shoot: root ratio		
		Vegetative	Early reproductive	Pod filling	Vegetative	Early reproductive	Pod filling
T9	C	86.33±0.09	85.96±0.27	82.84±0.33	10.73±0.59	14.90±0.37	16.64±0.64
	D	77.47±0.77	70.40±1.20	65.04±0.58	6.88±0.76	11.02±0.22	14.36±0.07
KU 301	C	89.27±0.27	88.54±0.08	84.05±0.11	11.65±0.46	11.04±0.23	12.62±0.49
	D	72.21±1.09	62.30±0.85	61.02±0.12	5.14±0.23	7.91±0.22	9.67±0.41
Pratap	C	88.39±0.17	85.53±0.25	84.16±0.21	14.89±0.64	17.57±0.53	18.35±0.49
	D	75.97±1.19	65.55±0.32	64.73±0.52	7.12±0.65	13.95±0.39	14.56±0.25
SG 21-5	C	86.89±0.31	84.96±0.12	83.53±0.29	13.45±0.38	20.25±0.11	18.66±0.59
	D	68.06±0.67	60.04±0.97	60.66±1.49	6.29±0.50	12.60±0.29	13.59±9.18
CD (0.05)	G	1.28			0.64		
	T	1.57			0.79		
	G×T	2.56			1.29		

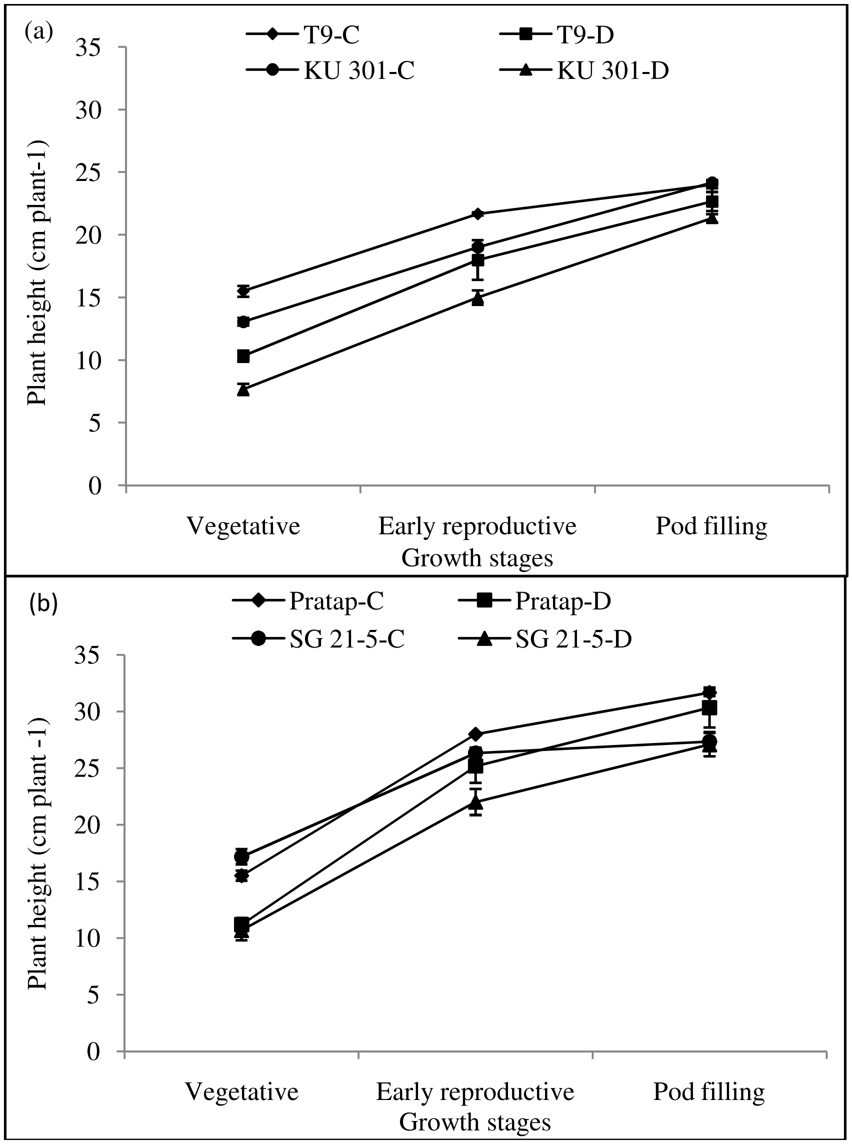

Figure-1
Effect of drought on plant height of (a) black gram and (b) green gram. Error bars indicate ± SE(C-control, D- drought)

