

Phytochemical analysis of *Trigonella foenum-graecum* under drought stress

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Abstract: Drought and Salt condition are main abiotic stresses that shows a on affect plants morphology and physiology, which finally leads in a decrease in crop yield. An experiment was carried to look into the effects of drought on a *Trigonella foenum-graecum* plants. A small number of characteristics were examined in both the stressed and control plants. The experiment's findings demonstrated a noteworthy distinction between stressed and healthy plants. In contrast to the stressed plant, the healthy plant had the highest shoot height. In plants that were stressed by the drought, the roots were more numerous. In the stressed condition, there was a decrease in chlorophyll content. Stressed plants had higher levels of proline, flavonoids, and carbohydrates than healthy plants.

Key Words: stress, carbohydrate, flavonoids and proline.

1. INTRODUCTION:

Any unfavourable substance or environment can cause stress, which is a hindrance to a plant's growth, development, and metabolism. (1). Plant stress is the result of a sudden change from an ideal or normal state to an unfavourable one, which has an adverse effect on the physiology of the plant. Plant stress can be of two types-Abiotic and Biotic stress. Abiotic stress majorly consists of cold, drought, salt and heavy metals that largely affects the plant growth and productivity. Biotic stress includes infection by microbes, herbivores, injuries etc. As plants are sessile, they can't escape from the adverse conditions of environmental. The constantly changes in the environment, the abiotic stress is the major factor by which the plant growth and productivity is affected. Abiotic stress plays a major role in limiting crop productivity and also the distribution of crops across different environmental conditions. These stresses sometimes overlap that effects the plant growth and productivity. Abiotic stress includes salinity, drought and high temperature situation.

***Trigonella foenum-graecum*:** -The seeds are primarily used as a relish with unleavened bread or as a condiment to flavour curries made of rice, pulses, flour, and meat. The flavourful leaves are consumed raw. The seeds have aphrodisiac, tonic, and carminative properties. The plant is indigenous to Asia and southern Europe. It is mostly grown in India's north. It is an annual legume that has long, slender pods with a prominent beak and white flowers. The crop is ready to be harvested in April after being sown in October or November.

Drought stress is one of the major damaging environmental stressors among abiotic stressors. A drought is a complex stress that affects the physiology, morphology, molecular state, and biochemical that regulate the plant growth, quality, and, ultimately, productivity (2). Crop yields may be hampered by drought stress, which can be from moderate and brief to extremely severe and prolonged.

Drought effect on morphology: -During the early stages of crop growth and establishment, a limiting factor is drought stress because it impacts both elongation and expansion growth. A common negative consequence of water stress on plants is a reduce in the amount of dry and wet biomass produced (3).

Drought effect on secondary metabolites: -Dry season Conditions diminishes the concentration of the saponins in *Chenopodium quinoa* from 0.46% of the dry weight in crops developing beneath direct water shortage circumstances to

0.38% in more water shortfall plant (4). Anthocyanins have been shown to accumulate during droughts and at low temperatures. Anthocyanin-rich plant tissues are typically drought-resistant (5,4). Flavonoids offer preventive properties during drought stress. Flavonoids have been linked to providing protection to plants growing in soils rich in harmful metals such as aluminium (6,4).

Secondary metabolite: The secondary metabolite are molecules that has no involvement in the maintaining the life, but it plays a vital role in adaptation and defence when the plant interacts with its surroundings. They help plants defend themselves against diseases, herbivores, and environmental stressors. Secondary metabolite production is extremely modest and is determined by the plant's physiology and development.

2. REVIEW OF LITERATURE:

Drought impacts incorporate development, yields, film guideline, colour substance, osmotic alteration water relations and photosynthetic exercises (7,8). The powerless plants to dry spell push change in reliance of stretch degrees, diverse occurring push calculate, edit species, and their development stages (9).

When dry season condition is there, the root: shoot proportion of plants increments since roots are less touchy in compared to shoots development repressed by less water potential. (10).

