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Morphological and Biochemical Changes in Moth Bean during Drought Stress

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The current investigation on the repercussions of drought stress on the growth parameters of the Moth bean (*Vigna aconitifolia*) has been carried out in a semi-field condition. Two sets of local moth bean cultivars (RMO-40: fifteen in each) were prepared, and one was subjected to terminal drought stress. The rest was maintained with proper watering as a control set. The treated plants were thoroughly evaluated based on leaf length, root length, and relative leaf water content in comparison with the control ones. The findings of this study reveal a significant impact of drought stress on moth bean's growth and physiological performance. Under drought conditions, treated plants exhibited considerable reductions in leaf length and root length compared to their well-watered counterparts. Additionally, the relative leaf water content declined under drought stress, indicating decreased water uptake and retention within the plants under stress. These outcomes underscore the vulnerability of moth bean to drought stress, emphasizing the urgency of developing efficient water management strategies and drought-resistant varieties to safeguard food security in regions where this legume serves as a staple crop. This research highlights the importance of understanding the adverse effects of drought stress on moth bean's growth parameters for sustainable agriculture and food production.

Keywords: Moth bean; relative water content; Membrane stability index; drought; legume; morphological changes.

1. INTRODUCTION

Moth bean [*Vigna aconitfolia* (Jacq.) Marechal] is an annual legume crop that belongs to the subfamily Papilionaceae of the Fabaceae family [1]. It is an herbaceous creeping annual that creates soil coverage when fully grown and conserves soil moisture significantly. It is known by several names at different places and linguistic zones including moth bean, mat bean, matki, and dew bean. It has wide social acceptance and geo-Figuric adaptation and is considered the native pulse of India. Ecologically, it is an annual legume of dry and warm regions and the most drought-hardy species in arid regions. Moth bean has a deep and fast penetrating root system, which helps them to survive up to 30-40 days in open fields without any irrigation[2,3]. Moth bean plants generally grow 15-40 cm tall, having short internodes. The primary branches are as large as 1.5 m possessing papilionaric flowers [3]. The Indian arid zone is characterized by a harsh and fragile system, which influences the productivity (both quantitative and qualitative) and socioeconomic status of the inhabitants. In order to combat the adverse effects of drought and to ensure food safety it is essential to develop water-deficit stress-tolerant genotypes [4]. Moth bean is highly resilient to water scarcity and can withstand prolonged periods of drought [5]. This remarkable adaptive trait makes it an essential crop in regions where water availability is limited and it contributes to food security in arid areas. Moth bean has a relatively short life cycle [6] with early maturation compared to many other legume crops. This characteristic allows for quick harvesting and multiple cropping, which is advantageous in regions with limited growing seasons. Moth bean is nutritionally valuable, and rich in protein (about 23-25%) and dietary fiber, making it an excellent source of essential amino acids and micronutrients [7], It is particularly beneficial for vegetarian diets [8] and contributes to combating malnutrition and protein deficiency. In addition to its nutritional significance, moth bean has medicinal properties. It is believed to possess antioxidant and anti-inflammatory properties [9] and traditional medicine systems often use it for various health conditions. Moth bean plays a crucial role in enhancing food security in regions where conventional crops may struggle to survive under water scarcity. Its ability to produce a reasonable yield even with minimal water resources ensures a stable food supply for communities in arid and drought-prone areas. Moth bean is an excellent rotational crop [10], particularly in cereal-based cropping systems. As a legume, it can fix atmospheric nitrogen [11] through a symbiotic relationship with nitrogen-fixing bacteria (rhizobia).In the face of climate change, this adaptability becomes even more crucial for sustaining agriculture and supporting the livelihoods of farming communities.

Drought stress restricts the availability of water in the soil, leading to reduced water uptake by plant roots [12] As a result, plants may experience water deficits and struggle to maintain their essential physiological processes. In response to water scarcity, plants typically close their stomata, which are small pores on the leaf surfaces responsible for gas exchange. While this reduces water loss through transpiration, it also limits the entry of carbon dioxide, essential for photosynthesis. Consequently, the rate of photosynthesis and carbon assimilation declined [13]. As photosynthesis is impaired under drought stress, plants produce less energy and assimilate fewer nutrients. This leads to reduced growth rates, lower biomass accumulation, and ultimately decreased crop yield [14]. The imbalance in water availability can create osmotic stress in plants. The concentration of solutes increases within the plant cells, leading to cellular dehydration and disruption of cellular functions. Drought-stressed plants may undergo premature leaf senescence as a survival strategy. This process involves the degradation of cellular components, chlorophyll breakdown, and leaf shedding, further reducing the photosynthetic capacity of the plant [15]. Drought stress can induce changes in plant morphology, including reduced plant height, leaf size, and root length. These morphological adjustments are attempts by the plant to cope with water scarcity and optimize water use.

The research objective of studying morphological and biochemical changes in moth bean (*Vigna aconitifolia*) during drought stress is to understand the plant's adaptive responses and physiological mechanisms in response to water scarcity. Understanding the morphological adaptations can provide valuable insights for crop breeders and agricultural scientists seeking to develop drought-tolerant varieties of moth bean and other legume crops. Additionally, this knowledge can help to optimize agronomic practices for improving crop productivity and water-use efficiency in regions prone to drought. Implementing sustainable agricultural practices based on the current investigations can lead to better water-use efficiency, reduced environmental impact, and increased agricultural sustainability. This knowledge can aid in developing strategies to adapt agriculture to changing climatic conditions, mitigating the impacts of climate-related challenges. The insights gained from studying morphological and biochemical changes in moth bean can apply to other legume crops and even other plant species. This research can provide a foundation for improving various crops' drought tolerance and overall performance under adverse environmental conditions. Enhancing the drought tolerance of moth bean and promoting sustainable agricultural practices can have positive ecological impacts. Reduced water consumption, improved soil health, and enhanced biodiversity conservation are potential outcomes of adopting drought-tolerant crop varieties and better agronomic practices. Overall, investigating the morphological and biochemical changes in moth bean during drought stress is of significant scientific and practical importance. It can contribute to the advancement of agricultural science, the development of climate-resilient crops, and the promotion of sustainable agriculture in the face of increasing water scarcity and climate uncertainties.

