

QUANTIFYING PROLINE AND TOTAL PHENOLIC CONTENT AS BIOMARKERS FOR DROUGHT RESILIENCE IN MUNGBEAN (*VIGNA RADIATA L.*) CULTIVARS.

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**Abstract:** Mungbean (*Vigna et al.*) belongs to the Fabaceae family. It has two planting seasons (spring and summer). Mungbean is a crop in arid and barren areas. These areas are more prone to water stress. The present study evaluated water stress's effect on green gram to find its resistance to drought stress. A controlled experiment was conducted under factorial CRD with three replications and two factors varieties and water levels (A set of 15 genotypes was obtained from the NIAB research institute, Faisalabad. Firstly, the total phenolic content (TPC) test analyzed these genotypes. The five varieties that showed high TPC were used for experiments such as NM-98, Abbas Mung, NM-121-25, NM-2016, and NM-92) at the experimental area of Plant Breeding and Genetics, University of Agriculture, Faisalabad, during the spring season of 2023. Three levels of water stress were applied. Data were recorded for different traits under water stress at different growth stages of mungbean. Analysis of variance was performed to see the significant difference among treatments, Tukey's HSD test was applied for the mean comparison test using Statistix 8.1 statistical software, and correlation analysis was performed to check the relationship between different traits. An increase in root length, proline content, and total phenolic content positively affected drought tolerance. The performance of NM-121-25 was better than that of others, and the interactive values of NM-2016 were better than those of others at different drought levels. Pod length, leaf number per plant, relative water content, membrane stability index, leaf area, and proline content showed significant positive results with plant yield.

**Keywords:** Correlation, Proline, Phenolic content, RWC, MSI, Yield per plant, Leaf area, Root length, Pod length.

## Introduction

Legumes are the chief source of food after cereals. Agronomically, legumes are well adapted to crop patterns and produce high yield, have high economic value due to high nutritional value, and have the ability to improve soil fertility by the processes of N fixation, which is present in the atmosphere with the help of bacteria such as Rhizobium. Legumes are an essential source of protein for the human diet (1). It is necessary to the daily diet because it has high protein content and vitamin balance. Pulses are sometimes referred to as humans' lifeline due to the balanced amino acid content of grains and protein combination, which coincides with milk protein. *Vigna radiata L.* is also known as golden bean, mugda, mungbean, or green gram. After chickpeas and pigeon peas, it is the third most crucial pulse crop. Mungbean belongs to the family Fabaceae; it is a self-pollinating crop. It is a diploid (2n=22) and fast-growing grain legume. Due to its short duration, it is the perfect legume for intercropping, catch cropping, and relay cropping (2). It is a vital source of protein for humans. Nutrients, including protein (24.5%), carbohydrates (59.9%), vitamins (3%), minerals, fibers, and antioxidants like phenolics and flavonoids, are present in high concentrations in the seeds of mungbean (3). In the Punjab province of Pakistan, Layyah, Bhakkar, Mianwali, and Rawalpindi are the central districts for mungbean

production. Kharif season, from July to October, is best for the growth of mungbean.

Mungbean is an economically important crop, but its productivity is affected (838kg/ha-1) by various weather conditions, which include biotic and abiotic stresses (4). In abiotic stresses, drought is an important limiting factor for the cultivation of green gram that reduces its growth and yield. Water stress affects various morphological, physiological, and biochemical processes, leading to severe yield losses. Water stress affects the germination of seed and impaired seedling growth due to affected cell elongation and cell division at the initial stage of growth which leads to the reduction in crop growth (5).

Reduction of soil moisture changes physiological and biochemical activities such as production of reactive oxygen species (ROS), proline, reduction in nutrient uptake, imbalance plant water relations, reduction of chlorophyll content, reduction in photosynthesis and pigment composition. Water stress affected the green gram at 25%, late vegetative at 39%, and the flowering stage at 59%. The flowering stage is more sensitive to water stress and is affected by 31-57% at the pod formation stage; it reduces by 26%, which reduces overall yield in areas where water availability is limited (6).