Effect of drought stress on yield and yield attributing parameters: Yield loss due to drought was more pronounced prominent in KU 301 and SG 21-5 than T9 and Pratap for all the treatments (Table -2). The percentage reduction in yield was highest in T_3 plants (T9-31.28%, KU 301- 48.52%, Pratap-37.12%, SG 21-5- 56.98%) and it was in the order of $T_3>T_4>T_2$. Drought had a pronounced impact on various yield indexes. Higher values of mean productivity (MP), rate productivity (RP), and drought tolerance index (DTI) were obtained for T9 and Pratap (Table-2 and Table-3). These two genotypes also recorded greater value of harvest index (HI) irrespective of treatments. Differences in seed yield were statistically significant due to genotypes, water stress treatments and their interactions ($p \leq 0.05$).

Under control condition, all the genotypes gave significantly higher seed yield than the drought treated plants (Table-2). This decline in yield traits under water deficit is related to disruption of leaf gas exchange properties which not only limits the size of the source and sinks tissues but the phloem loading; assimilate translocation and dry matter partitioning[17]. In the present study, higher reduction in leaf number and area of genotypes KU 301 and SG 21-5 under stress condition gave reduced source size leading to lower photosynthesis and lesser yield than rest of the genotypes. Yield loss caused by drought was highest in plants receiving stress during early reproductive stage. At this stage, the development of reproductive organs are under the control of photo-assimilate production and partitioning by the source tissues. Hence, water stress has a pronounced effect on grain development in the genotypes of black gram and green gram.

