Original Research Article

Proline accumulation and drought tolerance in green gram (*Vigna radiata* L.)

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ABSTRACT

Green gram (*Vigna radiata* L.), a key legume crop in Asia, contributes to sustainable agriculture by fixing atmospheric nitrogen and providing valuable nutrients. Despite its importance, productivity is hindered by various factors including drought, affecting key physiological and biochemical processes, leading to reduced yields. This study aimed to identify drought-tolerant green gram genotypes by evaluating 50 accessions for root, shoot, and biochemical parameters under controlled moisture stress.

An increase in root length and diameter under stress was observed, which aids in nutrient uptake and osmoregulation. Conversely, shoot length and dry weight decreased due to moisture limitations, with genotypes like VBN 3 and PLM 38 showing resilience by maintaining higher dry weights. Biochemical analysis revealed that proline accumulation, which correlates positively with drought tolerance, increased in most genotypes, particularly in IC 395518 and ML 1415. This suggests its role in maintaining cell turgor and mitigating stress effects. Chlorophyll content decreased under stress, while total phenolic content rose in some genotypes, further indicating drought tolerance.Correlation and path analysis revealed strong positive relationships between root traits and proline content, emphasizing their importance in drought tolerance.

The study concludes that genotypes with robust root systems and higher proline accumulation are more capable of withstanding drought, highlighting the need for breeding programs targeting these traits for improved green gram productivity under changing climates.

Keywords: *moisture stress, biochemical parameters, root length, shoot length, dry weight*

1. INTRODUCTION

Green gram (*Vigna radiata* L.) holds significant importance as a grain legume in Asia. Among the 13 food legumes cultivated in India, it stands as the third most crucial pulse crop, following chickpea and pigeon pea [1]. This crop is characterized by being diploid, self-pollinating, fast-growing, and having short growth duration [2].

Green gram possesses wide adaptability and demands minimal input resources [1]. Its robust root system architecture actively participates in fixing atmospheric nitrogen (30-50kg/ha) through symbiosis with *Rhizobium* bacteria [3], contributing significantly to soil fertility enhancement and sustainable agricultural yields. Additionally, as a rich source of vegetable proteins, micronutrients, and antioxidants like flavonoids and phenolics, green gram serves various purposes including food, animal feed, fodder, and green manure [4].

Despite its economic importance, the productivity of green gram remains stagnant due to unpredictable weather patterns and various environmental stresses. Among these stresses, drought poses the greatest challenge to green gram cultivation, hindering its growth and development [5].

Drought stress impacts various physiological processes crucial for growth and molecular functioning, resulting in reduced pod yield [6]. Initially, drought stress hampers seed germination and disrupts seedling establishment by affecting cell division and elongation, thereby impeding crop growth. It also disrupts assimilate balance, reduces sucrose content, and ultimately decreases dry matter allocation [7].

The characters like plant height, seed weight, root architecture and crop yield are significantly reduced under the drought stress conditions in green gram and other legumes. Zare *et al*. (2013)[8] reported a significant yield reduction of 51% to 85.5% due to drought stress in green gram, with flowering and post-flowering stages being more sensitive than the vegetativestage.Hence, there is an utmost need to develop drought tolerant varieties to improve crop productivity especially under the changing climate.

2. MATERIALS AND METHODS

The experimental material consisted of 50 different green gram (*Vigna radiata*) accessions collected from National Bureau of Plant Genetic Resources, New Delhi, Tamil Nadu Agricultural University, Coimbatore, SardarkrushinagarDantiwada Agricultural University, Gujarat, National Pulse Research Centre Vamban, RARS Pattambi under Kerala Agricultural University and various other local accessions. The experiments were conducted at College of Agriculture, Vellayani located 8.5° N, longitude of 76.9°E and an altitude of 96 m above mean sea level. The study was conducted during February to April 2024 in Completely Randomized Design in three replications. Four seeds of each accession were raised in pot. The moisture stress was imposed in the pot by withdrawing irrigation for 15 days at critical stages of growth *viz*., flowering and podding stage of the crop (reproductive stage). The soil moisture was also measured during this period by following gravimetric method. One control with all genotypes was maintained under irrigated conditions. Observations were recorded after 15 days of drought on various characters namely, seedling shoot length (cm), seedling root length (cm), seedling dry weight (g), root diameter (cm) and root dry weight (g). Various biochemical parameters indicating drought tolerance were also estimated.

