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Morpho-physiological responses in different mungbean genotypes under drought stress

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Abstract

The present experiment was performed with the objective of studying the morpho-physiological differences in seven mungbean genotypes namely, PDM 54 (V1), PDM39 (V2), IPM99-125 (V3), PDM11 (V4), IPM2-14 (V5), IPM2-3 (V6) and Pratap (V7) under water deficit environment. Some known parameters with their essential roles in drought stress like plant height, leaf no., leaf area, fresh and dry shoot and root biomass, leaf proline content and total chlorophyll were considered for the study under drought both in vegetative and reproductive stages. Results fed showing significant effects of water deficit conditions in all the seven mungbean genotypes. However, the detrimental effects of drought stress were more prominent in genotype IPM99-125 (V3), and left fewer changes in genotype Pratap (V7) which eventually depicts their differential behaviours under drought stress and the difference in their tolerance.

Keywords: Mungbean, drought, genotype, morpho-physiological responses, tolerance.

Introduction

Food productivity has emerged as a major concern worldwide owing to the detrimental effects of abiotic as well as biotic factors. This has necessitated study of stress and minimizing its loss has become essential to ensure the same. Environmental abiotic stress like cold, high temperature stress, drought, heavy metals, salinity etc. has been continuously impairing plant growth and imposing threat to the food production worldwide.

Drought is however one of the most potential devastating event impairing plant growth, development and their performance, amongst all other environmental factors[1]. Plants succumb to drought condition either when the transpiration rates are too high or when the supply of water to the roots is sub-optimum. Furthermore, considering the climate change models, scientists have come up stating that with the depletion of water resources, crop productivity would be even more highly accentuated in the recent times.

Mungbean, being known as an essential tropical grain legume, an important cash crop with high protein content and high nitrogen fixing capability brings into focus the research it needs. In contrast, with erratic rainfall, the production of mungbean is continuously threatened with the expanding drought stressed zones. This encourages researchers to know more about mungbean drought tolerance which thereby necessitates adetailed screening of the morphological differences lying beneath.

Plants and their response to drought are generally monitored by analysing the basic morphological and physiological parameters, already known as potential indicators of drought tolerance in a huge number of studies[2]. Some of them include plant growth, leaf characters, stomatal properties, and water relations[3]. In this study we are basically trying to make an assessment on the morpho-physiological differences of seven mungbean genotypes and further study how if they behave differentially under water deficit conditions.

Materials and methods

Plant material and growth conditions: We conducted the experiment in earthen pots with seven popular mungbean genotypes in Tezpur University which is located at north bank plain zone of Assam (26º14´ and 92º50´), Tezpur, India during September to December, 2014. The earthen pots were then kept under a temporary rain shed to avoid rainfall which was constructed with polyvinyl chloride (PVC) film of about 0.15 mm thickness and 85% transmittance. Mungbean seeds of these seven genotypes viz., PDM 54 (V1), PDM39 (V2), IPM99-125 (V3), PDM11 (V4), IPM2-14 (V5), IPM2-3 (V6) were supplied by Indian Institute of Pulses Research (IPRI), Kanpur, India and the seventh genotype Pratap denoted as V7 was obtained from Regional Agricultural Research Station (RARS), Shillongoni (Nagaon), Assam, India. Two treatments had been arranged for each genotype: One set of plants were maintained as control plants with regular watering. T1 - treatment plants where watering was withdrawn for 10 consecutive days during the vegetative stage which is almost 21 days after germination. T2-treatment plants where watering was withdrawn for 10 consecutive days during reproductive stage. The experiment was conducted in randomized block design with three replications.

Biochemical analysis: Analysis of some already known biochemical marker traits for drought tolerance was carried out in the second leaf from the top during vegetative stage of the crop. Leaves obtained from control and stressed plants were used to calculate relative leaf water content according to the method deciphered by Yang et al. with the following formula[4]:

$$RWC\ (\%) = [(FW-DW)/(TW-DW)] \times 100\%.$$

Anderson and Boardman's method was utilised to ascertain the total chlorophyll content of the genotypes[5]. Proline content of the leaves was determined by the method described by Bates and his group of researchers and was expressed as μ mol g^{-1}fresh weight[6]. Morphological parameters namely leaf number, leaf area, plant height, dry root and shoot biomass were measured every week. Parameters were measured before and after application of stress. The morphological parameters were measured on one particular plant throughout the experiment. Leaf number was measured by counting the numbers of the leaves. Laser leaf area meter (CI-203, USA) was employed to measure the leaf area. Plant height was measured using a thread and a scale.

Results and discussion

Effect of drought stress on leaf number: Leaf number was recorded for each genotype both before and after treatment. Drought has been found to decline the leaf number in the treated mungbean genotypes in comparison with the control plants. Drought stress both in vegetative as well as reproductive phases brought more defoliation in genotype V3 as compared to the other genotypes. On the contrary, drought during the vegetative stage gave genotype V5 highest reduction in leaf number, while genotype V6 gave the least when exposed to drought during reproductive stage (Figure-1a).

Effect of drought stress on leaf area: During initial stages of growth, a decreasing trend in leaf area was seen in all the genotypes after water was withdrawn due to senescence (Figure-1b). However, highest reduction in leaf area was seen in genotype V3 during water deficit conditions at vegetative stage, while the lowest was recorded in genotype V7 by maintaining a higher leaf area (Table-1). Drought on the other hand, caused highest reduction in leaf area in genotype V3 whereas lowest was observed in genotypes V1 when given during reproductive stage (Table-2). The reduction in leaf area recorded during drought in all the genotypes from the present experiment is due to decline in cell division and cell enlargement which finally leads to drop in cell number and deteriorates cell expansion and then reducing the average cell size as documented by researchers in several crops under drought[7,8]. However, the significantly lower reduction documented in genotypes such as V3 in vegetative stage and V4 and V7 in reproductive stage might be due to the inter-specific differences among them.