2. MATERIALS AND METHODS

2.1 Growing of Plants

The healthy seed of local moth bean variety RMO-40 was procured from the ARS, Bikaner, and sown in the seedling tray (pro tray) with appropriate growing medium (coco peat + perlite and vermiculite). One seed per cell was sown and covered with medium. Coco peat with 300 to 400 per cent moisture was used and hence no immediate irrigation was required until germination. Two sets of plants were established, *i.e.,* one as control (regular watering) and the rest as treated (subjected to drought). The trays are kept in a plant growth chamber (Lab Companion, Republic of Korea), where optimum temperature (30℃) and relative humidity (60%) were maintained under 16/8 h light: dark period. Spraying of 0.3 per cent (3 g/liter) water soluble fertilizer using poly feed 5 days after sowing to enhance the growth of the seedlings. 5 mL of MS media was also applied per plant after 10 days of sowing. The seedlings were ready for applying drought stress in about 15 days.

2.2 Applying drought Stress

For applying drought stress, fifteen days old seedlings were taken and drought stress was applied by withholding of water. One tray was taken as a control seedling and the other set was subjected to drought stress. The plants were kept in glasshouse conditions at 30°C with 60% relative humidity and 16 h light and 8 h dark (Fig. 1). The control samples were maintained by applying MS media and water parallelly.

2.3 Morphological, Physiological, and Biochemical Data Analysis

2.3.1 Physiological data analysis

(A) Relative Water Content (RWC)

It is generally accepted that the maintenance of the integrity and stability of membranes under water stress is a major component of drought tolerance in plants **(**Dastborhan and Ghassemi, 2015**).** Leaf segments were initially weighed and floated over the distilled water for 4 and 6 hours and the turgid weight was recorded. Dry weight was obtained after drying the leaf segments at 5 °C for 48 hours. The relative water content was calculated by the given formula [16].

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

(B) Membrane stability index

Membrane stability index (MSI) in randomly selected plants from treatment and controlled samples was determined according to the method of Sairam et al.17] For estimation of membrane stability index 100 mg leaf material, in two sets from randomly selected two plants of treatment and control samples, is taken in test tubes containing 10 ml of double distilled water. One set is heated at 40 °C for 30 min in a metabolic water bath then cooled at room temperature and the electrical conductivity of the solution is recorded on a conductivity bridge (C_1). The second set is boiled at 100 °C in a boiling water bath for 10 min, then cooled at room temperature, and its conductivity is measured on a conductivity bridge (C_2). The membrane stability index (MSI) is calculated as

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

2.3.2 Morphological data analysis

Morphological data analysis was done to identify, structure, and investigate the total set of possible relationships contained in a given plant. The traits used, their codes, and the procedures followed are detailed in the Table 1.

2.3.3 Correlation regression, and PCA analysis

Correlation-regression analyses were conducted to determine the relationships between different plant parameters. Correlation regression and PCA analysis of plant physiological as well as biochemical properties were done using relevant statistical packages on Rstudio.

Table 1. Traits and their description used in the present study

Sl. No.	Trait code	Trait name (units)	Description
1	PH	Plant height (cm)	The height of the plant was measured using a meter scale from the base of the plant to the tip of the Panicle
2	LW	Leaf Weight (g)	The weight of the leaf was measured, using a weighing machine
3	RL	Root length (cm)	The height of the plant root was measured, using a meter scale from the base of the plant to the tip of the root
4	WL	Width of leaf	The width of the leaf was measured, using a meter scale from one end to another end of the leaf
5	NL	Number of leaves	The number of leaves was counted
6	LL	Leaf length	The length of the leaves was measured, using a meter scale from one end to another end of the leaf
7.	RW	Root weight	The weight of the root was measured by a weighing machine

3. RESULTS AND DISCUSSION

3.1 Validation of drought Stress Occurrence on Moth Bean

Validating the occurrence of drought stress on moth bean (*Vigna aconitifolia*) involves assessing various physiological, morphological, and biochemical indicators of stress. Drought stress affects plants at multiple levels, from cellular changes to whole-plant responses. Drought occurrence in plants is shown in Fig. 2. Here's a step-by-step guide on how to validate drought stress occurrence on moth bean:

3.1.1 Morphological observations during drought stress

(A) Root length

A wide range of significant variability in root length was found in moth bean during drought stress in control as well as in drought-stressed plants. It varied from 7.83 cm to 15.75 cm with a mean range of 10.49 cm in the case of control plants. Whereas, it ranged from 3.60 to 8 cm with a 5.09 cm mean in drought-stressed plants (Table 2 and Fig. 2).

(B) Plant height

A significant change in moth bean plant height was observed due to drought stress in moth bean. The range of variation in plant height was observed from 13.30 to 18.70 cm with a mean value of 16.02 cm. In the case of drought-treated plants, the plant height ranged from 11 cm to 13.20 cm with a mean value of 12.27 cm (Table 3 and Fig. 3). Similarly, the reduced plant height in treated plants is also indicated by the occurrence of drought in moth bean plants.