Green gram faces water stress because it grows at 27-30 degrees Celsius with low humidity and average rainfall ranging from 60-80 centimeters. Mungbean also can

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develop under water stress, while mungbean genotypes show varying responses depending upon the stress duration, variety of the crop, and growth stage (7). Considering the current water shortage situation, there is a need for time to screen mungbean varieties under water stress (8).

Available agricultural land is reduced due to habitat use and changes in climate because the world's population is increasing daily. Therefore, it is essential to use water-stressed areas to meet the increasing demands of world energy and food (9). Annually, 50% of yield losses occur because 45% of the total agricultural land is under water stress. Therefore, it is essential to use drought-tolerant mungbean genotypes to overcome these situations (Abdelrahman et al., 2018). Water stress can be avoided through various management options such as proper irrigation scheduling and supplemental irrigation, water conservation practices such as using plant growth regulators (PGRs), mulching, and an adequately established farming system. However, sometimes it is impossible for farmers due to the cost (10). Therefore, it is essential to select water-stress-tolerant varieties to overcome the moisture-stress areas and enhance yield.

Therefore, to develop an effective phenotypic screening method for proper crop growth and development, it is essential to understand the crop response under different levels of water stress (11). In this way, evaluating different parameters and their correlation under water stress is essential to develop different tolerant varieties, which can be further used in breeding programs and help in water stress tolerance (12). The main objectives of the present study were to determine the influence of water stress on mungbean varieties at different developmental stages, identify water stress tolerant genotypes of mungbean, Examine morphological, physiological, and biochemical changes under water stress, identify potential morphological and biochemical marker for future research programs.

## Methodology

### Experimental Location

A controlled experiment was conducted at an experimental area of Plant Breeding and Genetics in a glass house at the University of Agriculture, Faisalabad, which is situated at 31.43o North latitude and 73.06o East longitude at a height of 185 meters above average sea level.

### Experimental Material

A set of 15 genotypes was obtained from the NIAB research institute, Faisalabad. Firstly, the total phenolic content (TPC) test analyzed these genotypes. The five varieties that showed high TPC were used for the experiment, such as NM-98, Abbas Mung, NM-121-25, NM-2016, and NM-92.

### Experimental design and layout

Under factorial conditions, the controlled experiment was conducted following a completely randomized design (CRD). Each and every pot was filled with 8 Kg of soil. Healthy seeds were planted in soil-filled plastic pots (6 seeds in each pot) during the spring season dated 07 March 2023. After seeding establishment, thinning was performed after 21 days of germination and three plants of equal magnitude were left in every single pot. The experiment was conducted at three levels of treatments: control (no water stress), vegetative stage, and reproductive stage. For water stress imposition at the vegetative stage, the plants were

fully watered until 25 days of emergence (true leaf stage), and then water stress was applied for 20 days. For drought imposition at the reproductive stage, the plants were thoroughly watered for 35 days after emergence (first flower bud appearance), and then water stress was applied for the same 20 days. The control plants were fully irrigated throughout the experiment. After 20 days without irrigation pots were regularly watered to evaluate parameters. Hand weeding was used to keep the pots weed-free. All agronomic treatments were kept the same and uniform. The crop was manually collected when 90% of the pods had matured and turned brown; plants were first sun-dried for four days in the field before manually threshed.

The data were recorded for the following morpho-physiological and biochemical parameters;

### Morphological Variables:

All the data were taken randomly from three randomly selected plants for all treatments. The data for leaf number per plant was counted manually, and shoot length was taken in centimeters by measuring tape. Yield-related variables were observed after harvesting, such as the number of pods per plant, pods per cluster, cluster per plant, number of seeds per pod, hundred seed weight, root length, yield per plant, and pod length.