2.1. Estimation of Proline (µmol g^{-1})

Proline levels were determined using the acid ninhydrin method proposed by Bates *et al.* in 1973 [9]. To create the sample extract, 0.5 grams of fresh leaf were blended in 10 ml of 3% aqueous sulphosalicylic acid. After filtering the mixture, 2 ml of the filtrate was combined with 2 ml each of acetic acid and acid ninhydrin in a test tube. The mixture underwent heating at 100°C for an hour in a water bath. Subsequently, the reaction was halted by immersing the test tubes in an ice bath for 10 minutes. After adding 4 ml of toluene and thorough stirring, the toluene-containing chromatophore was gathered, brought to room temperature, and its absorbance at 520 nm was measured using toluene as a reference. By preparing a range of proline standards using L-proline powder and constructing a standard curve, the proline content in the sample was determined.

$$\text{Proline content } (\mu\text{mol g}^{-1}) = (\mu \text{ proline/ml} \times \text{ml toluene}) \times \frac{5}{115.5 \text{ g sample}}$$

2.2. Estimation of chlorophyll (mg g^{-1})

The chlorophyll content in leaves was determined following the procedure of Yoshida *et al.* in 1971 [10]. A 0.5 g sample from the third fully expanded leaf was finely chopped and placed into a test tube. These samples were then allowed to incubate overnight with a 10 ml mixture of 80% acetone and DMSO in a 1:1 volume ratio. The resulting solution was transferred to a measuring cylinder and diluted to a total volume of 25 ml with the 80% acetone and DMSO mixture. Absorbance measurements were taken at 480 nm, 510 nm, 645 nm, and 663 nm against a blank consisting of only the 80% acetone and DMSO mixture. The chlorophyll content was calculated in mg g^{-1} using the provided equations.

$$\text{Chlorophyll a (mg g}^{-1}) = (12.7 \times A_{663} - 2.69 \times A_{645}) \times \frac{1 \times V}{1000 \times \text{fresh weight}}$$

$$\text{Chlorophyll b (mg g}^{-1}) = (22.9 \times A_{645} - 4.68 \times A_{663}) \times \frac{1 \times V}{1000 \times \text{fresh weight}}$$

$$\text{Total chlorophyll (mg g}^{-1}) = (20.2 \times A_{645} + 8.02 \times A_{663}) \times \frac{1 \times V}{1000 \times \text{fresh weight}}$$

Where, A = absorbance at specific wavelength,

V = final volume of chlorophyll extract in 80% Acetone: DMSO mixture;

W = fresh weight of tissue extracted

2.3. Total phenol content (mg g-1)

The phenol content in seeds was determined following the method recommended by Sadasivam and Manickam in 1996 [11]. Initially, 0.5 g of leaf was homogenized in 5 ml of 80% ethanol. The resulting homogenate was then centrifuged at 10,000 rpm for 20 minutes, and the supernatant obtained was evaporated to dryness. The residue was subsequently dissolved in 5 ml of distilled water. A 2 ml aliquot was pipetted into test tubes and the volume was adjusted to 3 ml with distilled water. To this solution, 0.5

ml of Folin-Ciocalteau reagent was added. After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added to each tube and thoroughly mixed. The tubes were then placed in boiling water for 1 minute, cooled, and absorbance readings were recorded at 650 nm against a reagent blank. The phenol content of the sample was determined from a standard curve prepared with various concentrations of catechol.

$$\text{Phenol content (mg g}^{-1}\text{)} = \frac{\text{concentration of catechol in mg/ml x volume of extract in ml}}{\text{weight of plant extract in g}}$$

All the observations were subjected to standard statistical procedures using GRAPES software version 1.1.0 [12].

3. RESULTS AND DISCUSSION

3.1. Assessment of variability

The mean values of 50 genotypes for the characters namely, root length, shoot length, root diameter, total plant dry weight, root dry weight, proline content, total chlorophyll content, total phenol content are presented in table 1.The average soil moisture content of treatment pot was 24.39% and 1.72% at 8th and 15th day respectively. While the control pot recorded an average moisture content of 27.32% and 30.8% at 8th and 15th day respectively.