Effect of drought stress on plant height: Control plants of all the mungbean genotypes maintained a higher plant height as compared to the treated ones. Once water was withdrawn, the genotypes showed a significant though linear decline in plant height during both vegetative and reproductive stages. However, the decline was highest and lowest in genotypes V3 and V7 respectively (Figure-1c). The reduced height in all the mungbean genotypes can be well related to loss of cell turgor pressure which leads to impairment of cell growth and development[7]. Similarly, the decline in plant leaf number might be due to the inhibition of mitosis, and newer cell formation.

Effect of drought stress on Relative leaf water content (RLWC): Substantial reduction in RWC of leaves was recorded for all the genotypes under drought while, the highest percentage reduction (54%) was seen in genotype V3 for both the stages. Significant differences were recorded between the genotypes under control and drought (Figure-1d). Relative leaf water content is another parameter that is affected by the drought resulting in decrease in cell size[9]. From our experiment we documented that genotype V3 recorded the maximum reduction in relative leaf water content and genotype V4 noted the higher recovery irrespective of the growth stages. The previously estimated content of proline and sugar justifies the lower RLWC in genotype V3 as the plant is unable to draw water from the soil.

Effect of drought stress on leaf chlorophyll: Lower total chlorophyll content was observed in all genotypes after drought treatment. Recorded values indicated significant differences under water stress between the genotypes V2, V3 and V7 which contrasted with observations under control. Whereas, genotypes V1 and V6 were similar under control and responded significantly different under drought in the vegetative stage. Furthermore, genotype V3 and V7 was seen to show its maximum susceptibility and maximum tolerance respectively under stress in both the stages. Adding and reconfirming the former results, sharp drop in CSI was notedin genotype V3, while the highest CSI was observed in genotype V7 (Figure-1e). Chlorophyll content of all the studied genotypes are severely affected under drought stress by alteration of the chlorophyll content, changes in photosynthetic components and thereby damaging the photosynthetic apparatus[10]. In the present experiment it has been found that plant undergoing drought stress suffered loss in chlorophyll content which is a confirmatory with various research findings[11]. Lower chlorophyll content during drought stress can be majorly attributed to damage of photosynthetic apparatus that is chloroplasts caused by active oxygen species[12]. Researches also emphasized that both chlorophyll a and chlorophyll b shows a decreasing trend when plants undergo drought stress[13]. The recorded reduction in chlorophyll content (chlorophyll-a, chlorophyll-b and total chlorophyll) of green gram genotypes under water scarcity could be due to the detrimental effects of drought stress on enzymes responsible for chlorophyll biosynthesis and may be due to degradation of the chlorophyll pigment. The highest reduction in the chlorophyll content in genotype V5 indicates its sensitivity towards drought more particularly at vegetative stage. On the other hand, the maintenance of relatively higher chlorophyll content in genotype V7 showed its ability to conserve the same event under water stress condition at both vegetative and reproductive stage. This reduction in chlorophyll content is also associated with decreased assimilation rate and the activity of the enzyme systems[14].

Effect of drought stress on leaf proline: Water stress significantly increased proline content in all the genotypes. Genotypes V3 and V7 have shown significantly different behaviour both under controlled and drought stress conditions. Coinciding their differences, genotype V7 responded more sharply for accumulating highest proline content (62%), while the least value (42%) of proline production was recorded in genotype V3 under both the stages (Figure-1f). During osmotic stress, plants accumulate proline which is one of the essential amino acids. Proline is known to regulate several essential plant functions during water stress by various osmotic adjustments, production of several osmo-protectants, scavenging free radicals & anti-oxidants, protecting macromolecules from degradation & denaturation, regulating cytosolic acidity & by sustaining a reserve for carbon & nitrogen post-stress[15-18]. Accumulation of proline under drought stress has been reported in many plants such as chick pea, and corn[19,20]. Production of ethylene is also known to be induced by drought dominance and thereby inducing senescence[21]. The documented higher proline content in genotype V7 during both vegetative and reproductive stage justifies its ability to provide maximum cell osmoprotection during water scarcity. In contrast the lowest accumulation in proline in genotype V3 indicates its least ability of osmoprotection during water stress. Thus the higher proline concentration of genotype V7 during stress provides better recovery.

Effect of drought stress on shoot and root biomass: Drought at vegetative stage showed highest reduction of fresh biomass in genotype V2 and lowest in genotypes V7. While, when recorded the same in reproductive stages, it was found to be higher in genotype V3 and less in genotype V6 after drought treatment (Fig: 1g). On contrary dry biomass was recorded for all the mungbean genotypes during drought and after drought recovery. Water stress led to significantly decreased rate of production of dry biomass with the highest decrease in genotype V2 and the least in genotype V6 at vegetative stage (Figure-1h). Drought stress during reproductive stage highly reduced the dry biomass, causing its highest reduction in genotype V1 and maximum recovery in genotype V4. These research findings are also supported by earlier works, who observed inter specific differences between two varieties of populous plant in total leaf area and total biomass under drought[22]. Again, the maintenance of higher plant biomass under water limited condition is a desirable character as the reduction of plant biomass has been often noted as a negative effect of drought on crops[23]. Simultaneously, the recorded reduction in plant biomass is due to simultaneous reduction of plant growth, plant height and leaf number. However, the change in resource pool (water) affects the rate of photosynthesis and distribution of photosynthate resulting lower plant biomass. The documented reduced leaf area, plant height and leaf number from our experiment support this statement. The highest reduction of plant biomass in genotype V2 indicates its sensitivity to drought, whereas by maintaining relatively higher biomass, genotype V7 showed its ability to resist the applied drought. The ability of genotype V7 to maintain higher leaf number, plant height and leaf area even during drought compared to the rest genotypes under consideration also shows accordance with our results. However, the better recovery seen in genotype V4 indicates its capability to recover after a moderate water stress of about 10 days.