Table 2. Root length of control and treated plant

S. No.	Control Plant (cm)	Treated Plant (cm)
1	15.00	4.50
2	10.00	4.00
3	9.00	8.00
4	10.00	5.50
5	8.70	3.80
6	14.25	4.30
7	10.50	3.90
8	9.90	7.70
9	9.00	5.40
10	8.26	3.80
11	15.75	4.50
12	11.00	4.10
13	7.83	7.80
14	9.13	5.50
15	9.00	3.60
Mean	10.49	5.09
SEm±	2.49	1.547

Fig. 1. Pro-tray display of drought-stressed plantlets along with the control ones (after 15 days of treatment)

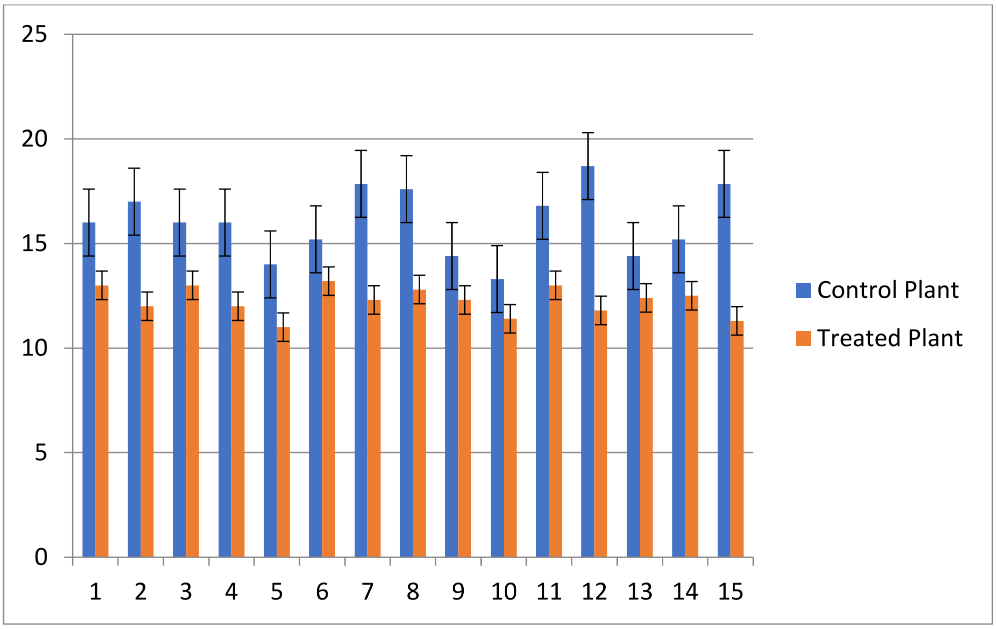

Fig. 2. Root length of control and treated plant including degree of error

(C) Leaf length

The variation in leaf length of moth bean was significantly affected due to drought stress. It ranged from 2.90 cm to 4.40 cm in control plants with a mean of 3.62 cm and from 1.90 cm to 3.3 cm with an overall mean of 2.63 cm in drought-stressed samples (Table 4 and Fig. 4).

Table 3. Plant hight of control and treated plant

S. No.	Control Plant (cm)	Treated Plant (cm)
1	16.00	13.00
2	17.00	12.00
3	16.00	13.00
4	16.00	12.00
5	14.00	11.00
6	15.20	13.20
7	17.85	12.30
8	17.60	12.80
9	14.40	12.30
10	13.30	11.40
11	16.80	13.00
12	18.70	11.80
13	14.40	12.40
14	15.20	12.50
15	17.85	11.30
Mean	16.02	12.27
SEm±	1.60	0.681

(D) Leaf width

A moderately significant variability in leaf width ranged from 1.9 cm to 3.32 cm with a mean value of 2.44 cm was observed in control plants. In drought-treated plants, leaf width was reduced and varied from 1.15 to 2.09 cm with1.52 cm mean as compared to control plants (Table 5 and Fig. 5). The variation in leaf length and width of control and stressed plants has thus suggested the occurrence of drought in moth bean plants.

Table 4. Leaf length of control and treated plant

S. No.	Control Plant (cm)	Treated Plant (cm)
1	3.70	3.30
2	3.70	2.00
3	4.20	2.50
4	3.50	3.20
5	3.00	2.20
6	3.80	3.30
7	3.60	2.00
8	4.40	2.50
9	3.50	3.20
10	2.90	2.20
11	3.70	3.20
12	3.70	1.90
13	4.20	2.50
14	3.30	3.20
15	3.10	2.20
Mean	3.62	2.63
SEm±	0.43	0.543

(E) Root weight

For control plants, wide variability in root length ranged from 0.08 to 0.18 gm with a mean of 0.11 gm recorded in moth bean.

As compared to control plants, the root length ranged from 0.01 gm to 0.03 gm with a mean of 0.02 gm in drought-treated plants of moth bean (Table 6 and Fig. 6). The significant variability in root length between control and drought-stressed plants occurred due to drought stress in moth bean.