Table 1. Differences between green gram germplasm with respect to morpho-physiological and biochemical characters under moisture stress

Sl. No.	Genotypes	RL	SL	RD	TPDW	RDW	PRO	TC	PHE
1	Andhra local	30.16	10.00	0.46	2.30	0.40	15.11	0.07	3.40
2	TM 96	27.59	34.78	0.44	0.17	0.25	0.85	0.41	3.35
3	IPM 205 7	18.64	22.74	0.43	2.75	0.33	10.90	0.53	1.80
4	Thiruvalla local	29.67	8.48	0.49	3.33	0.32	30.29	0.81	2.56
5	EC 396143	20.37	10.33	0.54	2.17	0.35	15.44	0.14	2.30
6	IPM 031	25.69	35.77	0.37	1.13	0.51	6.89	0.69	1.89
7	C4 PDM 139	25.97	11.93	0.45	2.69	0.95	26.90	1.08	2.65
8	ML 1415	34.37	5.20	0.38	3.76	0.32	35.60	0.97	5.18
9	Co GG 912	7.31	34.67	0.37	1.31	0.15	23.98	2.51	6.27
10	Co 8	32.33	12.36	0.42	3.00	2.76	28.45	0.87	2.79
11	IC 395518	43.00	5.54	0.43	3.29	0.45	37.61	0.14	3.83
12	GM 4	26.54	28.11	0.34	2.35	0.62	9.06	0.44	1.46
13	VBN 4	13.25	44.33	0.39	3.40	0.22	4.24	2.58	1.00
14	IPM 312 20	19.66	32.46	0.43	2.21	0.27	16.86	1.21	1.82
15	IC 148530	48.40	6.58	0.54	3.40	0.66	34.12	1.41	2.90
16	Co 9	15.90	38.67	0.49	0.27	0.40	0.65	0.68	0.54
17	VBN 2	12.98	48.22	0.42	5.41	0.35	5.05	0.75	5.11
18	VBN 3	9.87	45.33	0.44	6.26	0.32	0.26	0.40	0.04

19	PLM 963	15.30	37.33	0.41	3.78	0.30	7.68	1.23	3.72
20	VBN 1	18.58	4.69	0.48	2.61	0.28	23.22	0.60	2.20
21	IC 553601	28.77	37.48	0.41	3.55	0.61	18.95	1.94	2.28
22	EC 398884	32.19	29.33	0.46	1.60	0.76	3.64	0.63	3.39
23	C5 SML 668	28.13	8.65	0.33	2.77	0.37	31.84	1.68	5.17
24	C2 IPM2 14-1	16.76	39.35	0.39	2.94	0.32	5.28	3.07	1.36
25	GM 6	16.76	39.35	0.45	2.94	0.62	7.91	0.52	1.14
26	GM 7	20.77	27.16	0.45	2.75	0.54	15.20	0.47	2.18
27	GM 8	23.62	39.30	0.45	2.27	0.42	6.56	1.16	1.30
28	IC 148531	24.73	24.83	0.45	0.85	0.51	9.98	2.74	2.35
29	IC 597670	13.91	36.79	0.4	2.94	0.11	0.85	0.24	0.22
30	IC 607183	9.59	39.22	0.37	1.08	0.10	8.40	0.28	9.47
31	VBN 5	28.33	9.42	0.61	3.19	0.58	29.00	0.93	2.31
32	C2 IPM2 14-2	6.85	30.55	0.35	1.66	0.09	10.60	1.06	8.24
33	Kayamkulam local	23.48	6.50	0.52	2.62	0.29	25.22	0.90	2.92
34	GM 9	22.24	33.31	0.44	2.62	0.65	13.16	0.52	1.18
35	Trivandrum local	21.41	35.49	0.37	2.39	0.75	14.26	1.99	3.78
36	Kozhikode local	23.22	7.33	0.63	0.67	0.22	5.36	2.68	1.86
37	IC 148516	17.40	27.50	0.45	0.96	0.18	7.91	3.31	1.48
38	EC 396142	20.37	10.33	0.72	2.17	0.47	15.44	0.14	2.30
39	HDM 12	32.11	38.55	0.35	0.78	0.37	1.98	0.46	3.43
40	EC 314302	13.91	36.79	0.38	2.94	0.19	24.28	1.26	1.93
41	IC 520034	12.02	28.37	0.35	2.08	0.23	2.49	0.94	0.13
42	EC 165632	14.53	55.00	0.43	5.12	0.41	19.56	1.64	2.45
43	PLM 38	14.53	55.00	0.76	5.12	0.39	23.00	1.86	2.68
44	PLM 794	21.92	4.33	0.60	1.19	0.46	2.59	2.73	2.13
45	IC 548369	32.95	35.38	0.36	0.10	1.13	0.30	1.26	2.69
46	IC 606545	19.90	15.96	0.47	1.12	0.41	14.2	1.99	1.94
47	IC 418452	32.93	8.27	0.61	2.67	0.56	24.7	0.36	2.81
48	EC 272458	11.61	49.33	0.38	3.17	0.32	2.75	0.29	7.39
49	IC 488962	35.79	26.17	0.38	4.03	0.84	2.83	0.43	1.28
50	C1 IPM02 3	21.79	34.16	0.38	3.16	0.40	6.80	0.95	1.36
	Mean	22.84	26.27	0.44	2.48	0.47	13.59	1.13	2.76
	SE(d)	3.96	3.01	0.03	0.49	0.12	3.01	0.56	0.51
	CD (5%)	7.49	3.39	0.06	0.95	0.26	4.49	1.12	1.02
	CV	21.28	14.07	9.23	24.24	32.36	27.16	60.68	22.83