Table-1: Two-way ANOVA results demonstrating the mean squares of the parameters and the significance ($P \leq 0.05$) within the mungbean genotypes in the vegetative stage.

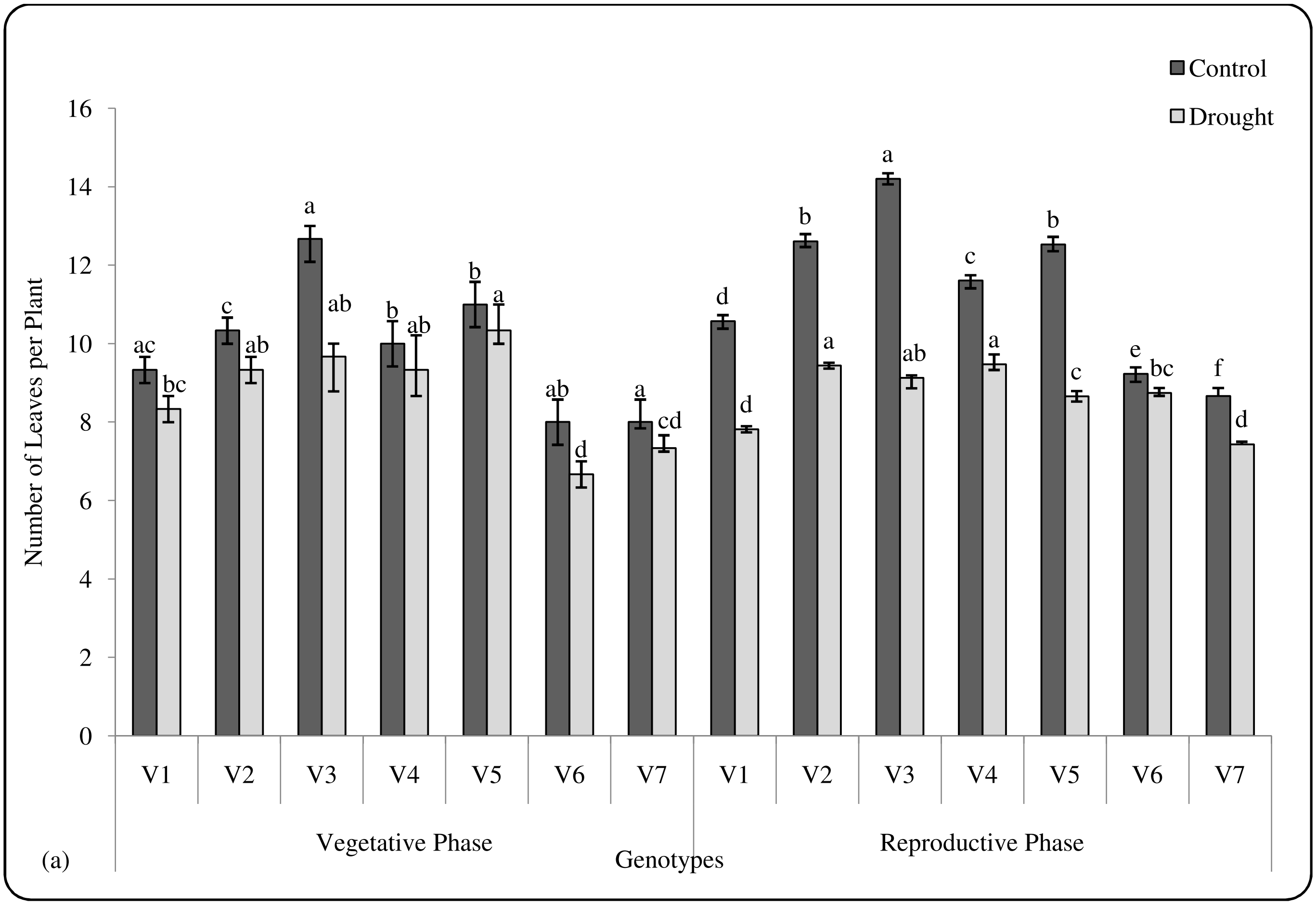
Parameters	Treatment	Variety	TxV	Significance S/NS	Error
RWC (%)	2896.305	227.336	248.964	S	0.098
Chlorophyll (mgchl g^{-1}fr. wt.)	9.613	65.146	7.342	S	0.092
Proline (µmole g^{-1}fr. wt.)	95.452	1.254	1.131	S	0.024
Leaf number	12.524	14.881	1.048	S	0.738
Leaf area (cm^2)	2904.433	10092.360	941.804	S	5.340
Plant height (cm)	92.097	111.720	14.290	S	0.311
Fresh matter (g $plant^{-1}$)	0.072	0.252	0.029	S	7.905E-6
Dry matter (g $plant^{-1}$)	0.012	0.046	0.015	S	0.00