### Physiological Parameters:

Relative water content (RWC) was measured by collecting three leaf samples of selected plants under both controlled and water stress conditions. Fresh leaf weight was calculated with the help of electronic balance. Later, leaves were soaked in water for a whole night for a turgid leaf weight. After turgid weight recording, the leaves samples were dried for an hour at room temperature. The samples were stored for 24 hours at 65 c to achieve dry weight. Relative water content was calculated by using Bars and Weatherly's formula (1962).

$$RWC = \frac{(\text{Fresh weight} - \text{Dry weight})}{(\text{Turgid weight} - \text{Dry weight})} \times 100$$

The membrane stability index (MSI) for different treatments was calculated by following the protocol proposed by Sairam (1994). The 100 mg sample was put into two sets of test tubes containing 10 mL of distilled water. One set was heated at 40 c for thirty minutes, while the other was heated at 100 c for 10 minutes. C1 and C2 were recorded (electrical conductivities).

The following formula calculated MSI:

$$MSI (\%) = 1 - (C1/C2) \times 100$$

Leaf area (LA) was calculated by measuring the maximum width and length, and then the leaf area of each plant in each treatment was calculated according to the formula.

$$\text{Leaf area} = \text{maximum leaf length} \times \text{maximum leaf width}$$

### Biochemical Variables

Total phenolic content (TPC) was determined by the FC method (Folin-Ciocalteu) proposed by Naaz et al. (2016). 0.5 mg of plant extract was mixed with 700 µl distilled water, and then 100 ml Hcl was added and placed at 82 degrees Celsius for 24 hours. It broke down the cell wall, and 200 ml ethanol was added. Then, the sample was prepared for further analysis. After that 5 ml Fc reagent and 4 ml sodium bicarbonate were added. The sample was placed in an incubator. The absorbance was noted after 1 hour at 765 nm, and a calibration curve was plotted by taking absorbance as a concentration function using a spectrophotometer.

It was calculated by following the formula

$$T = C \times V / M$$

Where,

T = total contents of phenolic compound in mg GAE/g plant extract.

C = the concentration of gallic acid calculated from the calibration curve in mg/mL.

V = the volume of extract in ml.

M = the weight of plant extract in grams.

Proline content (PRO) was determined by following the protocol proposed by Bates et al. (1973). Homogenize 0.5g of tissue in a pestle and mortar with 10 ml of 3% aqueous sulphosalicylic acid and filter. To 2ml of filtrate, add 2ml each of glacial acetic acid and ninhydrin and mix. Keep in boiling water bath for 1h and then terminate reaction by placing on an ice bath. Add 4ml of toluene mix vigorously for 20-30sec. Aspirate the chromophore (toluene) layer and warm to room temperature. Measure the absorbance of the red color at 520 nm against a reagent blank. Calculate the amount of proline in the sample using a standard curve prepared from pure proline using a spectrophotometer.

#### Statistical Analysis

Two-factor factorial ANOVA was performed for each parameter using Statistix 8.1 software. Moreover, Tukey's HSD test was used to compare the means. Correlation analysis was done to study the relationship between multiple traits. MS Excel sheet was used to form a graph to represent the data.

### Results and discussion

All traits exhibited a highly significant variation among the five mungbean varieties at a P value of 0.01. Morphological characters had an adverse effect against water stress, such as all the characters related to yield reduction under water stress. Morphological characteristics, such as root length, are increased under water stress. Mean squares of all parameters are given in Table 1, which shows that all have highly significant results under water stress. This is probably due to the water absorption capacity being low during the vegetative stage due to a shortage of soil water; consequently, grain yield and growth will be decided by the ability to grow vigorously and accumulate as much dry weight as possible before flowering (Uddin et al., 2013; Baroowa & Gogoi, 2016). The number of pods per plant is an important mungbean parameter related to seed yield. Maximum pods were observed under fully irrigated conditions, and fewer pods were observed in stressed conditions. Similar findings were observed by Parvez et al. (2013). Similar findings were also observed in soybean Lie et al. (2003). The number of pods per cluster is an important parameter of mungbean. There were a more significant number of pods in control conditions, and fewer pods were observed in stressed conditions it is because, with the increase of moisture stress, there was the abscission of pods, which was supported by Hossain et al. (2010). Water stress adversely affected cell growth during both growth stages; vegetative and reproductive ultimately reduced the yield. Water stress affected the cluster per plant. There was a lower number of clusters per plant under drought conditions; therefore, minimum yield was observed. At the reproductive stage, when water is required for the development of water, the limited supply of water makes abnormal pods and a lower number of clusters per plant;