RL – Root length (cm); RD – Root diameter (cm); SL – Shoot length (cm); TPDW – Total plant dry weight (g); RDW – Root dry weight (g); PRO – Proline content (µmolg^{-1}); PHE – Total phenol content (mg g^{-1}); TC – Total Chlorophyll (mg g^{-1})

3.1.1. Root parameters

A wide variation was recorded among the genotypes with respect to various parameters, indicating ample scope of selection. A significant difference was noted among genotypes for root length

which ranged from 6.85 to 48.40 cm. Root length was found to be higher in majority of genotypes under moisture stress condition than control. This was in conformity with the results of Prakash *et al.* (2018) [13] in black gram. The genotypes IC 148530 and IC 395518 recorded longer root length under stress conditions. Under moisture stress condition, an increase in root diameter was recorded in majority of the genotypes compared to control condition. This was in accordance with reports of Prakash *et al.* (2018) [13] and Zhou *et al.* (2018) [7] in green gram. Thicker root helps plants to acquire nutrients faster and increase the reserve of non-structural carbohydrates which inturn help in osmoregulation and osmoprotection.In this study, PLM 38 and EC 396142 recorded greater diameter under moisture stress condition. Genotypes with superior root architecture will be able to avoid drought. Similar observation was made by Amarapalli (2022) [14] in green gram.

3.1.2. Shoot parameters

A reduction in shoot length was recorded under moisture stress condition compared to control condition in all genotypes. Similar observations were made by Ranawake*et al.* (2011) [15] in green gram and Pandiyan *et al.* (2017) [16] in black gram and green gram. The decrease in shoot length could be attributed to deeper root growth, which is promoted by shorter plant height and enables the plant to absorb more moisture under water stress conditions. Genotypes with longer shoot lengths relative to their roots tend to be more sensitive to moisture stress.In the present study, longer shoot lengths were recorded by PLM 38, VBN 3, EC 272458, IC 606545, IC 520034, C2 IPM2 14-2, IC 597670 and Co 9 which also had lower root length making them sensitive to moisture stress condition.

3.1.3. Dry weight

Seedling dry weight reduced under moisture stress condition compared to control in majority of the genotypes which were in confirmatory with the observations of Kaur *et al.* (2017) [17] in mungbean and Meena (2017) [18] in chickpea.The decrease in the plant dry weight could be attributed to the reduction in shoot and root length, which may result from inhibited cell division and differentiation under conditions of moisture stress. The genotypes with higher root and shoot length namely VBN 3, IC 553601 and PLM 38 recorded higher plant dry weight compared to control in moisture stress conditions. Corresponding observation was made by Kumar *et al.* (2011) [19] in pigeon pea.An increase in root dry weight was observed in some genotypes in moisture stress condition compared to control pots. The genotypes TM 96, IPM 031, C4 PDM 139, Co 8, IC 553601, Trivandrum local and IC 548369 recorded higher root dry weight. This was in confirmation with findings of Prakash *et al.* (2018) [13] and Santos *et al.* (2020) [20] in green gram. Increased root dry weight may be due to increased allocation of dry matter to roots under stress condition.On contradictory to this, C2 IPM 2 14-2, Andhra local, ML1415, GM 6, IPM 2057, VBN 1, GM 8, GM 7, and IC 148516 showed decrease in root dry weight under stress condition. A similar observation was made by Dien *et al.* (2017) [21] in rice.