Cytokinin, Abscisic acid, malate, ethylene and another unknown factor have been involved in the root shoot signals. Due to misfortunate of turgor pressure in cell there is 50 times increase in the level of ABA under the drought condition (11).

The capacity of plants to adjust to diverse natural condition is in coordinate or circuitous affiliation with the capacity to adjustment at level of photosynthesis (12).

Stomatal closures denies the takes off with carbon di oxide and the photosynthetic carbon consolidation is brought down in favour to the photorespiration. Restraint of photosynthesis is due to diminish within the stomatal opening by not influencing ATP union. The diminish within the stomatal opening causes the decrease in net carbon di oxide take-up by the takes off, hence repressing photosynthesis (13).

The action of Calvin cycle chemicals such as Rubisco; diminished emphatically in dry season focused plants. The diminish in CO₂ digestion is credited to brought down in chemical action included within the RuBP recovery (14). Chlorophyll is one of the most chloroplast component for the photosynthesis, and the relative chlorophyll substance incorporates a positive relationship with the photosynthetic rate. The decrease within the chlorophyll substance may be a sign of the oxidative push and it may lead to colour photo-oxidation and chlorophyll weakening. Drought stretch produces the alter in the proportion of chlorophyll 'a' and 'b' and carotenoid (15). Both Chlorophyll a and b are vulnerable to soil lack of hydration (15). Carotenoids have numerous extra parts and somewhat offer assistance the edit to outlive foes of water lacking condition.

A common adverse effect of water push on plants is the decrease in damp and dry biomass generation (15). The osmotic direction can keep up the turgor weight of cell for survival or to offer assistance plant development beneath the dry season.

The proteolytic possibilities of cell have expanded basically within the vacuolar sap beneath dry spell push. The tall action is watched for all the parts within the delicate Phaseolus and Vigna cultivars may be dependable, at slightest incompletely, for the fast diminish of takes off and chloroplasts protein beneath dry season (16).

Proline can act as a flag particle, which comes about in tweaking mitochondrial capacities, cell multiplication or cell passing is affected and triggers the certain quality expression, which can be basic for plant development recuperation from stretch (17).

Flavonoids plays as defensive work amid water inaccessibility. Flavonoids are involved to supply assurance to crops developing in Soil that are wealthy in poisonous metals such as aluminium (18)
Drought actuates oxidative stretch in plants by era of responsive oxygen species (ROS) (19)

3. MATERIALS AND METHODS:

Measurement of Shoot

The measurement of shoot length (devoid of root) was taken using scale to study the comparative account between the control, drought stressed plant.

Measurement of Root

The measurement of root (devoid of shoot) was taken using scale to study the comparative account between the control, drought stressed plant.

Measurement of Root Biomass:

The biomass of root is here inferred as total fresh weight of plant (devoid of shoot). The weight of control, drought stressed plant was taken separately.

Estimation of Chlorophyll content –

The below protocol for determination of total chlorophyll content was obtained (20).

Principle: The chlorophyll from different leaf samples is extracted using an organic solvent (acetone). Then the concentration of chlorophyll in different samples are estimated by measuring their absorbance values in a spectrophotometer.

Estimation of Carbohydrates:

The below protocol for estimation of carbohydrates was obtained (21,22)

Principle: Glucose dehydrates to hydroxymethyl furfural in a hot acidic media. This produces a green product with phenol and has an absorbance maximum at 490 nm.

Estimation of proline –

The below proline estimation protocol (23).

Principle: Proteins precipitate as complexes during selective extraction with aqueous sulpho-salicylic acid. Other interfering elements are most likely eliminated by absorption into the protein sulpho-salicylic acid complex. In acidic circumstances (pH 1.0), the extracted proline reacts with ninhydrin to generate the chromophore (red colour), which is then read at 520nm.

- i. Standard graph: A standard graph of Proline concentration ($\mu\text{g/mL}$) (X-axis) versus Absorbance (at 520nm) (Y-axis) was plotted, that was used to find out the amount of proline present in the leaf extracts used in the experiment.