these findings correlate with Uddin et al. (2013). The number of seeds per pod is an important characteristic of mungbean and is related to yield. There were many seeds per pod in control conditions, and all seeds were healthy. The minimum number of seeds per pod was observed in stressed plants. Water is required for the seed filling per pod; therefore, minimum seeds per pod were observed in the reproductive stage. These findings correlate with previous studies by Allahmoradi et al. (2011). Water stress significantly affects the weight of the seed. Under control conditions, healthy seeds were achieved; therefore, their weight was high. At the reproductive stage, water is required for the pod filling, but in the absence of sufficient moisture, there were abnormal seeds developed under stressed conditions; therefore, their weight was also reduced. With the increase in water stress, there was a reduction in the weight of the seed. These findings correlate with the findings of Naresh et al. (2013). Root length is the adaptation of plants to tolerate water stress. Control plants have shorter roots as compared to stressed plants. The increase in root length in dry soil and the establishment of a root network that goes deep into the soil for absorption of moisture efficiently enables green gram to survive under water stress similar findings were found by Jaleel et al. (2008). Water stress greatly impacted the shoot length by impairing cell division and expansion, leading to the eventual loss of cell turgor. At both the vegetative and reproductive growth stages, water-stressed plants' shoot lengths varied, indicating that their reduced water absorption capacity was the cause of their high shoot length in the control plants., it was also supported by Allahmoradi et al. (2011) and Ratnasekera and Subhashi (2015). Yield/plant is an important parameter for mungbean. The maximum yield was obtained under control conditions as there was a normal supply of water. Yield per plant is affected by water stress through the intensity and duration of stress. With the increase in stress, yield reduction and minimum yield were observed in treatment 3. Similar findings were found by Baroowa and Gogoi (2016). Water stress has a significant effect on the yield of mungbean; yield loss occurs due to the abortion of pods. The maximum pod length was observed under irrigated conditions and the lowest pod length was observed in stressed conditions. Variations in pod length were also observed by Parvez et al. (2013) and Lie et al. (2003). Irrigated pots were observed to have a higher leaf number per plant than stressed pots both at the vegetative and reproductive stages. Similar findings were also observed by Raza et al., 2012 who confirmed that different irrigations have significant effects on the number of leaves per plant. Water stress has a direct effect on the leaf's number per plant supported by Ranawake et al. (2011) It was observed that drought stresses markedly affected the physiological characteristics of mungbean varieties; however, the yield reduction was less than what was expected from the impact on physiological characteristics. This variation in morpho-physiological traits might be due to the varying nature and duration of stress, as many genes govern these traits. Physiological characteristics such as relative water content, membrane stability index, and leaf area were also negatively affected. All these characteristics reduced as water stress increased. Relative water content (RWC) is the key indicator of drought stress. Water stress negatively affects the RWC as it decreases the leaf water potential. Complete irrigated plants showed the highest

