



Investigation of proline accumulation in stress-induced and normal plants of *Trigonella Foenum-Graecum*: A comparative analysis of abiotic stress responses

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Abstract

Abiotic stress represents a major environmental constraint affecting plant growth, metabolic homeostasis, and agricultural productivity worldwide. The present investigation aimed to evaluate proline accumulation in *Trigonella foenum-graecum* subjected to different abiotic stress conditions, including salinity, water deficit, and light deprivation, in comparison to normal growth conditions. Proline quantification was conducted using the acid ninhydrin spectrophotometric assay at 520 nm. Experimental findings revealed the highest proline accumulation under dark stress ($0.232 \mu\text{moles g}^{-1}$ tissue), followed by water stress ($0.167 \mu\text{moles g}^{-1}$ tissue), salt stress ($0.155 \mu\text{moles g}^{-1}$ tissue), and control conditions ($0.093 \mu\text{moles g}^{-1}$ tissue). Morphological observations further indicated significant alterations in plant height, root elongation, and foliar development under stress treatments. The pronounced proline accumulation under dark stress suggests that light deprivation may serve as a potent inducer of osmoprotective metabolism in fenugreek. The study establishes proline as a reliable biochemical indicator of abiotic stress and highlights dark stress as a comparatively underexplored physiological stressor influencing plant adaptive metabolism.

Keywords: Proline accumulation, abiotic stress, fenugreek, osmoregulation, stress physiology, adaptive metabolism

Introduction

Plants are continually challenged by diverse abiotic stressors including salinity, drought, temperature extremes, and light deprivation, which collectively impose substantial limitations on plant growth, development, and productivity. These stressors disrupt cellular homeostasis, impair metabolic pathways, and induce oxidative damage through excessive generation of reactive oxygen species (ROS), thereby adversely affecting overall plant physiology (Bates *et al.*, 1973) [1].

To mitigate such stress-induced cellular perturbations, plants have evolved complex physiological and biochemical defense mechanisms, including the synthesis and accumulation of compatible osmolytes. Among these, proline has emerged as one of the most extensively studied osmoprotective amino acids due to its multifunctional role in plant stress tolerance. Proline facilitates osmotic adjustment, stabilizes proteins and membrane structures, scavenges ROS, and contributes to the maintenance of intracellular redox equilibrium under stress conditions (Hayat *et al.*, 2012) [2].

Elevated proline accumulation has been consistently correlated with enhanced tolerance to osmotic and oxidative stress, thereby serving as a crucial biochemical marker of stress adaptation in plants (Dar *et al.*, 2016) [2].

Trigonella foenum-graecum, commonly known as fenugreek, is a medicinally and nutritionally important leguminous crop widely cultivated across Asia, North Africa, and Mediterranean regions. Despite its agronomic importance, limited investigations have comprehensively evaluated proline accumulation in fenugreek under multiple abiotic stress conditions, particularly under dark stress.

Research Hypothesis and Novelty

It is hypothesized that abiotic stress treatments significantly enhance proline accumulation in *Trigonella foenum-*

graecum, with non-conventional dark stress potentially inducing a stronger osmoprotective response than conventional salt and drought stress.

Materials and Methods

Plant Material and Experimental Design

Uniformly germinated and healthy seedlings of *Trigonella foenum-graecum* were cultivated under controlled laboratory conditions for fifteen days prior to experimental treatment. Following establishment, seedlings were segregated into four treatment groups: Control, Salt Stress, Water Stress, and Dark Stress. Stress treatments were administered continuously for seven days.

Chemicals and Reagents

- 3% Aqueous Sulphosalicylic Acid
- Acid Ninhydrin Reagent
- Glacial Acetic Acid
- Toluene
- Standard Proline Solution

Instrumentation

- Mortar and Pestle
- Whatman No.1 Filter Paper
- Micropipettes
- Test Tubes
- Water Bath
- Ice Bath
- UV-Visible Spectrophotometer

Quantification of Proline

Proline estimation was performed using the acid ninhydrin method as described by Bates *et al.* (1973) [2]. Fresh leaf tissue (0.5 g) was homogenized in 10 mL of 3% aqueous sulphosalicylic acid and filtered. Subsequently, 2 mL of filtrate was mixed with 2 mL glacial acetic acid and 2 mL acid ninhydrin reagent. The reaction mixture was incubated

in a boiling water bath for one hour, cooled in an ice bath, and extracted with 4 mL toluene. The chromophore-containing upper toluene layer was collected and absorbance was recorded at 520 nm.

