ISSN (Online): 2349-1183; ISSN (Print): 2349-9265
TROPICAL PLANT RESEARCH
The Journal of the Society for Tropical Plant Research

10(2): 76–81, 2023
DOI: 10.22271/tpr.2023.v10.i2.009

Research article

Effect of water stress (drought) on growth, photosynthetic pigments, nitrogen and protein content of *Ocimum sanctum* L. (Tulsi)

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Abstract: Water stress, induced by drought, is a significant environmental factor that adversely affects the growth and physiological processes of plants, including Tulsi *Ocimum sanctum* (Tulsi). In this study, we investigated the effect of water stress on the growth, photosynthetic pigments, nitrogen content, and protein content of Tulsi plants. To assess the impact of water stress on Tulsi, three sets of plants were subjected to different irrigation regimes: well-watered (control group), moderate and severe (experimental group). The water-stressed plants were subjected to reduced water supply to mimic drought conditions, while the control group received optimal watering throughout the experiment. The results revealed that water stress significantly impacted the growth of Tulsi plants. The water-stressed plants exhibited reduced shoot and root growth, as well as decreased leaf area, compared to the well-watered plants. Additionally, the photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotenoids, were significantly lower in the water-stressed plants, indicating an impairment of photosynthetic activity. In conclusion, water stress caused by drought has a detrimental effect on Tulsi plants, leading to reduced growth, impaired photosynthetic pigments, decreased nitrogen content, and lower protein levels. Understanding the physiological responses of Tulsi to water stress can aid in devising appropriate strategies for conserving and managing water resources in agricultural practices while sustaining the growth and productivity of this valuable medicinal plant.
Keywords: Tulsi - Water stress - Drought - Morphological - Physiological.

[Cite as: Dev D, Kannaojiya O & Sunita K (2023) Effect of water stress (drought) on growth, photosynthetic pigments, nitrogen and protein content of *Ocimum sanctum* L. (Tulsi). *Tropical Plant Research* 10(2): 76–81]

INTRODUCTION

Ocimum sanctum L. (Tulsi) native to Asia, is a ubiquitous, versatile plant venerated in Hinduism and has a place in front of every Hindu home. In Ayurveda texts it has been called as "Mother medicine of nature", "The incomparable one", "The queen of herbs" etc. This herb, a member of the Lamiaceae family, is well known worldwide for its spiritual, religious, and medicinal properties.

The plant has an unpleasant, bitter, steaming, light, and dry flavor, indicating the presence of medicinal properties. Because of its beneficially diversified characteristics, it is widely known and has been utilized for generations in Ayurvedic and Greek treatments. The presence of phytochemicals in leaves such as flavonoids, phenol, terpenoids, alkaloids, eugenol, and others confers the potential to combat infection by target cells, according to researches.

Ocimum sanctum minimizes stress, boosts stamina, decreases inflammation, lowers cholesterol, eliminates toxins, protects against radiation, avoids gastric ulcers, lowers fevers, promotes digestion, and has a high concentration of antioxidants and other minerals. Tulsi is notably beneficial to the heart, blood vessels, liver, and lungs, as well as regulating blood pressure and blood sugar. In addition to these benefits, it also treats diarrhea, dysentery, back pain, ringworm, insect, snake and scorpion bites. Research points to Tulsi, like many modern medications, may be a cyclooxygenase-2 inhibitor because to its high quantity of eugenol (1-hydroxy-2-

Received: 27 April 2023 Published: 31 August 2023
https://doi.org/10.22271/tpr.2023.v10.i2.009

methoxy-4-allylbenzene).

The herb called holy basil is seen as a manifestation of the goddess Lakshmi (Tulsi), the primary spouse of the deity Vishnu, in Hinduism. Vaishnavites (Vishnu worshippers) hold the plant in high regard, and Lord Krishna, an avatar of Vishnu, is said to wear a garland of holy basil leaves and blossoms around his neck. The plant is grown in many Hindu homes, usually in the courtyard of a four-sided structure, and its presence is supposed to increase piety, promote meditation, purify, and protect. Water steeped with the leaves is commonly given to the dying to help raise their souls, and funeral pyres are typically adorned with holy basil branches in the hope that the deceased would achieve redemption.

The plant is hardly enough to grow in any soil except those that are extremely saline, alkaline, or wet. Sandy loam soil with a high organic matter concentration, on the other hand, is preferable. The crop is quite adaptable and can grow in both tropical and subtropical climates. Long days with high temperatures have been shown to benefit plant growth and oil production.