RWC compared to stressed plants, as with the increase of water stress, relative water content decreased at both vegetative and reproductive growth stages. Similar results were found by Parvin et al. (2014), Shanazari et al. (2018), and Nazran et al. (2019). Membrane stability index (MSI) is the first line of defense in genotypes, and it is reduced under water stress due to the excessive accumulation of reactive oxygen species, which damages its phospholipid lipid and fatty acid compositions. In control conditions, mungbean maintains its MSI, but as the water increases, it decreases MSI at different growth stages. Similar findings were found by Ahmadzadeh et al. (2011), Sibel and Birol (2007), and Ratnasekera and Subhashi (2015). The leaf area is significantly affected by water stress, and it is decreased when water stress increases. Similar results were found by Sangakkara et al. (2001). The leaf area is reduced due to reduced cell division. Leaf area is reduced because, through this, there is less water loss via transpiration. Similar findings have been found in many plant species: Karademir et al. (2012), Avramova et al. (2015), and Larkunthod et al. (2018).

Biochemical characteristics such as total phenolic and proline content were increased during the stress period in both vegetative and reproductive stages because they enabled mung to survive under water stress. Total phenolic content is the secondary metabolite that increases under drought stress. Its accumulation under water stress is an indicator of drought resistance. Maximum total phenolic content was observed in stressed plants as compared to irrigated plants. Similar findings were observed in increased total phenolic content in thyme plants under water stress Emami Bistgani et al. (2017) and in sweat basil, where seed priming increased TPC Kim et al. (2005). Proline is an important indicator of drought tolerance as it maintains the osmotic balance in plant cells under water stress. Under water stress, proline accumulation is increased as it enables the plants to survive under water stress. Similar findings were found by Baroowa and Gogoi (2012), Bartels and Sunker (2005), Fahramand et al. (2014), Yaish (2015) and Bharadwaj et al. (2018).

#### Heritability and Genetic advance

Number of pods per plant had the low heritability 28.09 % and low GAM 17.66 %, number of pods per cluster have high heritability 78.50% and low GAM 18.28%, cluster per plant have high heritability 99.77% and moderate GAM 22.07%, number of seeds per pod had high heritability 85.69% and moderate GAM 17.51%, hundred seed weight had high heritability 96.40% and high GAM 20.46%, root length had high heritability 73.81% and high GAM 32.14%, shoot length had high heritability 80.13% and moderate GAM 10.72%, yield per plant had high heritability 92.97% and low GAM 4.27%, pod length had high heritability 79.71% and moderate GAM 13.63%, leaf number per plant had high heritability 71.35% and low GAM 5.51%, relative water content had high heritability 61.45% and moderate GAM 17.23%, membrane stability index had high heritability 92.86% and moderate GAM 11.72%, leaf area had high heritability 67% and low GAM 3.97%, total phenolic content had high heritability 98.14% and high GAM 47.82%, Proline content had high heritability 99.79% and high GAM 85.20%. Results indicated that variables such as hundred seed weight, root length, total phenolic content, and proline content had high heritability and high

GAM; therefore, these parameters could be used for further breeding research programs (Table 2).

#### Correlation

Correlation is a process that establishes the relationship between two variables. There are some types of correlation: positive correlation (values of variables move in the same direction), negative correlation (values of variables move in opposite direction), and no correlation (when there is no linear dependence or no relation between the two variables). Correlation studies revealed that the number of pods per plant showed a highly significant and positive correlation with the number of pods per cluster, cluster per plant, seeds per pod, root length, shoot length, yield per plant, leaf's number per plant, relative water content, membrane stability index, leaf area, and proline content. The number of pods per cluster showed a highly significant and positive correlation with cluster per plant, seeds per pod, root length, shoot length, yield per plant, pod length, leaf number per plant, relative water content, membrane stability index, leaf area, and proline content. Cluster per plant showed a highly significant and positive correlation with the seed per pod, root length, shoot length, yield per plant, leaf number per plant, relative water content, membrane stability index, and proline content. The number of seeds per pod showed a highly significant positive correlation with hundred seed weight, root length, shoot length, yield per plant, leaf number per plant, relative water content, membrane stability index, leaf area, and proline content. Root length showed a highly significant positive correlation with shoot length, yield per plant, leaf number per plant, relative water content, membrane stability index, leaf area, and proline content. Shoot length showed a highly significant positive correlation with yield per plant, pod length, leaf number per plant, relative water content, membrane stability index, and proline. Yield per plant showed a highly significant positive correlation with pod length, leaf number per plant, relative water content, membrane stability index, and proline. Pod length showed a highly significant positive correlation with leaf number per plant, relative water content, membrane stability index, and proline content. Leaf's number per plant showed a highly significant positive correlation with relative water content, membrane stability index, and proline content. Relative water content showed a highly significant positive correlation with the membrane stability index and leaf area, and the membrane stability index showed a highly significant and positive correlation with proline content; showed a significant positive correlation with leaf area (Table 3).