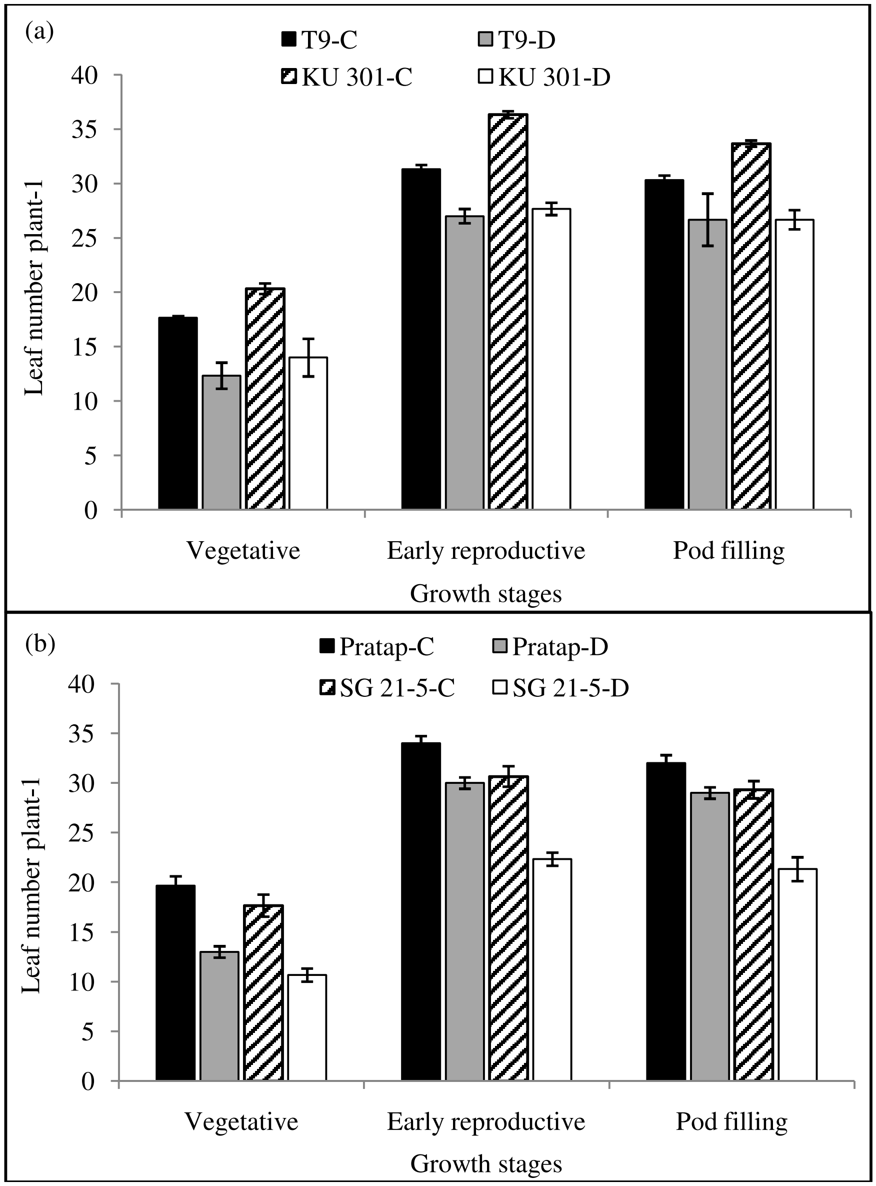

Figure-2
Effect of drought on leaf number of (a) black gram and (b) green gram. Error bars indicate ± SE(C-control, D- drought)

Table-2
Seed yield, mean productivity (MP) and rate productivity (RP) of black gram and green gram (mean± standard error, different letters indicate significant differences between treatments based on DMRT at P = 0.05)

Genotypes	Seed yield (q/ ha)				MP	RP
	T_1	T_2	T_3	T_4		
T9	11.14±0.02a	9.95±0.03ab	7.65±0.12c	8.08±0.04bc	9.21	0.77
KU 301	10.75±0.04a	8.34±0.03b	5.53±0.03c	6.05±0.07b	7.67	0.62
Pratap	12.04±0.09a	10.07±0.04b	7.57±0.05c	8.24±0.06c	9.48	0.72
SG 21-5	12.26±0.02a	9.15±0.06b	5.28±0.02c	7.65±0.02c	8.58	0.60