3.1.4.Biochemical parameters

Proline levels are a key factor in enhancing water stress tolerance in plants. An increase in proline content was observed in majority of the genotypes compared to the control condition. This was in agreement with the findings of Naidu *et al*. (2008) [22] Bangar *et al*. (2019) [23] in green gram. The increase in levels of proline content can be used by plants to combat moisture stress condition by maintaining cell turgor and preventing electrolyte leakage thus keeping reactive oxygen species (ROS) levels normal.In the present study, IC 395518, ML 1415, IC 148530 and C5 SML 668 recorded higher proline content under stress condition. This suggests their ability to tolerate moisture stress indicating that varieties with elevated proline levels are more capable of withstanding the negative impacts of moisture stress and can achieve higher yields.

A reduction in chlorophyll content was observed in majority of the genotypes under moisture stress condition than control. Similar results were reported by Pandiyan *et al.* (2017) [16] in green gram and black gram and Jincy *et al.* (2020) [24] in green gram. The decrease in chlorophyll content during moisture stress might be caused by photo-oxidation and degradation of chlorophyll. The genotypes IC 148516, C2 IPM2 14-1, IC 148530, PLM 794, Kozhikode local, VBN 4, and Co GG 912 recorded higher chlorophyll content indicating their level of tolerance to drought.

Green gram is rich source of polyphenolics, the major phenolic constituent in green gram are phenolic acids. Under moisture stress conditions, an increase in phenol content was observed in some of the genotypes. This was in accordance with reports of Varela *et al.* (2016) [25]. In the current study, the genotype IC 607183 recorded higher total phenol content reflecting their ability for drought tolerance.

3.2. Correlation studies

Correlation coefficient measures the extent and direction of association between characters and thus helps in effective selection.The genotypic correlation matrix with respect to the various characters have been estimated and is presented in table 2 and figure 1. The character root length had maximum positive correlation with root diameter (0.949) followed by proline content (0.700) and root dry weight (0.636) whilea significant negative correlation was observed with shoot length (-0.331).Root diameter expressed a significant positive correlation with all the characters considered except for shoot length which had a nonsignificant correlation. The proline content of the drought affected seedlings were positively correlated with root diameter (0.747), root length (0.700), total plant dry weight (0.424), phenol content (0.356), root dry weight (0.325) and total chlorophyll (0.155) while a significant negative correlation was recorded for shoot length (-0.465). The root diameter (0.509) of the affected plants alone exhibited a significant positive correlation with the biochemical parameter, total chlorophyll content. The phenol content of the affected plants also did not show a very high correlation with any of the biometric parameters. Proline content of the drought affected plants can be considered as a reliable indicator of drought tolerance. Proline accumulation in stressed plants was earlier reported by Anaytullah*et al*. (2007)[26] and Baroowa and Gogoi (2012) [6]. Fahramand*et al*. (2014) [27] observed the increased

proline accumulation in tolerant genotypes than that of other amino acids; therefore, proline can be used as a criterion for screening drought tolerant varieties.

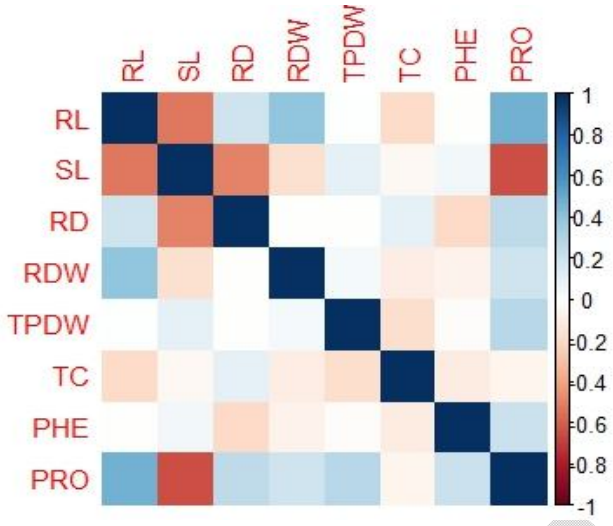
A notable connection of chlorophyll content with proline was earlier reported by Bangar *et al*., 2019 [23].Prakash *et al*. (2018) [13] evaluated black gram genotypes for drought tolerance based on root dynamics and observed higher values in root parameters viz. root length and dry weight of root under severe water stress.Chlorophyll content exhibited a notable connection with proline and protein content. Plant height showed a strong correlation with leaf area, seed count per pod, and pod count per plant. Leaf area displayed a negative correlation with proline but demonstrated positive associations with RWC, protein content, and yield components [23].Santos *et al*. (2020) [20] reported that when subjected to moisture stress, drought tolerant cowpea genotypes recorded increased root dry weight of 24.57%.Sivakumar *et al*. (1998) [28] reported that proline could be used as biochemical marker for drought tolerance.Proline accumulation in stressed plants was reported by Anaytullah*et al*. (2007) [26].Baroowa and Gogoi (2012) [6] observed an accumulation of proline in leaves during stressed period and decreased in the subsequent recovery stages.Fahraman*det al*. (2014) [27] observed the increased proline accumulation in tolerant genotypes than that of other amino acids; therefore, proline can be used as a criterion for screening drought tolerant varieties.According to Dutta and Bera (2016) [29] proline content increased with decreasing water potential at all stages of observation irrespective of cultivars tested.