#### Cluster analysis

The cluster analysis grouped the genotypes into 5 clusters. Cluster 1 contained the leaf area, hundred seed weight, and membrane stability index. (Fig.1). Cluster 2 contained the number of seeds per pod, the number of pods per cluster, relative water content, and pod length. Cluster 3 contained shoot length, cluster per plant. Cluster 4 contained yield per plant and number of pods per plant. Cluster 5 contained proline content, root length, and total phenolic content. Cluster analysis has been used to assign the morphological, physiological, and biochemical parameters into homogenous groups based on similar responses.

#### Conclusion



Water stress adversely affected mungbean, such as it affects it morphologically, physiologically, and biochemically. Under water stress, genotypes such as NM-121-25, NM-92, and NM-98 performed well. The findings of current studies indicated that yield reduction increases with the increase of

water stress and membrane stability index, total phenolic content, proline content, relative water content, and other yield-related traits are the parameters of drought-resistant varieties.

**Table 1: Mean squares for all the characters of mungbean under water stress**

S.O.V	DF	PP	PC	CP	SP	HSW	RL	SL	YP	PL	NL	RWC	MSI	LA	TPC	PRO
Genotypes	4	14.786 5	1.8901	0.8196	3.5179	5.4068 5	4.771	0.8676	3.3115 7	0.3238 8	43.898	158.59	90.21	1363.4 2	12484. 5	1.4726 7
Treatments	2	32.695 7	86.312 1	15.714 6	18.947 5	0.4118 4	725.66 9	28.305 2	9.3767 5	3.3281 5	315.28 8	1897.3 6	2689.3 5	2387.8 2	665.8	7.1290 4
Geno x trt	8	2.2972	0.789	1.4143	0.1355	0.0042	3.774	0.6566	0.1176 7	0.1315 7	13.219	31.39	49.51	153.49	27.6	0.2821 3

PP = Number of pods per plant, PC = Number of pods per cluster, CP = Cluster per plant, SP = Number of seed per pod, HSW = Hundred seed weight, RL = Root length, SL = Shoot length, YP= Yield per plant, PL = Pod length, NL = Leaf number per plant, RWC = Relative water content, MSI = Membrane stability index, LA = Leaf area, TPC = Total phenolic content, PRO = Proline content

**Table 2: Genetic components**

Characters	h <sup>2</sup> (%)	GAM (%)
PP	28.02	17.66
PC	78.50	18.28
CP	99.77	22.07
SP	85.69	17.51
HSW	96.40	20.46
RL	73.81	32.14
SL	80.13	10.72
YP	92.97	4.27
PL	79.71	13.63
NL	71.35	5.51
RWC	61.45	17.23
MSI	92.86	11.72
LA	67.00	3.97
TPC	98.14	47.82
PRO	99.79	85.20

Heritability = high > 60, % moderate (31-60), and low (0-30) Johnson et al. (1955). GAM = high >20%, moderate = 10-20%, and low < 10 % Johnson et al (1955). PP = Number of pods per plant, PC = Number of pods per cluster, CP = Cluster per plant, SP = Number of seeds per pod, HSW = Hundred seed weight, RL = Root length, SL = Shoot length, YP= Yield per plant, PL = Pod length, NL = Leaf number per plant, RWC = Relative water content, MSI = Membrane stability index, LA = Leaf area, TPC = Total phenolic content, PRO = Proline content.