Table 5. Leaf length of control and treated plant

S. No.	Control Plant (cm)	Treated Plant (cm)
1	2	2.00
2	2.2	1.30
3	2.4	1.20
4	1.8	1.70
5	1.9	1.40
6	3.32	2.06
7	2.06	1.25
8	2.60	1.15
9	3.04	1.62
10	2.24	1.41
11	3.19	2.09
12	2.05	1.31
13	2.42	1.19
14	3.23	1.70
15	2.13	1.46
Mean	2.44	1.52
SEm±	0.52	0.323

3.1.2 Physiological parameters

(A) RWC

The Relative Water Content (RWC) was calculated during drought stress in moth bean and it was compared between control and stressed plants. The RWC ranged from 92.53 to 103.30% in control plants with an overall mean was 98.12 whereas it varied from 34.03 to 48.43% with a mean of 42.42 in drought-stressed plants (Table 8 and Fig. 8). The RWC is directly proportional to the water content in the plant during drought-stressed conditions. It was always found near 100% in normal and healthy plants whereas it was decreased in stressed plants. The present analysis suggested the occurrence of the drought in moth bean plants because it was found lesser as compared to the healthy control plants.

(F) Number of leaves

A significant variability ranging from 12 to 20 cm with a mean value of 16 and a standard error of 2.84 was observed in control plants. Whereas, in drought-treated plants, the number of leaves varied from 7 to 9 with a mean value of 8, and the standard error mean was 0.676. The data are shown in Table 7 and Fig. 7.

Table 6. Root weight of control and treated plant

S. No.	Control Plant (gm)	Treated Plant (gm)
1	0.10	0.02
2	0.17	0.01
3	0.10	0.02
4	0.08	0.03
5	0.10	0.02
6	0.10	0.02
7	0.17	0.01
8	0.10	0.02
9	0.08	0.03
10	0.10	0.02
11	0.10	0.02
12	0.18	0.01
13	0.09	0.02
14	0.08	0.03
15	0.09	0.02
Mean	0.11	0.02
SEm±	0.03	0.0069

Table 7. Number of leaves of control and treated plant

S. No.	Control Plant (cm)	Treated Plant (cm)
1	17	9
2	20	9
3	20	9
4	15	8
5	13	7
6	17	8
7	19	9
8	19	9
9	15	8
10	13	7
11	16	8
12	20	8
13	20	8
14	15	8
15	12	8
Mean	16	8
SEm±	2.84	0.676