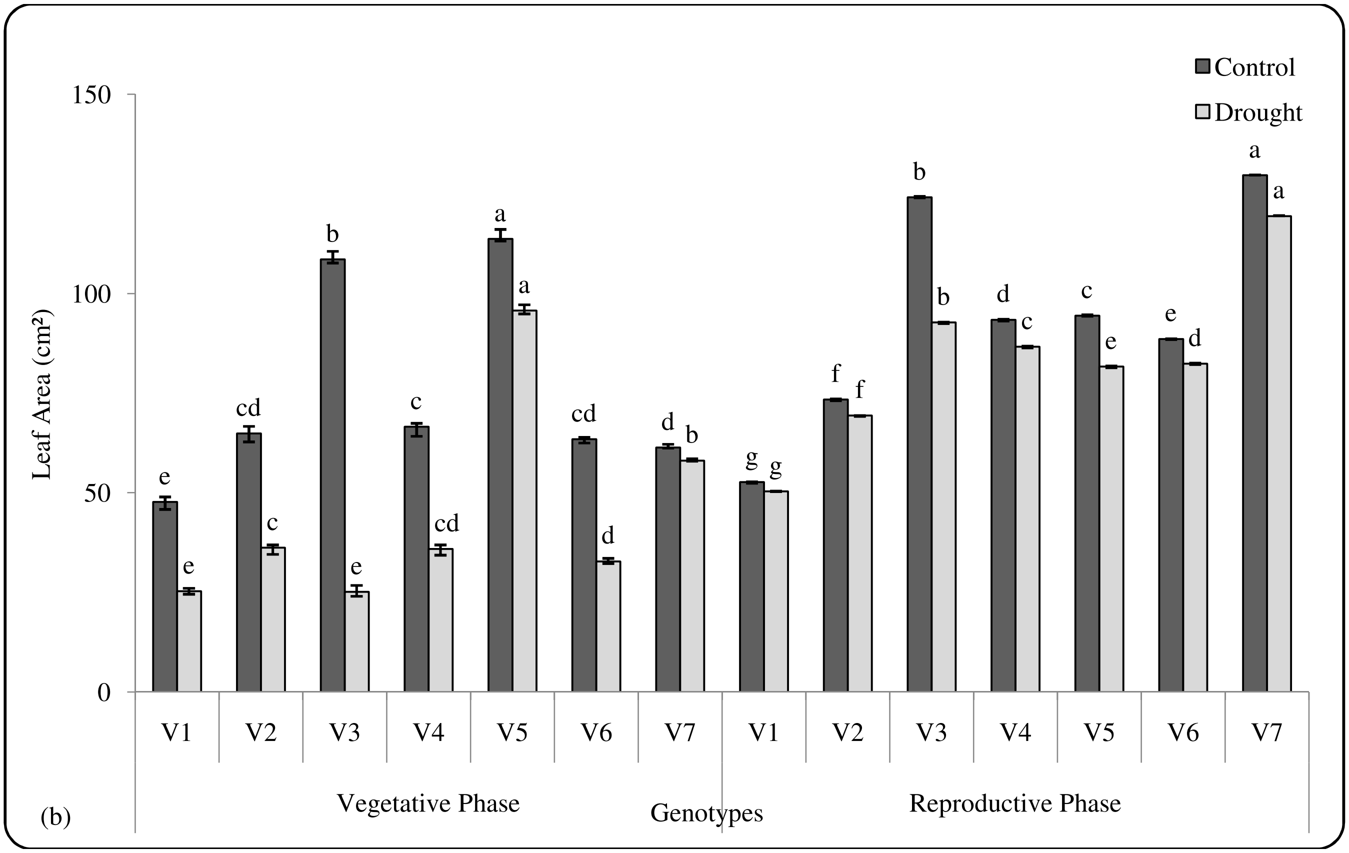
** = significant at 0.01 probability level.

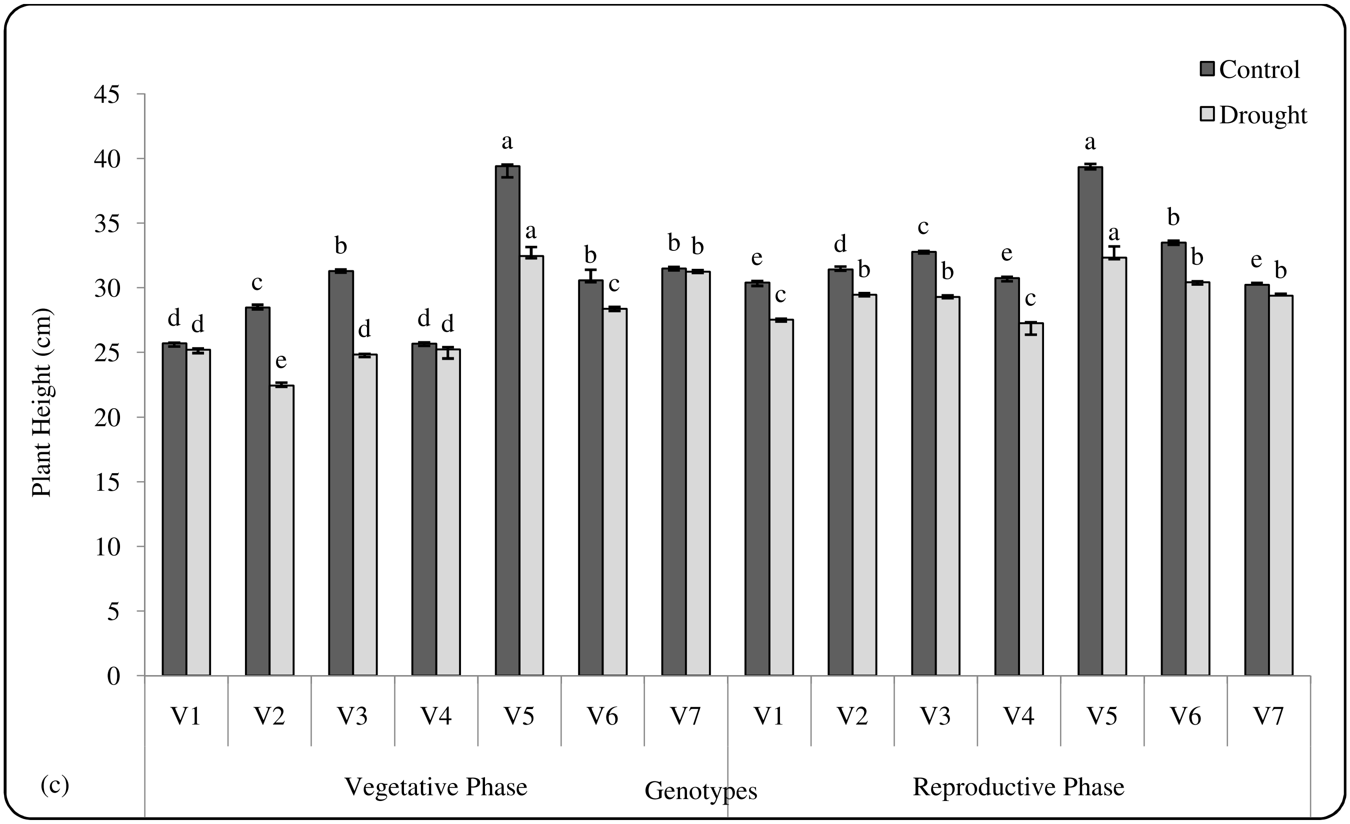
Table-2: Two-way ANOVA results demonstrating the mean squares of the parameters and the significance ($P \leq 0.05$) within the mungbean genotypes in the reproductive stage.