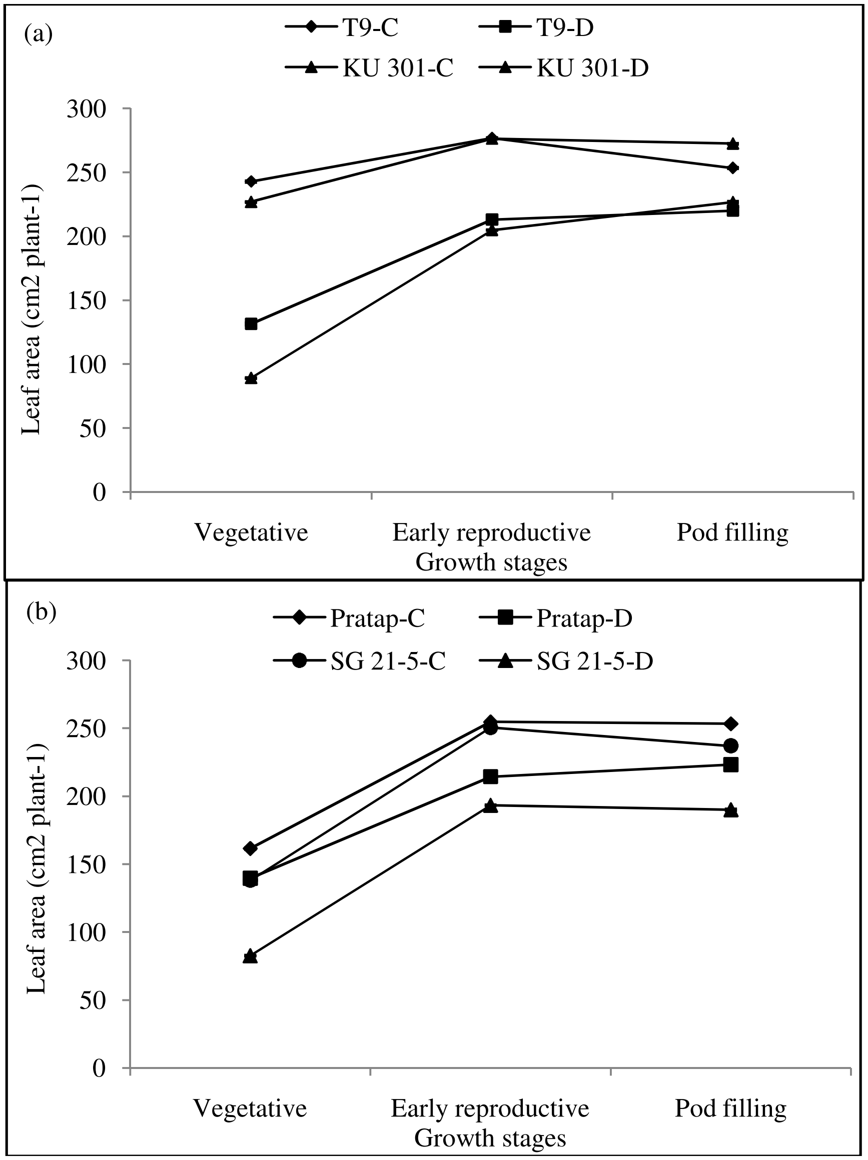

Figure-3
Effect of drought on leaf area of (a) black gram and (b) green gram. Error bars indicate ± SE(C-control, D- drought)

Table-3
Harvest index (HI) and drought tolerance index (DTI) of black gram and green gram

Genotypes	Harvest index (%)				DTI
	T_1	T_2	T_3	T_4	
T9	52.37	48.56	46.52	43.38	8.71
KU 301	51.86	42.06	36.59	37.30	6.52
Pratap	53.31	48.85	44.22	43.86	8.55
SG 21-5	51.05	41.80	31.38	39.87	7.42

Table-4
Correlation coefficient of seed yield with the studied traits

Parameters	**Seed yield**			
	T9	**KU 301**	**Pratap**	**SG 21-5**
RLWC	0.948**	0.982**	0.984**	0.961**
Plant height	0.008	0.120	0.095	0.084
Leaf number	1.181	0.230	0.041	0.238
Leaf area	0.460	0.380	0.164	0.288
Shoot: root ratio	0.521	0.597	0.386	0.516

**.Correlation is significant at the level 0.01 (2-tailed).

Harvest index were considerably decreased by water deficit (Table-3). Drought caused a disorder in the partitioning of carbohydrates to the pods and thus hampering in pod filling process[18]. As a consequence we obtained reduced pod weight and harvest index of the stressed plants. Earlier workers also observed higher sensitivity of reproductive growth to water stress compared to generative growth[19]. Beside producing higher seed yield, the genotypes T9 and Pratap also showed greater values of HI, MP, RP, and DTI. Similar findings were reported by other workers while working with chickpea and wheat[20,21].

Conclusion

A remarkable impact of drought had been observed on plant morpho- physiological and yield characteristics of these two crops. In water limited environment, this information will be helpful to provide a basis for development of strategies to stabilize their yields. Genotypes maintaining higher relative leaf water content gave better yield and hence it can be selected as an important stress marker. These morpho-physiological traits may be interesting for selection of drought tolerant genotypes for improved productivity in drought prone environments, as these are relatively simple to evaluate. Early reproductive stage had been proved to be more vulnerable stage in terms of yield loss. Therefore, at this stage proper irrigation should be provided. Among the studied genotypes, T9 and Pratap were identified as drought tolerant genotypes for Assam (India) and areas with similar environmental conditions.

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