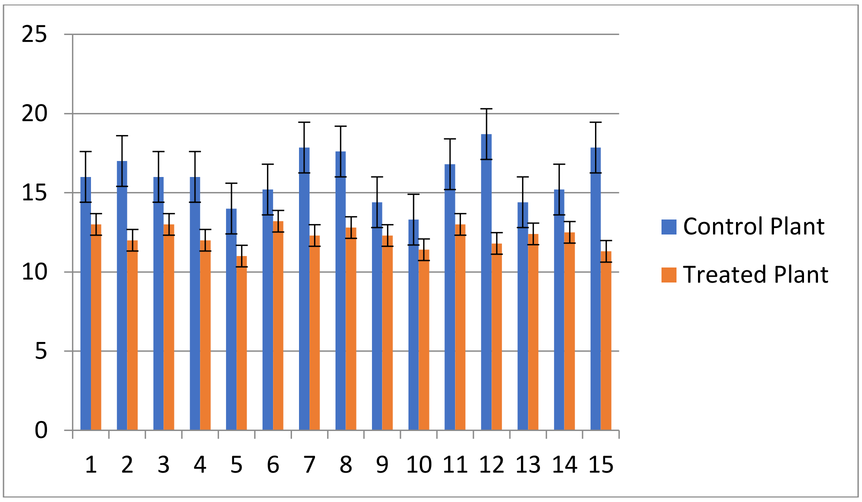

Fig. 3. Plant height of control and treated plant including the degree of error

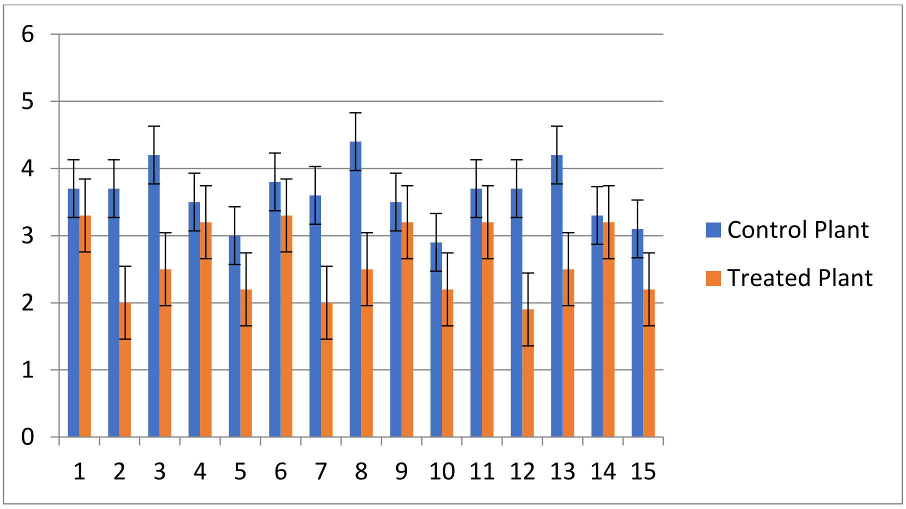

Fig. 4. Leaf length of control and treated plant including degree of error

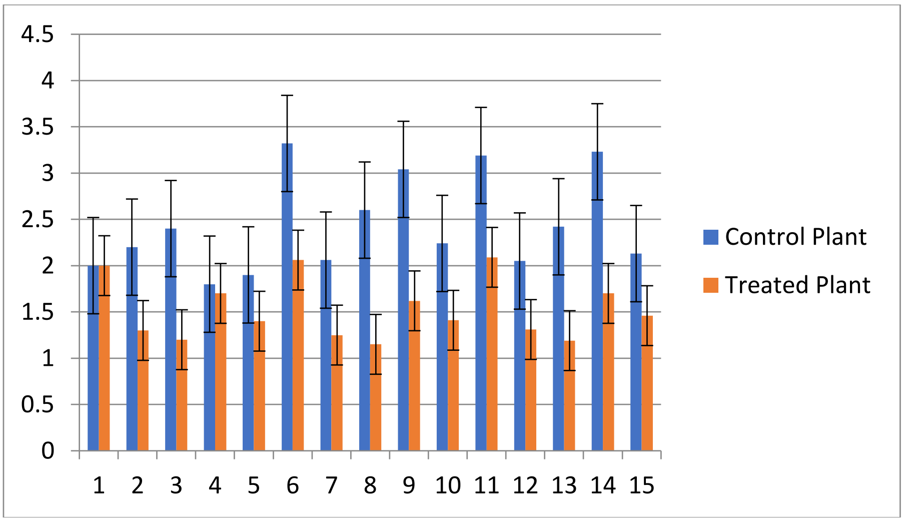

Fig. 5. Leaf width of control and treated plant

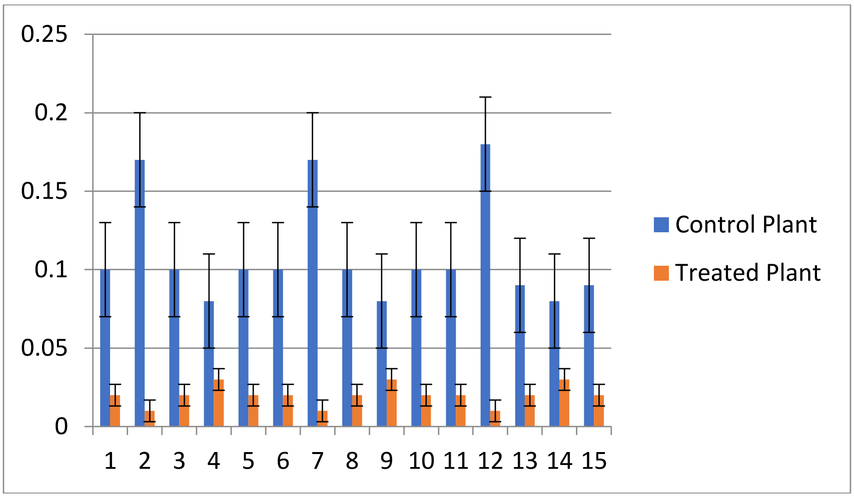

Fig. 6. Root weight of control and treated plant

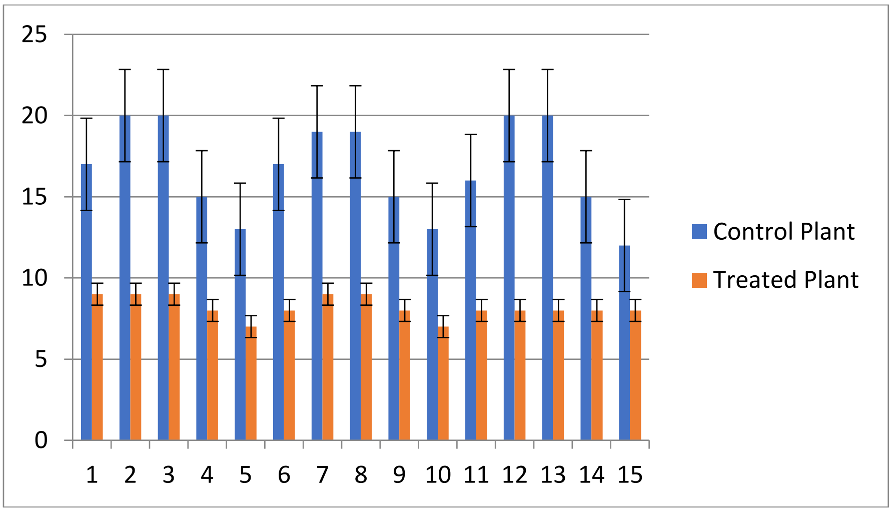

Fig. 7. Number of leaves of control and treated plant

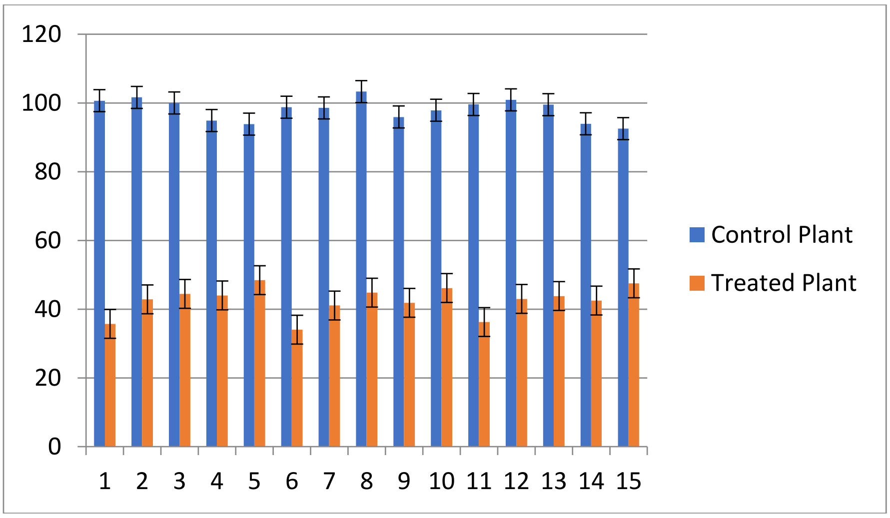

Fig. 8. RWC of control and treated plant

(B) MSI

A significant variation in the MSI was observed in stress-treated plants as compared to control plants during drought stress in moth bean. The MSI of control plants was 74.54% while stress plants showed 84.83% MSI during drought conditions in moth bean as described in Table 9 and Fig. 9. The higher MSI of stressed plants as compared to control plants indicated the occurrence of drought stress in moth bean.