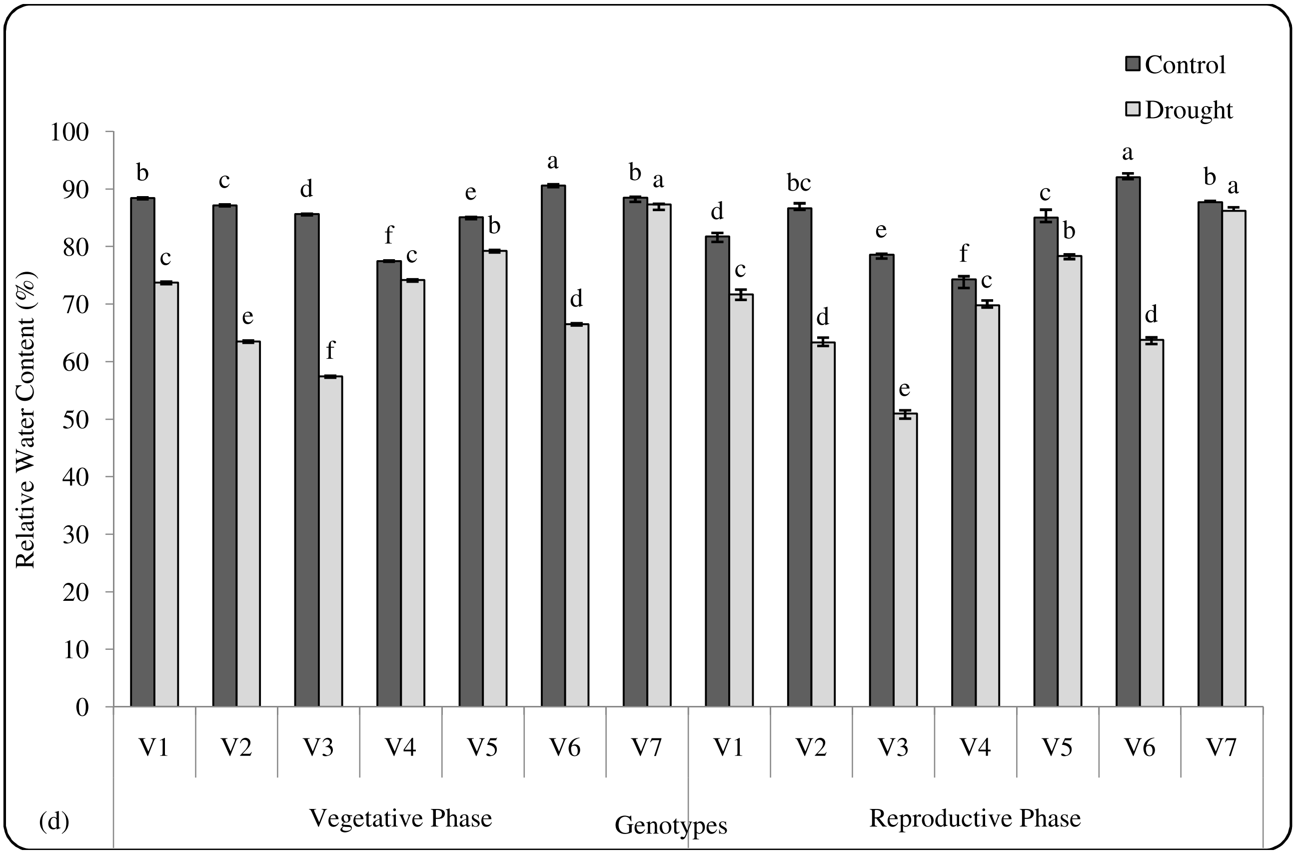
Parameters	Treatment	Variety	TxV	Significance S/NS	Error
RWC (%)	2223.138	297.557	197.235	S	1.648
Chlorophyll (mgchl g^{-1}fr. wt.)	52.260	11.601	5.902	S	0.040
Proline (µmole g^{-1}fr. wt.)	90.054	2.714	1.505	S	0.035
Leaf number	9.939	75.228	3.647	S	0.071
Leaf area (cm^2)	3371.015	1166.146	146.091	S	0.096
Plant height (cm)	34.161	110.646	5. 486	S	0.225
Fresh matter (g $plant^{-1}$)	0.043	0.225	0.034	S	0.00
Dry matter (g $plant^{-1}$)	0.020	0.051	0.010	S	8.890 E-5