Estimation of Phenolics by Folin - Ciocalteu method –

The below protocol for the estimation of phenolics was obtained from the paper on Total phenolic and oxidative content of *Marrubium peregrinum* L.(24)

Principle: The plants deliver phenols, which are fragrant benzene ring particles containing one or more hydroxyl bunches, for the most part to protect against stretch, and their concentration within the plant test can be calculated utilizing the FC reagent.

- i. A standard graph of Phenolics concentration ($\mu\text{g/mL}$) (X-axis) versus Absorbance (at 650nm) (Y-axis) was plotted, that was taken to determine the amount of phenolics present in the leaves extracts used in the experiment.

Estimation of Flavonoids

The protocol for the estimation of flavonoids Milan S. Stanković. (24). After fully mixing the solution once more, the absorbance level at 510 nm was measured comparison to a blank. The flavonoid content was calculated using the calibration curve and the linear equation.

4. RESULTS AND OBSERVATION:

Shoot length : Table 1- Measurement of Shoot length

Plant Sample		Shoot Length	Average
Control	Sample 1	22	22.4
	Sample 2	22.7	
	Sample 3	22.5	
Drought	Sample1	20	19.9
	Sample 2	19.7	
	Sample 3	20	

The length of the shoot of control, drought stressed was measured after removing the root. The measurement was as following:

Root length

The root length of the control, drought stressed was measured after removing the shoot. The measurement was as following:

Table 2- Measurement of Root length

Plant Sample		Root length	Average
Control	Sample1	15	14.47
	Sample2	15.4	
	Sample 3	13	
Drought	Sample 1	17	17
	Sample 2	17.8	
	Sample3	16.2	

Root Biomass

The weight of entire root of control, drought stressed plant was measured. The measurement was tabulated as following

Table 3- Measurement of Biomass of Root

		Root Biomass(gm)	Average
Control	Sample 1	0.251	0.251
	Sample2	0.250	
	Sample 3	0.252	
Drought	Sample 1	0.259	0.255
	Sample2	0.248	
	Sample 3	0.260	

Estimation of Chlorophyll content

The absorbance of the leaf extracts of control, drought stressed was noted at 645 and 663 nm. The value was tabulated as following:

Table 4.1- Absorbance value

Plant sample	Absorbance	
	645	663
Control Plant	0.432	0.693
Drought stressed	0.206	0.518

The amount of Chlorophyll a, Chlorophyll b and Total Chlorophyll Contents of the plants was calculated by using the formulae given below –

- mg of Chlorophyll a/gram of tissue = $12.7(A_{663}) - 2.69(A_{645}) \times V/1000 \times W$
- mg of Chlorophyll b/gram of tissue = $22.9(A_{645}) - 4.68(A_{663}) \times V/1000 \times W$
- mg of Total Chlorophyll/gram of tissue = $20.2(A_{645}) + 8.02(A_{663}) \times V/1000 \times W$

Where, the V is the final volume of the extract of chlorophyll, A = absorbance at certain wavelengths and W = weight of the tissues used to make the extract.

The Calculations of chlorophyll content in the plant's samples are given below:

Control

- i. mg of chlorophyll a/g of tissue=8.778 mg/g
- ii. mg of chlorophyll b/g of tissue =9.827 mg/g
- iii. total chlorophyll/g of tissue =8.837 mg/g

Plant under drought stress

- i. mg of Chlorophyll a/g of tissue=6.568 mg/g
- ii. mg of chlorophyll b/g of tissue=4.669 mg/g
- iii. Total chlorophyll/g of tissue =4.244 mg/g

A comparative result of the above calculations is as following

Table 4.2- Amount of Chlorophyll present

Plant sample	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
Control	8.778	9.827	8.837
Drought	6.568	4.669	4.244

In this manner, from over result it was clear that chlorophyll a, chlorophyll b and add up to chlorophyll were greatest in control and slightest in plant bearing drought stress.