Table 8. RWC of control and treated plant

S. No.	Control Plant (%)	Treated Plant (%)
1	100.67	35.71
2	101.61	42.85
3	100	44.44
4	94.89	44
5	93.84	48.43
6	98.76	34.03
7	98.56	41.05
8	103.30	44.80
9	95.93	41.84
10	97.88	46.15
11	99.56	36.25
12	100.90	42.98
13	99.50	43.82
14	93.94	42.50
15	92.53	47.51
Mean	98.12	42.42
SEm±	3.20	4.194

3.2. Cos2 Variable Dim

Cos2 is a measure used in Principal Component Analysis (PCA) to assess the quality of representation of a variable on a given principal component. The high value of RWC in the graph could indicate that the drought-treated plants had a higher relative water content compared to the control plants.

Drought response mechanisms in plants include morph-physiological, biochemical, cellular, and molecular processes that take place in plants under drought stress. These processes include improvement in the root system, leaf structure, osmotic adjustment, relative water content, and stomata regulation.

3.3 Correlation

The correlation matrix was visualized using a heatmap (Fig. 11). The matrix included 12 variables, labeled as “MSI”, “RWC”, “NL”, “RW”, “LW”, “LL”, “PH”, “RL”, “Treated”, “Control”, “RWC”, and “MSI”. The color scale ranged from -1.0 to 1.0, with blue representing a negative correlation and red representing a positive correlation. A few cells were white, indicating no correlation between the corresponding variables.

Table 9. MSI of control and treated plant

S. No.	Control Plant (%)	Treated Plant (%)
1	76.320	87.820
2	74.270	86.860
3	75.830	82.750
4	73.290	83.080
5	73.400	83.960
6	74.270	85.440
7	72.680	84.850
8	74.370	85.170
9	77.100	87.140
10	74.850	85.370
11	75.760	84.220
12	72.940	86.310
13	72.180	82.250
14	76.390	85.290
15	74.570	82.000
Mean	74.548	84.834
SEm±	1.488	1.788

3.4 PCA Biplots

A PCA biplot is a type of scatter plot used in Principal Component Analysis (PCA) to represent the original data using principal components that explain the majority of the data variance. The biplot has two components: scores and loading vectors. The scores are represented by points on the plot, while the loading vectors are represented by arrows or lines 1. This Fig. 12, appears to be a PCA biplot with two dimensions, Dim1 and Dim2. The x-axis is labeled “Dim1 (97.7%)” and the y-axis is labeled “Dim2 (2.2%)”, indicating that these two dimensions explain 97.7% and 2.2% of the data variance, respectively. The plot has a black grid and a green line connecting the points, which are colored according to a scale from yellow to green, with yellow being the lowest value and green being the highest value. The legend on the right side of the plot shows the scale and the labels “cos2” and “0.200” to “0.350”.The plot has several labels, including “RW”, “PH”, “RWC”, “MSI”, “RL”, “LL”, “LW”, “Treated”, and “Control”. These labels likely represent different variables or groups in the data. The points on the plot represent individual samples, with their position along Dim1 and Dim2 indicating their scores on these two principal components. The loading vectors are not shown in this image, so it is not possible to determine how strongly each variable influences the principal components. However, based on the position of the points and their color, it appears that there may be some separation between the treated and control groups along Dim1.