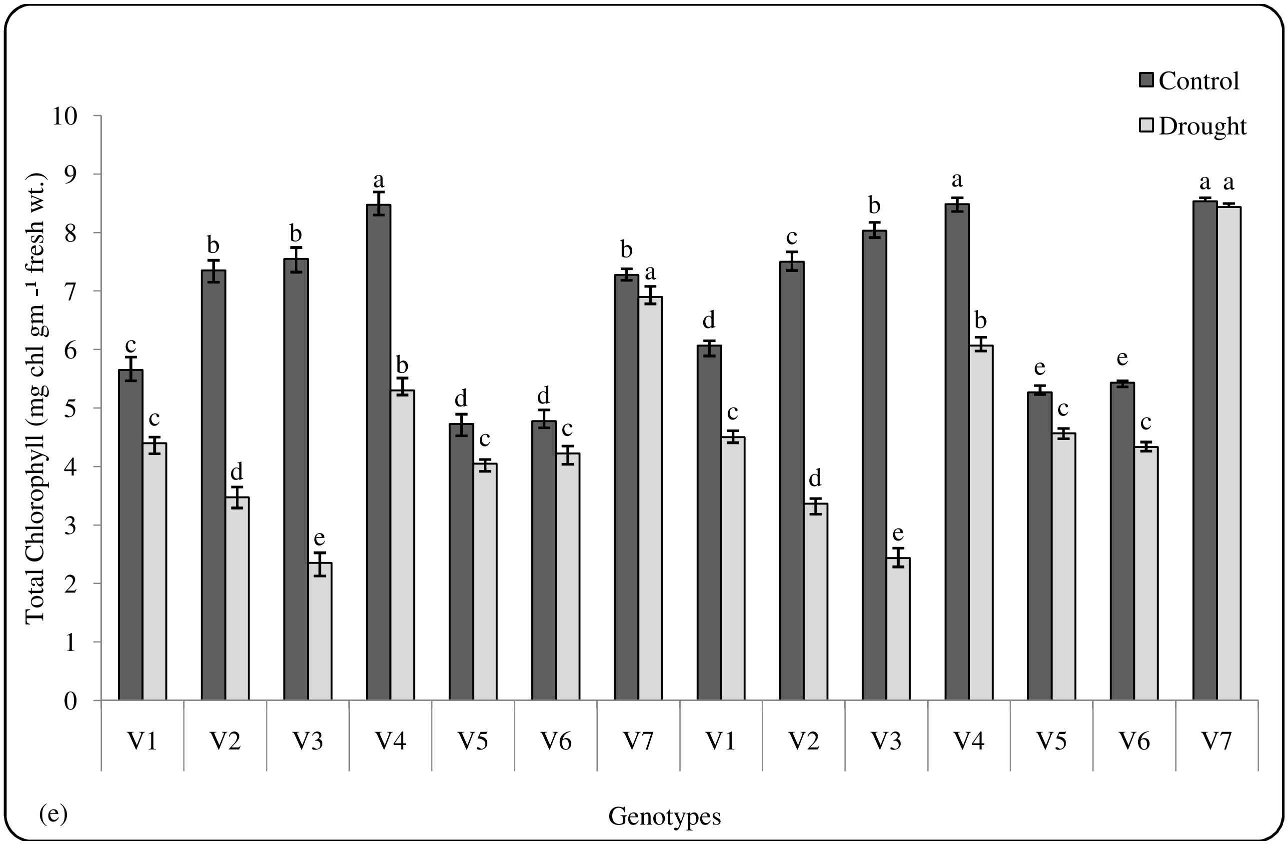
** = significant at 0.01 probability level.