Table 2. Genotypic correlation matrix of drought related characters in green gram

	RL	SL	RD	RDW	TPDW	PRO	TC	PHE
RL	1							
SL	-0.331**	1						
RD	0.949**	-0.061	1					
RDW	0.636**	0.035	0.387**	1				
TPDW	0.206*	0.331**	0.472**	0.196*	1			
PRO	0.7**	-0.465**	0.747**	0.325**	0.424**	1		
TC	0.004	0.105	0.509**	-0.014	-0.138	0.155*	1	
PHE	0.131	0.205*	0.192*	0.038	0.127	0.356**	0.037	1

RL – Root length (cm); RD – Root diameter (cm); SL – Shoot length (cm); TPDW – Total plant dry weight (g); RDW – Root dry weight (g); PRO – Proline content (µmolg^{-1}); PHE – Total phenol content (mg g^{-1}); TC – Total Chlorophyll (mg g^{-1})

Figure 1. Genotypic correlation of drought related characters in green gram



3.3. Path analysis

Path coefficient divides the correlation coefficient into direct and indirect effects and gives information about the influence of one variable upon another.Proline can be used as a criterion for screening drought tolerant varieties, hence the direct and indirect effects of the various characters on proline content was estimated and is presented in table 3.

The highest positive direct effect on proline content was recorded by total plant dry weight (0.5080) followed by phenol content (0.3790) and root diameter (0.2010). A very high negative direct effect was imposed by shoot length on proline content (-0.7080). Although total plant dry weight had a very high direct effect, it was negatively affected by shoot length by its indirect effect (-0.2350) on proline content. The character root length (0.0450) had very small direct effect on proline content but it exhibited indirect effect through shoot length (0.2320), root diameter (0.1890) and root dry weight (0.1060). The residual effect of the path analysis was 0.2683, indicating that almost 73% of the factors affecting proline content of the plant has been included in the study.

Proline accumulation as a mechanism to prevent adverse effects of drought in various plants have been earlier reported by Man *et al*. (2011) [30]; Saha*et al.* (2019) [31]; Furlan*et al.* (2020) [32]; Kijowska-Ober*cet al*. (2023) [33] and Nutthapornnitchakul*et al.* (2024) [34].

Table 3. Direct and indirect effects of drought related characters on proline content in green gram

	RL	SL	RD	RDW	TPDW	TC	PHE	Genotypic correlation with main variable
RL	**0.0450**	0.2320	0.1890	0.0830	0.1060	-0.0010	0.0490	**0.7030**
SL	-0.0150	**-0.7080**	-0.0110	0.0050	0.1680	0.0190	0.0780	**-0.4630**
RD	0.0430	0.0400	**0.2010**	0.0510	0.2400	0.0920	0.0740	**0.7400**
RDW	0.0290	-0.0260	0.0780	**0.1310**	0.1000	-0.0020	0.0140	**0.3250**
TPDW	0.0090	-0.2350	0.0950	0.0260	**0.5080**	-0.0250	0.0480	**0.4270**
TC	0.0000	-0.0760	0.1030	-0.0020	-0.0710	**0.1790**	0.0140	**0.1480**
PHE	0.0060	-0.1460	0.0390	0.0050	0.0650	0.0070	**0.3790**	**0.3540**