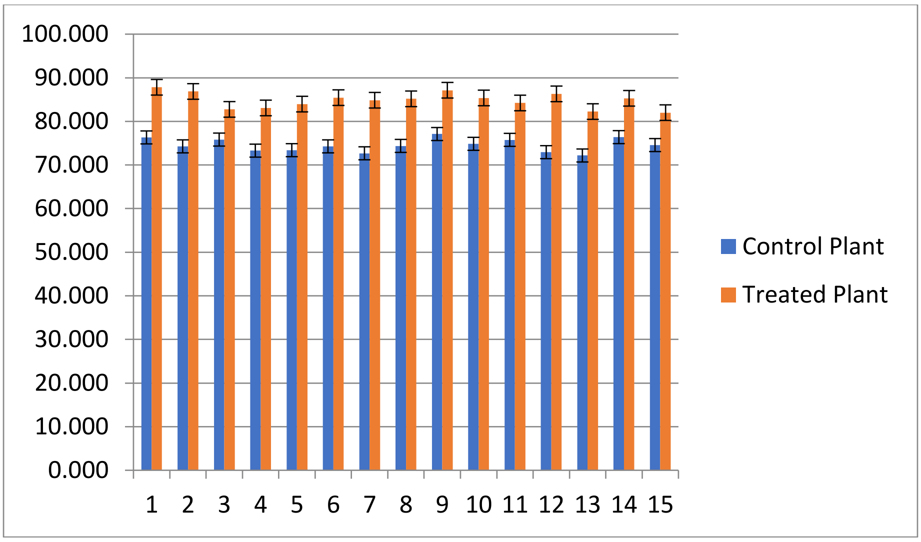

Fig. 9. MSI of control and treated plant

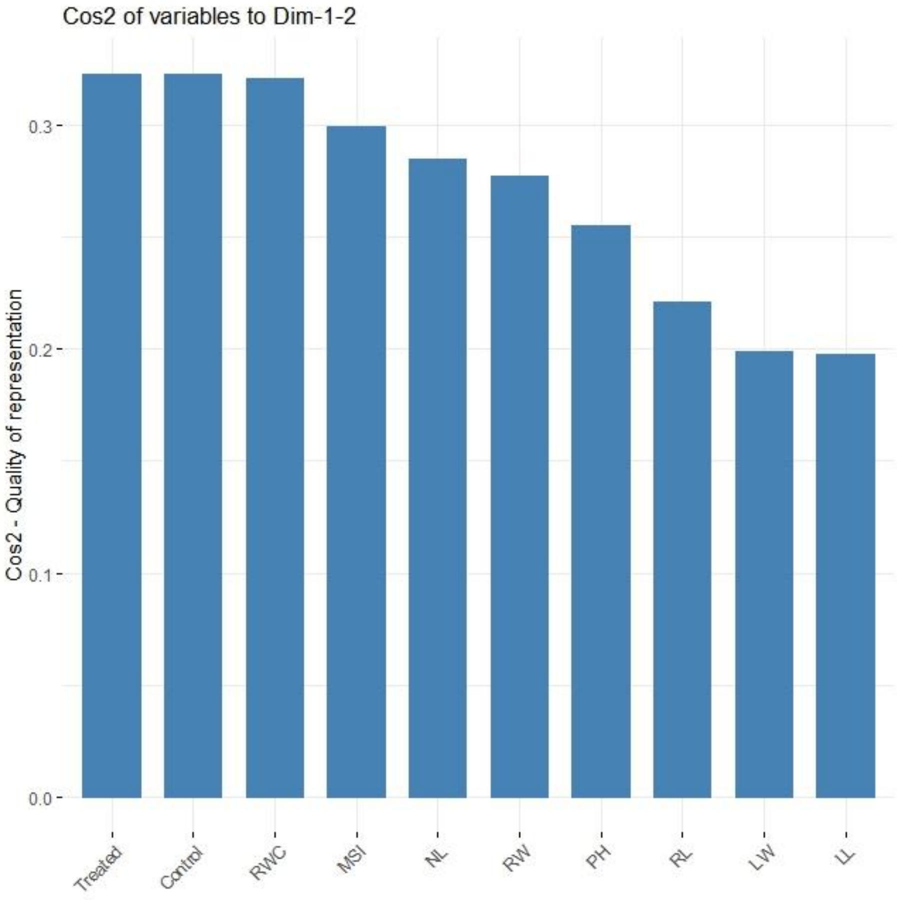

Fig. 10. Cos2 variable dim

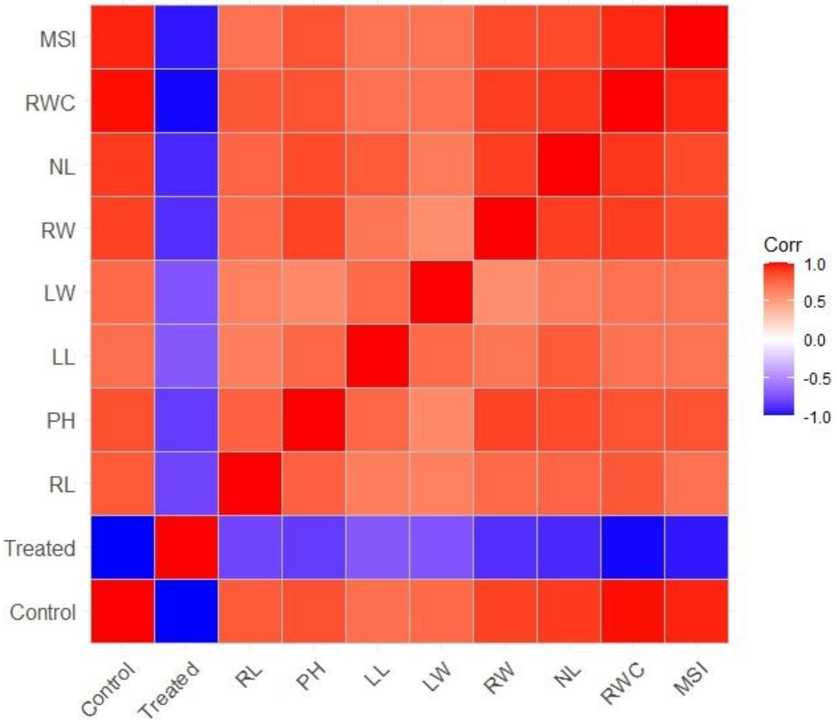

Fig. 11. Correlation

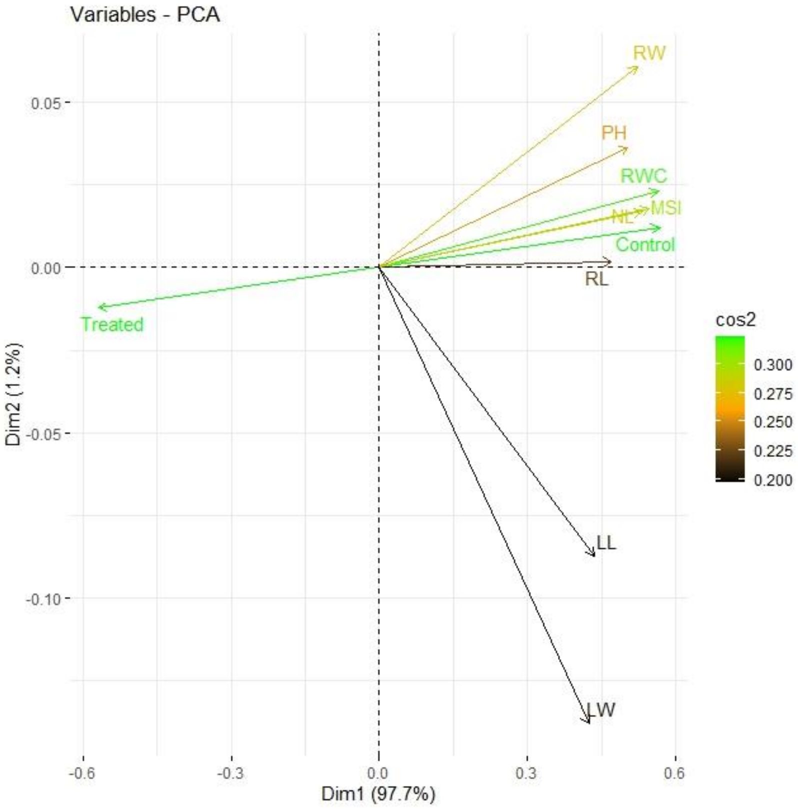

Fig. 12. nPCA Biplots

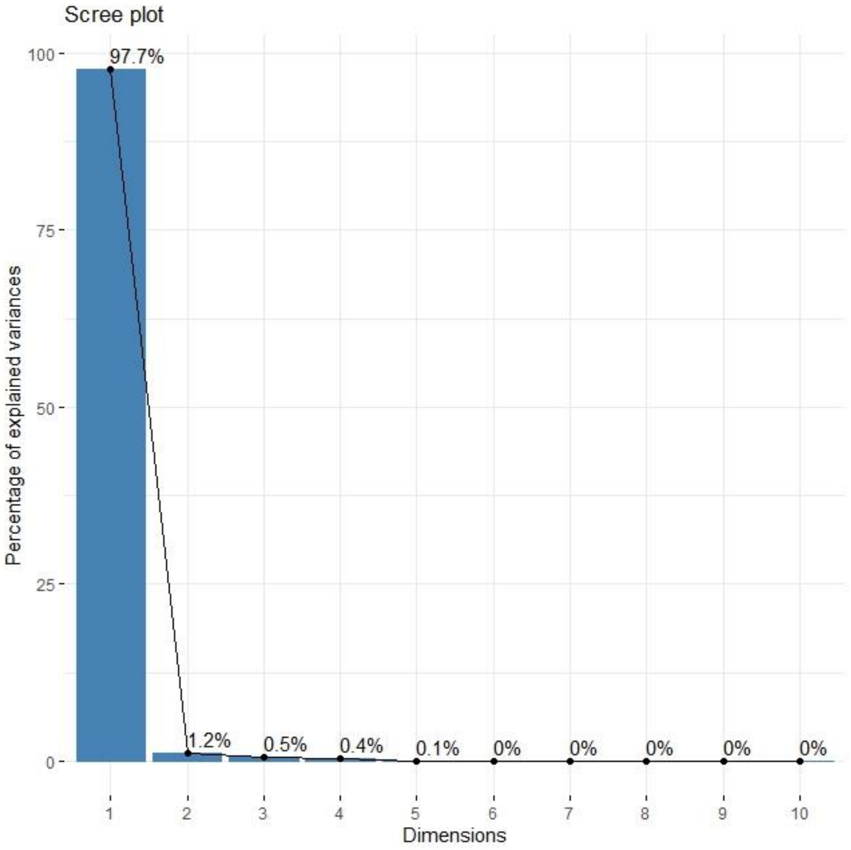

Fig. 13. Scree plot

The majority of the cells were red, indicating a positive correlation between the corresponding variables. For example, there was a strong positive correlation between the variables “MSI” and “RWC” (r = X, $p < 0.05$). This suggests that as the value of MSI increases, so does the value of RWC.

3.5 Scree Plot

A scree plot is a graphical representation of the percentage of explained variances by each dimension in a principal component analysis (PCA). The plot shows the eigenvalues (or percentage of explained variances) of each dimension in descending order. The purpose of a scree plot is to help determine the optimal number of dimensions to retain in a PCA. Based on the description of Fig. 13, it appears that the scree plot shows the percentage of explained variances for dimensions 2 to 10. The blue line starts at 97.7% at dimension 2 and decreases to 0% at dimension 10. This suggests that the first two dimensions explain most of the variance in the data. However, without more context or information about the data and analysis, it is difficult for me to provide a more detailed interpretation of this specific scree plot and its relation to drought-treated plants and control plants.

4. CONCLUSION

In this study, a pot experiment was conducted, subjecting *Vigna aconitifolia* (moth bean) to two distinct treatments: a control group with optimal watering conditions and a drought stress group with limited water availability. Our results reveal that plants have developed different types of tolerance mechanisms to fight against drought and plants will try to perform their normal metabolic activity even under abnormal environmental conditions [18]. This study showed significant alterations in the growth parameters of moth bean under drought stress [5]. Specifically, we noted a marked reduction in both leaf length and root length in the drought-stressed plants compared to the well-watered control group [1]. This adaptation suggests the plant's resource allocation strategy, geared towards water conservation and survival in the face of limited water resources. Additionally, our assessment of relative leaf water content indicated a substantial decrease in moisture retention within the leaves of drought-stressed plants, indicative of water deficit-induced stress [19]. These findings collectively emphasize the intricate and adaptive responses of *Vigna aconitifolia* to drought stress, offering insights into its capacity to modulate growth and physiological processes in challenging environmental conditions. Such insights are essential for devising strategies to enhance the resilience of moth bean, thus contributing to sustainable agriculture in water-scarce regions and ensuring food security.

COMPETING INTERESTS

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