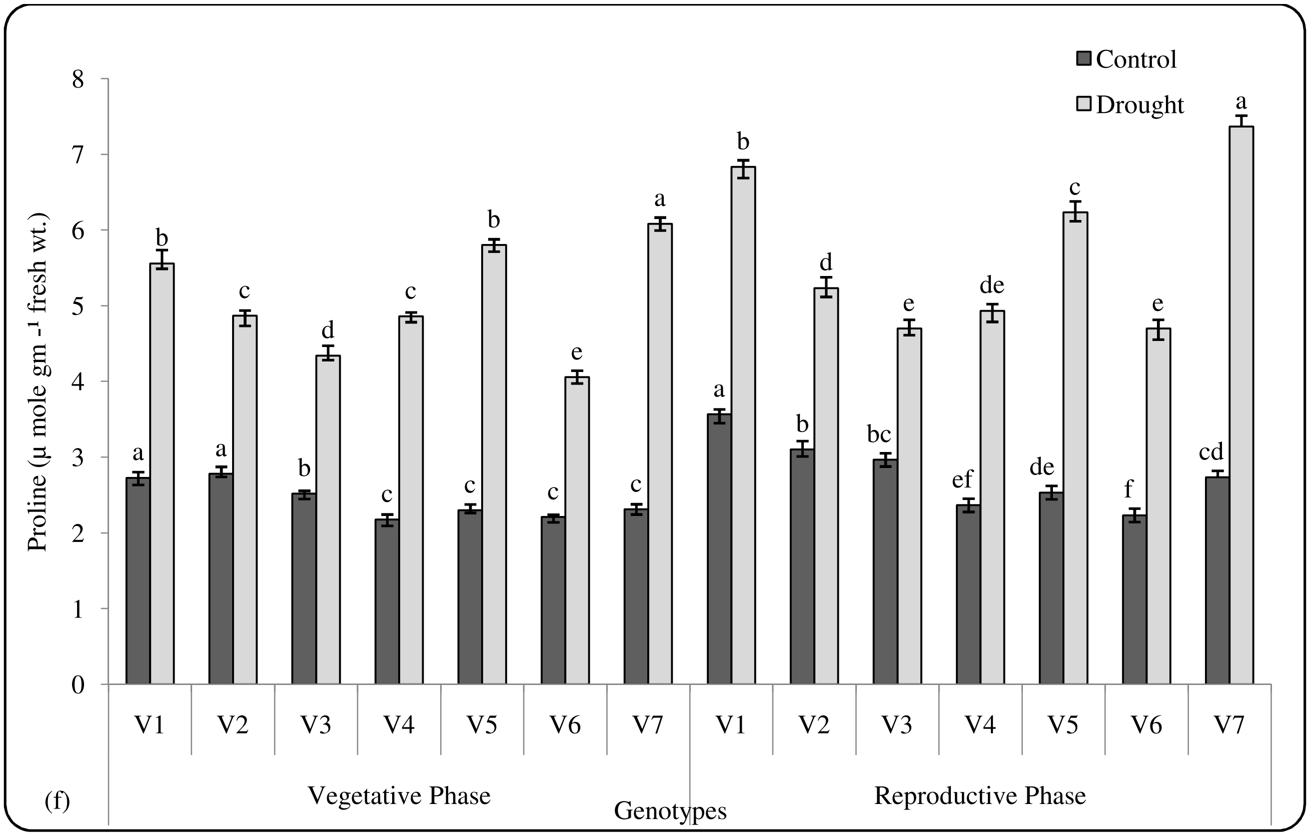


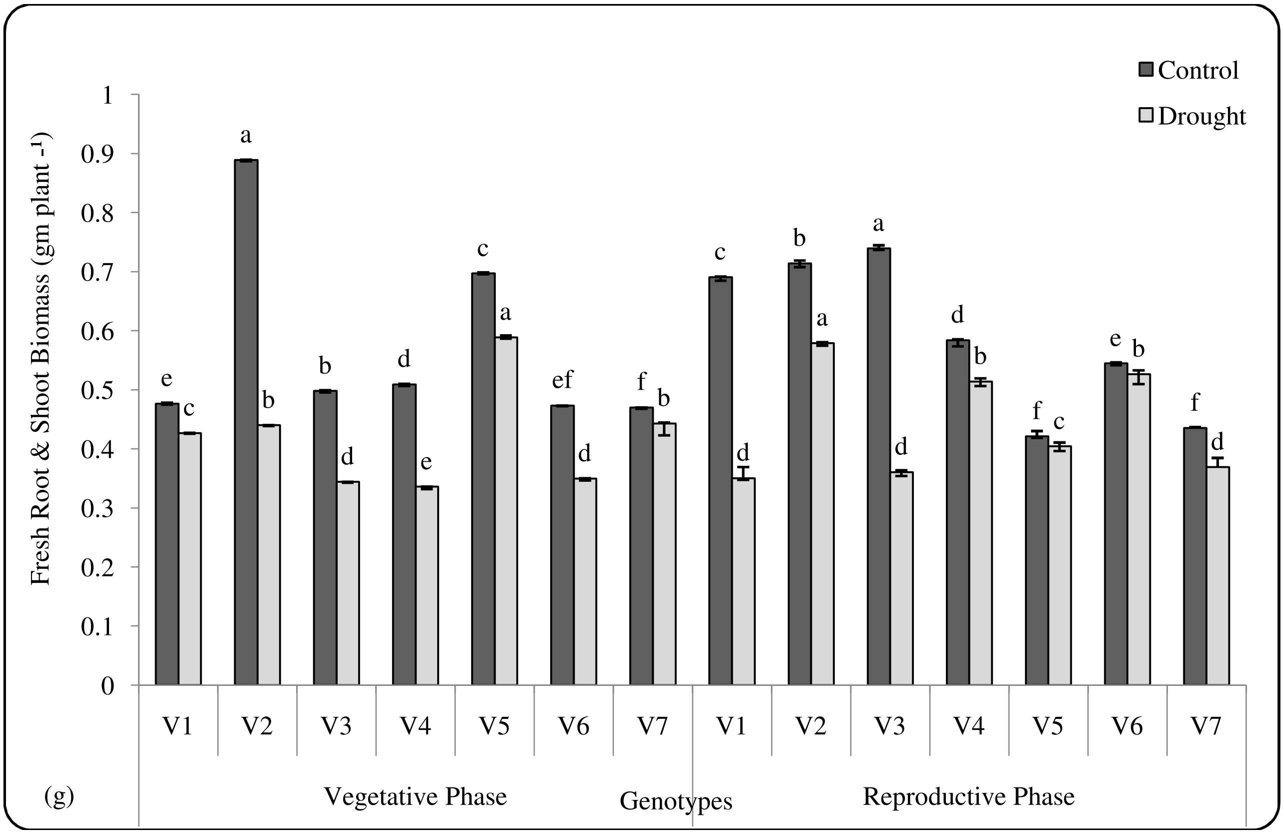

■ Control
□ Drought
150
100
50
0
Leaf Area (cm²)
e
e
cd
c
b
e
c
cd
a
a
cd
d
d
b
g
g
f
f
b
b
d
c
c
e
e
d
a
a
V1
V2
V3
V4
V5
V6
V7
V1
V2
V3
V4
V5
V6
V7
Vegetative Phase
Genotypes
Reproductive Phase
(b)


■ Control
□ Drought
45
40
35
30
25
20
15
10
5
0
Plant Height (cm)
d
d
c
e
b
d
d
d
a
a
b
c
b
b
e
c
d
b
c
b
e
c
a
a
b
b
e
b
V1
V2
V3
V4
V5
V6
V7
V1
V2
V3
V4
V5
V6
V7
Vegetative Phase
Genotypes
Reproductive Phase
(c)


Control
Drought
Relative Water Content (%)
100
90
80
70
60
50
40
30
20
10
0
b
c
c
e
d
f
f
c
e
b
a
d
b
a
d
c
bc
d
e
e
f
c
c
b
a
d
b
a
V1
V2
V3
V4
V5
V6
V7
V1
V2
V3
V4
V5
V6
V7
(d)
Vegetative Phase
Genotypes
Reproductive Phase