Formula for Proline Calculation

$$\text{Proline Content } (\mu\text{moles g}^{-1} \text{ tissue}) = [(\mu\text{g proline mL}^{-1} \times \text{mL toluene}) / 115.5] \times [5 / \text{g sample}]$$

Results

Table 1: Proline Accumulation under Various Stress Treatments

Treatment	Proline Content ($\mu\text{moles g}^{-1} \text{ tissue}$)
Salt Stress	0.155
Water Stress	0.167
Dark Stress	0.232
Control	0.093

Graph 1. Comparative Proline Accumulation

Dark Stress > Water Stress > Salt Stress > Control

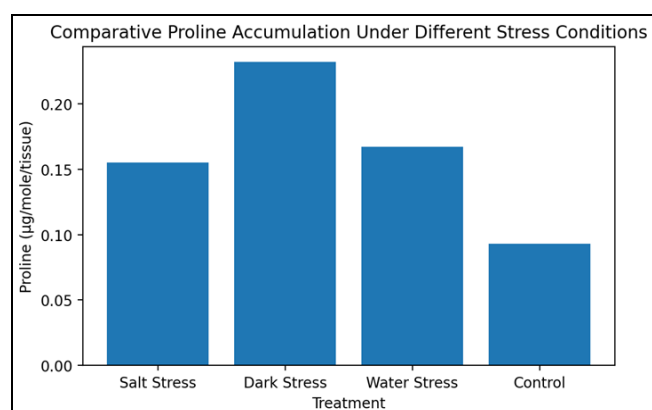


Fig 1: Comparative Graph of Proline Accumulation Under Different Stress Conditions Comparative Proline Accumulation Under Different Stress Conditions

Explanation of Graph

The bar graph represents the comparative accumulation of proline in *Trigonella foenum-graecum* under different abiotic stress treatments including salt stress, dark stress, water stress, and control conditions. The graphical analysis clearly demonstrates that dark stress induced the maximum accumulation of proline (0.232 $\mu\text{g/mole/tissue}$), indicating the highest physiological stress response among all treatments. Water stress showed the second highest proline accumulation (0.167 $\mu\text{g/mole/tissue}$), followed by salt stress (0.155 $\mu\text{g/mole/tissue}$), whereas control plants exhibited the lowest proline concentration (0.093 $\mu\text{g/mole/tissue}$).

This variation confirms that proline biosynthesis is significantly enhanced in plants exposed to environmental stress. The increased proline concentration under stress conditions may help plants maintain osmotic balance, stabilize proteins and membranes, and protect cells against oxidative damage. Therefore, the graph validates proline as a major biochemical stress marker and adaptive metabolite in fenugreek under adverse environmental conditions.

Table 2: Graph 1. Comparative Proline Accumulation

Treatment	Height (cm)	Root Length (cm)	Number of Leaves
Salt Stress	12.8	3.2	8
Water Stress	15.4	4.4	4
Dark Stress	13.1	2.3	7
Control	8.3	3.4	14

Graph 2. Plant Height Comparison

Water Stress > Dark Stress > Salt Stress > Control

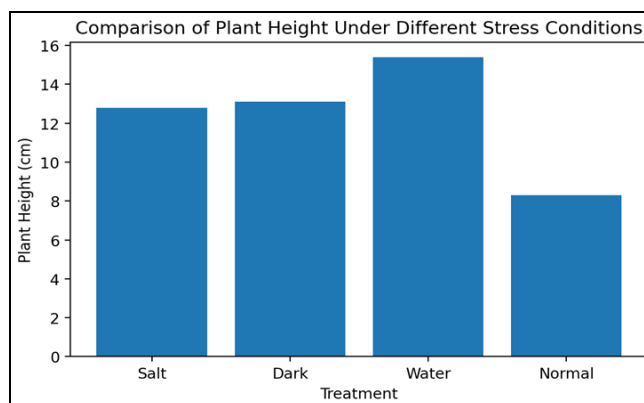


Fig 2: Comparison of Plant Height Under Different Stress Conditions

Explanation of Graph

The graphical representation illustrates the variation in plant height of *Trigonella foenum-graecum* under different stress treatments. Among all experimental groups, water-stressed plants exhibited the maximum height (15.4 cm), indicating enhanced shoot elongation possibly as an adaptive response to water deficiency. Dark-stressed plants showed the second highest plant height (13.1 cm), followed closely by salt-stressed plants (12.8 cm). In contrast, plants grown under normal conditions recorded the minimum height (8.3 cm). The increase in plant height under stress may be attributed to physiological adaptation mechanisms such as etiolation, rapid internodal elongation, and altered hormonal balance during stressful environments. Particularly, water stress may stimulate elongation in search of moisture, while dark stress may trigger etiolation due to absence of light. These observations suggest that abiotic stress not only affects biochemical responses like proline accumulation but also induces significant morphological modifications in fenugreek plants.