**Table 3: Correlation analysis between different traits of mungbean genotypes under different water stress levels**

	PP	PC	CP	SP	HSW	RL	SL	YP	PL	NL	RWC	MSI	LA	TPC
PC	0.701 2**													
CP	0.445 3**	0.716 7**												
SP	0.605 9**	0.808 5**	0.559 1**											

[Citation: Shaheen, A., Faridi, R., Khan, S.A., Malik, S., Parveen, A., Saleem, F., Farzand, M., Raza, A., Bibi, A., Walyat, S., Shabbir, F., (2024). Quantifying proline and total phenolic content as biomarkers for drought resilience in mungbean (vigna radiata l.) cultivars. *Biol. Clin. Sci. Res. J.*, 2024: 1074. doi: <https://doi.org/10.54112/bcsrj.v2024i1.1074>]

<b>HS</b>	0.148	0.285	-	0.556															
<b>W</b>	4	7	0.039	3**															
<b>RL</b>	0.622	0.933	0.775	0.808	-														
	7**	3**	2**	3**	0.160														
<b>SL</b>	0.494	0.880	0.760	0.721	0.128	0.935													
	1**	7**	1**	8**	7	**													
<b>YP</b>	0.806	0.702	0.630	0.625	0.100	0.661	0.541												
	**	**	2**	9**	2	7**	3**												
<b>PL</b>	0.595	0.799	0.738	0.794	0.320	0.783	0.707	0.759											
	6**	**	8**	2**	5*	6**	6**	4**											
<b>NL</b>	0.547	0.701	0.588	0.713	0.324	0.702	0.619	0.724	0.818										
	2**	1**	3**	6**	1*	2**	4**	2**	8**										
<b>RWC</b>	0.666	0.827	0.757	0.824	0.290	0.825	0.789	0.826	0.838	0.784									
	5**	7**	9**	4**	8*	1**	3**	2**	2**	8**									
<b>MSI</b>	0.695	0.802	0.715	0.832	0.204	0.867	0.771	0.759	0.819	0.786	0.900								
<b>I</b>	1**	2**	1**	2**	1	9**	9**	9**	4**	5**	5**								
<b>LA</b>	0.471	0.625	0.283	0.567	0.525	0.405	0.332	0.365	0.465	0.287	0.392	0.351							
	1**	7**	2	7**	3**	9**	4*	2**	3**	2	1**	8*							
<b>TPC</b>	0.214	-	-	-	-	0.155	-	0.179	0.018	-	-	-	0.16						
	7	0.106	0.113	0.124	0.140	3	0.350	3	6	0.006	0.211	0.023	68						
		6	2	6	1		8*			6	5	9							
<b>PRO</b>	0.548	0.694	0.637	0.586	-	0.804	0.812	0.491	0.560	0.512	0.721	0.768	-	0.35					
	2**	9**	3**	5**	0.064	7**	6**	7**	8**	3**	6**	3**	0.14	8*					
					6								38						

Significant = (\*) at 0.05 alpha level, Highly significant = (\*\*) at 0.01 alpha level, PP = Number of pods per plant, PC = Number of pods per cluster, CP = Cluster per plant, SP = Number of seed per pod, HSW = Hundred seed weight, RL = Root length, SL = Shoot length, YP= Yield per plant, PL = Pod length, NL = Leaf number per plant, RWC = Relative water content, MSI = Membrane stability index, LA = Leaf area, TPC = Total phenolic content, PRO = Proline content