Control
Drought
Total Chlorophyll (mg chl gm -¹ fresh wt.)
10
9
8
7
6
5
4
3
2
1
0
c
c
b
d
b
e
a
b
d
c
d
c
b
a
d
c
c
d
b
e
a
b
e
c
e
c
a
a
V1
V2
V3
V4
V5
V6
V7
V1
V2
V3
V4
V5
V6
V7
(e)
Genotypes


■Control
□Drought
Proline (µ mole gm -¹ fresh wt.)
0
1
2
3
4
5
6
7
8
a
b
a
c
b
d
c
c
c
b
c
e
c
a
a
b
b
d
bc
e
ef
de
de
c
f
e
cd
a
V1
V2
V3
V4
V5
V6
V7
V1
V2
V3
V4
V5
V6
V7
(f)
Vegetative Phase
Genotypes
Reproductive Phase


■Control
□Drought
Fresh Root & Shoot Biomass (gm plant -¹)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
e
c
a
b
b
d
d
e
c
a
ef
d
f
b
c
d
b
a
a
d
d
b
f
c
e
b
f
d
V1
V2
V3
V4
V5
V6
V7
V1
V2
V3
V4
V5
V6
V7
(g)
Vegetative Phase
Genotypes
Reproductive Phase

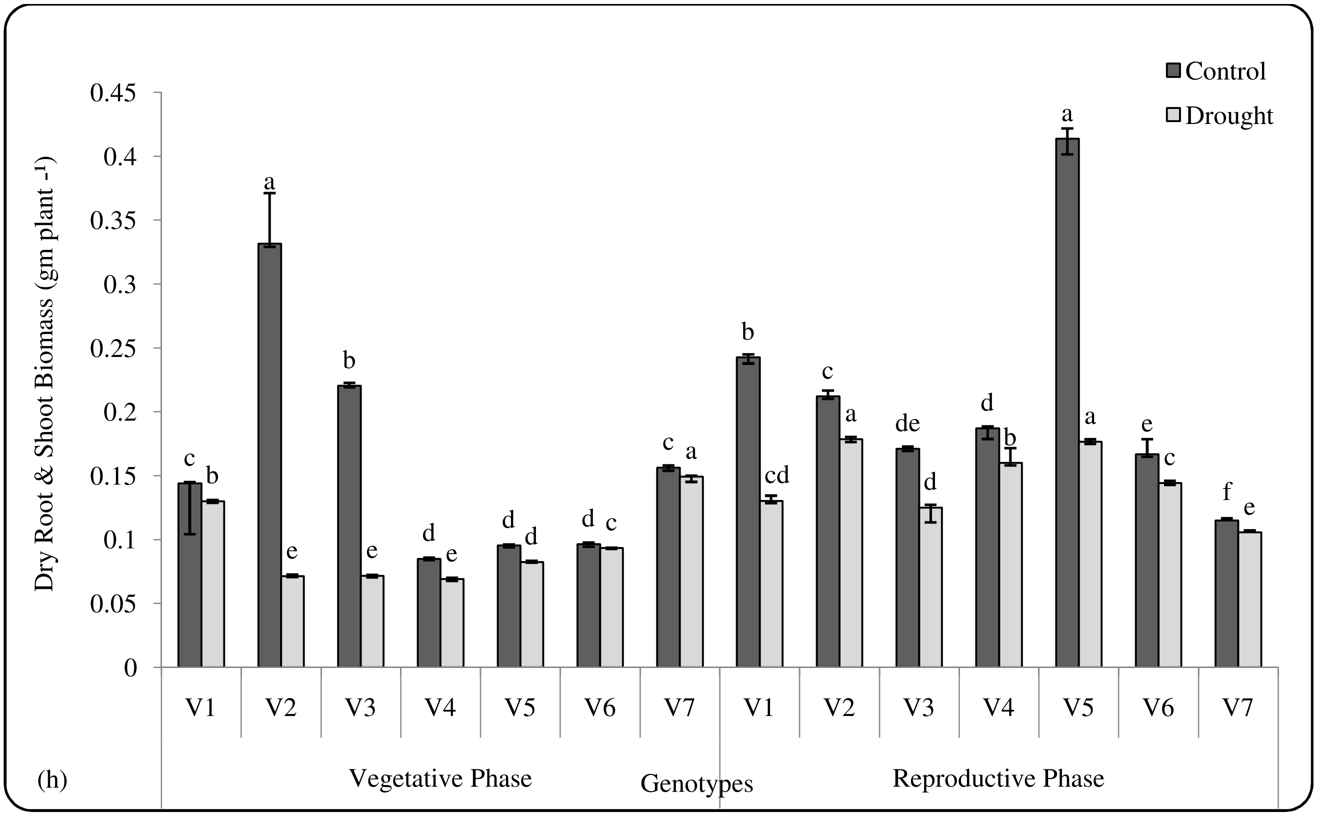


Figure-1: Effects of drought stress in seven mungbean genotypes during vegetative & reproductive phases in the following physio-morphological parameters: (a) Number of Leaves per Plant, (b) Leaf Area, (c) Plant Height, (d) RWC, (e) Chlorophyll, (f) Proline, (g) Fresh Matter & (h) Dry Matter. Error bars indicate ± SE.

Conclusion

In this study, the observed higher reduction of studied morphological parameters in genotype V3 (IPM99-125) indicates its susceptibility to water stress more particularly during vegetative stage. On the contrary, the least changes in the studied morphological parameters of genotype V7 (Pratap) showed its better tolerance towards drought among the seven studied genotypes of green gram. It can hence be employed in various crop-breeding programs to develop transgenics.

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