RL – Root length (cm); RD – Root diameter (cm); SL – Shoot length (cm); TPDW – Total plant dry weight (g); RDW – Root dry weight (g); PHE – Total phenol content (mg g^{-1}); TC – Total Chlorophyll (mg g^{-1})

Residual effect – 0.2683

4. CONCLUSION

This study highlights the significant variation in characters for screening against drought tolerance among 50 green gram genotypes, emphasizing the importance of root architecture and proline accumulation in withstanding moisture stress. Genotypes with longer roots and higher proline levels, such as IC 395518, ML 1415 and Co 8 showed greater drought resilience, suggesting their potential for breeding programs aimed at improving green gram productivity under adverse environmental conditions.The study's findings provide valuable insights for developing drought-tolerant varieties, crucial for enhancing crop yields in the face of unpredictable weather patterns and climate change, ultimately contributing to sustainable agriculture and food security.

7. REFERENCES

1. Nair RM, Schafleitner R, Kenyon L, Srinivasan R, Easdown W, Ebert RW. et al. Genetic improvement of mungbean. J. Breed. Genet. 2019;44:177-190.
2. Singh DP, Singh BB, Pratap A. Genetic improvement of mungbean and urdbean and their role in enhancing pulse production in India. Indian J. Genet. 2016; 76(4):550-567.
3. Allito BB, Nana EM, Alemneh AA. Rhizobia strain and legume genome interaction effects on nitrogen fixation and yield of grain legume: a review. Mol. Soil Biol.2015; 20:1-6.
4. Arnoldi A, Zanoni C, Lammi C, Boschin G. The role of grain legumes in the prevention of hypercholesterolemia and hypertension. Crit. Rev. Plant Sci. 2015;34(1-3):144-168.

5. BasuPS, PratapA, GuptaS, SharmaK, TomarR, SinghNP, et al. Physiological Traits for Shortening Crop Duration and Improving Productivity of Greengram (*Vigna radiata* L. Wilczek) under High Temperature. Front. Plant Sci. 2019;10:1508.
6. Baroowa B, Gogoi N. Effect of induced drought on different growth and biochemical attributes of black gram (*Vigna mungo* L.) and green gram (*Vigna radiata* L.).J. Environ. Res. develop. 2012; 6(3A).
7. Zhou R, Kong L, Wu Z, Rosenqvist E, Wang Y, Zhao L, Zhao T, Ottosen C. et al. . Physiological response of tomatoes at drought, heat and their combination followed by recovery. Physiol. Plant. 2019;165: 144–154
8. Zare M, Dehghani B, Alizadeh O, Azarpanah A. The evaluation of various agronomic traits of mungbean (*Vigna radiata* L.) genotypes under drought stress and non-stress conditions. Int. J. Farming Allied Sci. 2013; *2*: 764–770.
9. Bates LS, Waldren RP, Teare ID.Rapid determination of free proline for water-stresss tudies. Plant and Soil. 1973;39(1): 205-207.
10. Yoshida S, Forno DA, Cock JH. Laboratory Manual for Physiological Studies of Rice. International Rice Research Institute, 1971;2nd Ed.
11. Sadasivam S, Manickam A. Biochemical Methods for Agricultural Sciences. Wiley Eastern Ltd., New Delhi, 1996; 246p.
12. Gopinath, P.P., Parsad, R., Joseph, B. and Adarsh, V.S.GRAPES: General Rshiny based analysis platform empowered by statistics.2020. https:// www.kaugrapes.com/home.
13. Prakash M, Elangaimannan R, Sunilkumar B, Narayanan GS.Evaluation of black gram genotypes for drought tolerance based on root dynamics and gas exchange parameters. Legume Res. 2018;41(3): 384-391.
14. Amarapalli G. Studies on the effect of water stress on root traits in green gram cultivars. Legume Res. 2022; 1-7.
15. Ranawake AL, Dahanayaka N, Amarasingha UGS, Rodrigo W, Rodrigo UTD. Effect of water stress on growth and yield of mung bean (*Vigna radiata* L.). Trop. Agric. Res. Ext. 2011; *14*, 76–79.
16. Pandiyan M, Senthil N, Balaji T, Veeramani P, Savitha BK, Sendhilvel V, Gopilkrishnan, A. et al. Studies on performance of drought tolerant genotypes under drought and normal conditions through morpho, physio and biochemical attributes of black gram (Vigna mungo L.) and green gram (*Vigna radiata* L.). Int. J. Adv. Res.2017; 5(2): 489-496.
17. Kaur AJG, Devendra J. SSR marker assisted evaluation of Genetic Diversity in Mungbean (*Vigna radiata* L.) genotypes. Braz. Arch. Biol. Technol. 2017; 61: 1678-4324.
18. Meena H.Morpho-physiological attributes of drought tolerance in chickpea genotypes at seedling stage. M.Sc. (Ag) thesis, Sri Karan Narendra Agriculture University, Jobner, 2017;111p.
19. Kumar RR, Karjol K, Naik GR. Variation of sensitivity to drought stress in pigeon pea (Cajanus cajan [L.] Millsp) cultivars during seed germination and early seedling growth. World J. Sci. Technol. 2011;1(1): 11-18.
20. Santos R, Carvalho M, Rosa E, Carnide V, Castro I.Root and agromorphological traits performance in cowpea under drought stress. Agron. 2020; 10(10):1-20.
21. Dien DC, Yamakawa T, Mochizuki T, Htwe AZ.Dry weight accumulation, root plasticity, and stomatal conductance in Rice (*Oryza sativa* L.) Am. J. Plant Sci. 2017;8(12).
22. Naidu TC, Raju N, Yanan A. Screening of drought tolerance in greengram (*Vigna radiata* l. Wilczek) genotypes under receding soil moisture. Agricul. Food Sci. 2008.
23. Bangar P, Chaudhury A, Tiwari B, Kumar S, Kumari R, Bhat K.V et al. Morphophysiological and biochemical response of mungbean [*Vigna radiata* (L.) Wilczek] varieties at different developmental stages under drought stress. Turk. J. Biol. 2019;43(1): 58-69.
24. Jincy M, Prasad VBR, Senthil A, Jeyakumar P, Manivannan N. Physiological divergence in green gram [*Vigna radiata* (L.) Wilczek] genotypes for drought and high temperature stress tolerance during flowering phase. Legume Res. 2020; 1: 1-8.
25. Varela MC, Arslan I, Reginato MA, Cenzano AM Luna MV. Phenolic compounds as indicators of drought resistance in shrubs from Patagonian shrublands (Argentina). Plant Physiol. and Biochem. 2016;104: 81-91.