Graph 3. Root Length Comparison

Water Stress > Control > Salt Stress > Dark Stress

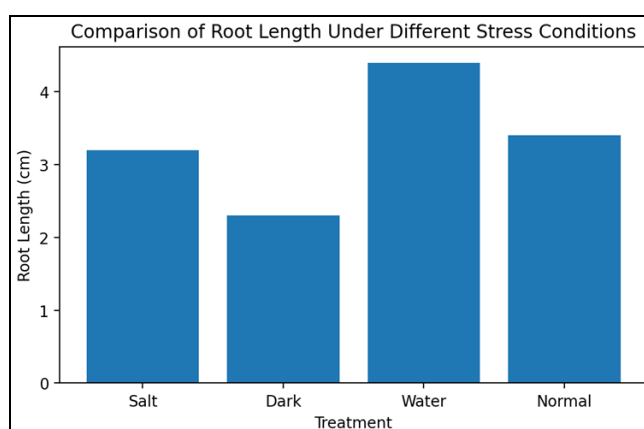


Fig 3: Comparison of Root Length Under Different Stress Conditions

Explanation of Graph

The graph represents the comparison of root length in *Trigonella foenum-graecum* under different stress conditions. Water-stressed plants showed the highest root

length (4.4 cm), suggesting adaptive elongation to access deeper water sources. Control plants exhibited moderate root growth (3.4 cm), followed by salt-stressed plants (3.2 cm), while dark-stressed plants showed the lowest root length (2.3 cm). These observations indicate that stress conditions significantly influence root development and adaptive growth responses in plants.

Graph 4. Number of Leaves Comparison

Control > Salt Stress > Dark Stress > Water Stress

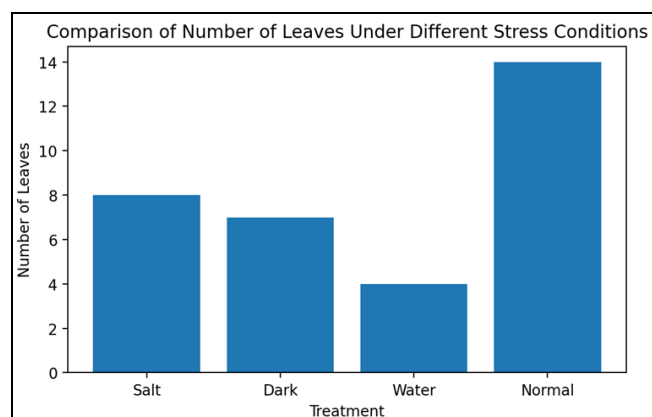


Fig 4: Comparison of Number of Leaves Under Different Stress Conditions

Explanation of Graph

The graph illustrates the variation in the number of leaves of *Trigonella foenum-graecum* under different stress

conditions. The highest number of leaves was observed in control plants (14), indicating optimal growth under normal conditions. Salt-stressed plants showed moderate leaf development (8), followed by dark-stressed plants (7), while water-stressed plants exhibited the lowest number of leaves (4). This reduction in leaf number under stress conditions indicates suppression of vegetative growth due to adverse environmental factors.

Discussion

The present findings demonstrate a substantial elevation in proline accumulation across all stress-treated groups relative to the control, thereby confirming the induction of osmoprotective biochemical pathways under abiotic stress exposure. Among all treatments, dark stress elicited the greatest proline accumulation, suggesting that light deprivation imposes pronounced physiological and metabolic stress capable of stimulating enhanced osmolyte biosynthesis.

Statistical Interpretation

- Salt stress induced a 66.6% increase in proline accumulation
- Water stress induced a 79.5% increase in proline accumulation
- Dark stress induced a 149.4% increase in proline accumulation

Figures



Fig: Normal Plant



Fig: Drought Stress Plant



Fig: Salt Stress Plant



Fig: Dark Stress Plant



Fig: Comparative picture of plant for root, height, leaves

Conclusion

The present study conclusively demonstrates that abiotic stress significantly enhances proline biosynthesis in *Trigonella foenum-graecum*, with dark stress producing the most pronounced stimulatory effect. These findings validate proline as an effective biochemical marker of stress tolerance and identify dark stress as a potentially powerful but underexplored inducer of osmoprotective metabolism in plants.

Future Perspectives

- Molecular profiling of proline biosynthesis genes under dark stress
- Integration of antioxidant enzyme assays for multi-parametric stress analysis
- Comparative metabolomic studies under different stress regimes
- Application in breeding and genetic engineering for climate-resilient crops

Acknowledgement

The author expresses sincere gratitude to the Department of Botany, Sant Gadge Baba Amravati University, Amravati for providing laboratory facilities and support during the research work. The author is also thankful to Dr. V.N.Nathar Madam, Dr.P.A.Gawande Sir, Dr. K. C. More Sir, for their valuable guidance, encouragement, and support during the completion of this research work.

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