Estimation of Carbohydrates

The amount of carbohydrate in control, drought stressed was estimated. The absorbance value(490nm) was tabulated as following:

Table 5-Absorbance value

Plant Sample	Absorbance(490 nm)
Control	0.277
Drought Stressed	0.632

The amount of carbohydrates in the plants was obtained by plotting the above absorbance values on a standard carbohydrates graph. The results are given below-

- Amount of carbohydrates in control plants =345 μ g/g of the leaf tissue
- Amount of carbohydrates in drought stressed plants = 854 μ g/g of the leaf tissue

Estimation of Prolines

The amount of Prolines in control, drought stressed were estimated. The absorbance value(520nm) was tabulated as following:

Table 6-Absorbance value

Plant sample	Absorbance(520nm)
Control	0.025
Drought	0.218

The amount of Proline in the plants was obtained by plotting the above absorbance values on a standard Proline graph. The results are given below-

- i. Concentration of Proline in control plants = 11 μ g/g of the leaf tissue.
- ii. Concentration of Proline in drought stress = 21 μ g/g of the leaf tissue.

Estimation of Phenolics

The amount of Phenol in control, drought stressed was estimated. The absorbance value(650nm) was tabulated as following:

Table 7-Absorbance value

Plant Sample	Absorbance(650nm)
Control	0.624
	0.626
Drought	0.785

The amount of Phenolic compounds in the plants was obtained by plotting the above absorbance values on a standard Phenolics graph. The results are given below

- i. Amount of Phenolics in control Plants,
 - Plant – 1 = 41 μ g/g of the leaf tissue.
 - Plant – 2 = 42 μ g/g of the leaf tissue.
- ii. Amount of Phenolics in plants under drought stress=49 μ g/g of the leaf tissue.

Estimation of Flavonoids

The amount of Flavonoids in control, drought stressed was estimated. The absorbance value(650nm) was tabulated as following:

Table 8-Absorbance value

Plant Sample	Absorbance (510 nm)
Control	0.453
Drought	0.993

The amount of flavonoids in the plants was obtained by plotting the absorbance values on a standard quercetin graph. The results are given below:

- i. Amount of Flavonoids in control plant-47 μ g/g of the leaf tissue.
- ii. Amount of Flavonoids in drought stressed plant=98 μ g/g of the leaf tissue.

5. DISCUSSION:

Abiotic natural components such dry spell is major stresses that influences the plant improvement and efficiency that eventually leads to the diminished rural abdicate. Subsequently, we were inquisitive about examining the impacts of dry season push on the plant by analyzing certain parameters like proline, flavonoids, chlorophyll substance, sum of carbohydrate, phenolics. Impact of push on the morphology of plant: Few characters were considered to ponder the morphological changes which included the root length, shoot length, leaf length and breadth.

Impact of dry spell stretch on the plant morphology: The focused plant had a shorter department length than the control plant due to a diminish in cell division and development. Roots are the essential organs that react to dry spell push. Plants with deeper root systems collect water from deeper soil layers, allowing the plant to avoid drought conditions. The root proliferated more in the stressed plant than in the control. This was also supported by in *Argania spinosa*(25). The length and breadth of the leaves decreased, indicating a drought-resistant mechanism. Reducing leaf area will reduce transpiration. The reduction in length and breadth of the plant could be attributed to lower cell expansion and division. Many additional species had leaf size reductions during drought conditions. Because of slower glucose metabolism, the plant accumulates more carbs under stressful conditions. (26). The drought-stressed plant had the largest glucose content, followed by the salt stressed plant. Control plants contain less glucose than stressed plants.

Proline can act as a chaperon particle secured from protein structures and make strides protein exercises (27,17). Agreeing to higher proline substance in touchy cultivar, Proline shows up to play no work in osmosis direction in brassica plants, and cultivars with tall seed yield in salt conditions were combined with extra osmoprotectants to move forward osmosis control or resistance to saltiness stretch in plants. The amount of phenolics is higher in drought stress followed by salt stress and the least in control. The amount of flavonoids is higher in drought stressed plants, followed by lower in control. Flavonoids in higher plants act as antioxidants in response to stressful conditions.

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