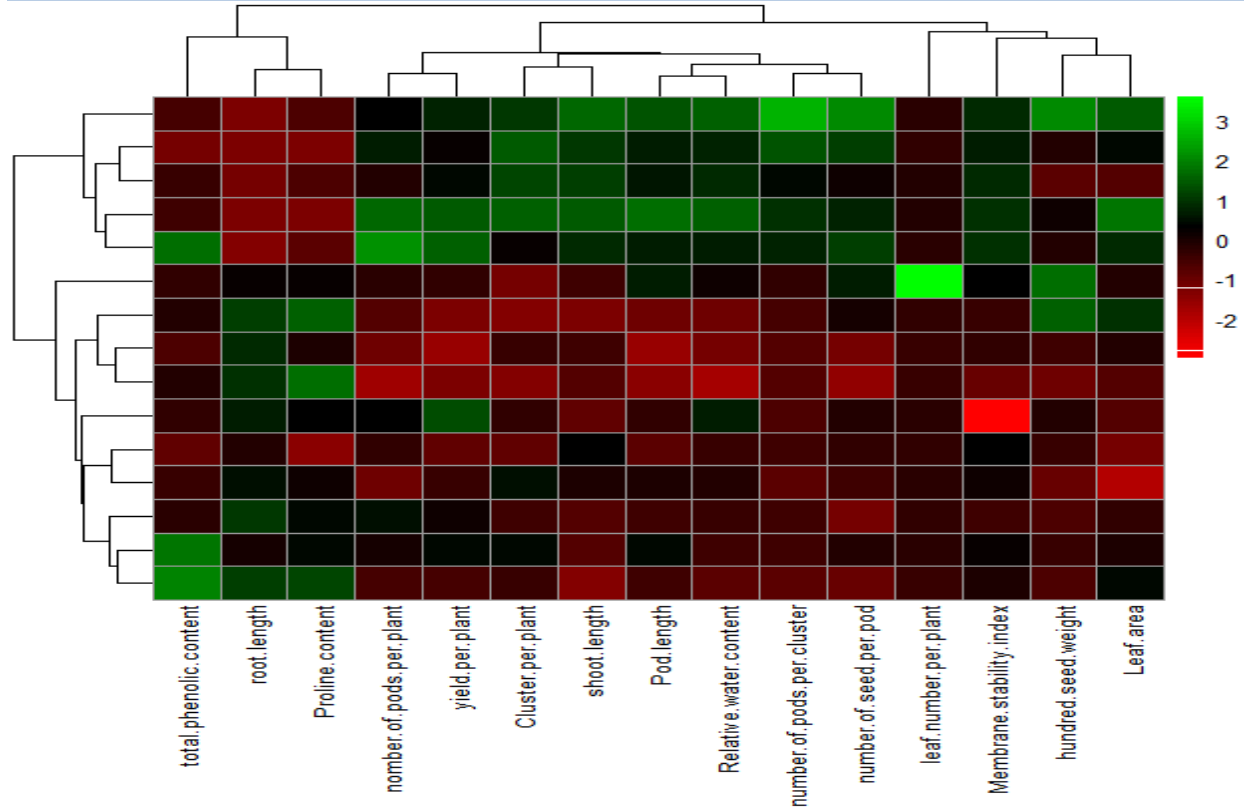


Fig.1 Cluster analysis between morphological, physiological, and biochemical attributes of mungbean. T1 = Control, T2 = water stress in the vegetative stage, T3= water stress in the reproductive stage. Quantifying proline and total phenolic content as biomarkers for drought resilience in Vigna radiate cultivars. Effect of water stress on the leaf area, hundred seed weight and membrane stability index, seed per pod, number of pods per cluster, relative water content, pod length, shoot length, cluster per plant, yield per plant, number of pods per plant, proline content, root length and total phenolic content of Vigna radiate.

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**Declarations****Data Availability statement**

All data generated or analyzed during the study are included in the manuscript.

**Ethics approval and consent to participate.**

It is approved by the department concerned.

**Consent for publication**

Approved

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The authors declared an absence of conflict of interest.

**Authors Contribution**

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