26. Anaytullah, Bose B, Yadav RS. PEG induced moisture stress: screening for drought tolerance in rice. Indian J. Plant Physiol. 2007; 12(1): 88-90.
27. Fahramand M, Mahmoody M, Keykha A, Noori M, Rigi K. Influence of abiotic stress on proline, photosynthetic enzymes and growth. Int. Res. J. Appl. Basic Sci. 2014; *8*, 257–265.
28. Sivakumar P, Sharmila P, Saradhi PP. Proline suppresses Rubisco activity in higher plants. Bioche. Biophys. Res. Commun.1998; *252*(2):428-432.
29. Dutta P, Bandopadhyay P, Bera AK. Identification of leaf based physiological markers for drought susceptibility during early seedling development of mungbean. Am. J. Plant Sci.2016; 7:1921–1936.
30. Man D, Bao Y, Han L, Zhang X. Drought Tolerance Associated with Proline and Hormone Metabolism in Two Tall Fescue Cultivars. Hort. Sci. *2011; 46*(7), 1027-1032
31. Saha S, Begum H, Nasrin S. Effects of Drought Stress on Growth and Accumulation of Proline in Five Rice Varieties (*Oryza Sativa* L.). J. Asiat. Soc. 2019; 45. 241-247.
32. Furlan AL, Bianucci E, Giordano W, Castro S, Becker DF. Proline metabolic dynamics and implications in drought tolerance of peanut plants. Plant Physiol. Biochem.2020; 151: 566-578
33. Kijowska-Oberc J, Dylewski Ł, Ratajczak E. Proline concentrations in seedlings of woody plants change with drought stress duration and are mediated by seed characteristics: a meta-analysis. Sci Rep. 2023; 13, 15157.
34. NutthapornnitchakulS, SonjaroonW, PutthisawongN, ThumthuanN, Tasanasuwan P, JantasuriyaratC. Effect of Drought Stress on Proline Gene Expression, Enzyme Activity, and Physiological Responses in Thai Mulberry (Morus spp.).J. Biosci. 2024; *31*(3): 559-571.