MATERIAL AND METHOD

The experiment was conducted in DDU Gorakhpur University, Gorakhpur campus during the month of February and March. Each set (3 seedlings) was subjected to different amount of water (Fig. 1). Different levels of soil water capacity (SWC 70–50–30%) resulted in significant changes in morphological and physiological parameters of *Ocimum sanctum* L. The three seedlings were designated as CONTROL (70% SWC), moderate stress (50% SWC) and severe stress (30% SWC). The entire plant specimens were observed in accordance to their day of plantation. Different morphological parameters like plant height, stem length, root length, number of leaves, number of branches, fresh weight and dry weight were measured and studied. In order to estimate the photosynthetic capacity of *Ocimum sanctum*, under moderate and severe stress, Chlorophyll a, chlorophyll b and carotenoid (of the leaves) of the after every 30, 45 and 60 DAT (Days after transplantation) were also measured.

Figure 1. Morphological, Physiological and Biochemical effect of water stress on plants.

The amount of Chl. a and Chl. b and total chlorophyll in Tulsi was measured in the primary leaves by the method of Arnon (1949) and calculated in terms of mg per ml fresh weight of leaves by the following formula:

$$\text{Chlorophyll a (mg ml}^{-1}\text{)} = [12.7 \text{ (A663)} - 2.69 \text{ (A645)}] \times V/1000 \times W$$

$$\text{Chlorophyll b (mg ml}^{-1}\text{)} = [22.9 \text{ (A645)} - 4.68 \text{ (A663)}] \times V/1000 \times W$$

$$\text{Total chlorophyll (mg ml}^{-1}\text{)} = [20.2 \text{ (A645)} - 8.02 \text{ (A663)}] \times V/1000 \times W$$

$$\text{Carotenoid} = [\text{O.D. } 440 \times \text{Volume}/196 \times \text{Weight}]$$

Where, A= Optical density observed for chlorophyll extract at the particular indicated wavelength. V= Final volume of the chlorophyll extract in 80% acetone. W= Fresh weight of leaves in mg.

Nitrogen content was estimated in dried leaves obtained from water stressed and control plants by the method of Doneen (1932). The absorbance of pale yellow color so developed was measured at 440 nanometer in a UV/VIS systronic spectrometer. The amount of soluble nitrogen was calculated in mg N2 g^{-1} dry weight of sample using a sample curve prepared from ammon ium sulfate. For measurement of protein content in dry leaves. The amount of insoluble nitrogen fraction as obtained by micro - kjeldahl method was multiplied by a factor 6.25

RESULT AND DISCUSSION

The effect of water stress on plants is significant, adversely impacting various aspects of plant physiology and growth. Water stress limits access to resources required for photosynthesis, impairs normal plant functionality, and induces morphological, physiological, and biochemical changes to compensate for water limitations (Forouzandeh *et al.* 2012). This can lead to reduced plant growth, productivity, and water use efficiency, as well as an increase in reactive oxygen species, affecting photosynthetic capacity and leading to stunted growth, leaf rolling, and other visible symptoms of stress (Boutraa *et al.* 2010, Osakabe *et al.* 2014, Sun *et al.* 2020, Seleiman *et al.* 2021). Additionally, plants affected by water stress have lower and weaker defences, making them more susceptible to pest and disease attacks. Understanding the molecular and physiological mechanisms involved in plant responses to water stress is essential to improve plant productivity during such conditions (Osakabe *et al.* 2014). Various approaches, such as potassium application, have been suggested to alleviate the adverse effects of water stress and maintain plant productivity (Seleiman *et al.* 2021).

Morphological parameter

i. Plant height and Root length: Plant height is one of the prominent visible change that can occur under water stress. It is measured throughout the length from max. plant tip to root via meter scale. There was a significant decrease in the root length of the plants in all the three sets. In each set the root length was longest and dense in the control plant followed by the moderate stressed plant while shortest in the severe stressed plant (Table 1). Shil & Dewanjee (2022) also studied that mild drought stress at 50% soil moisture level causes an increase in plant height, leaf area, number of branches and leaves, fresh and dry biomass of *Ocimum basilicum* L. while further increase in stress level causes a reduction in these growth parameters. Alishah *et al.* (2006) studied the effects of different levels of water stress applying 100, 90, 80, 70, 60% of field capacity on potted purple basil in a growth chamber. They reported that as the water stress increased, the plant height and herb yield decreased. The highest average plant height, fresh herb yield and dry herb yield were given as 56.46 cm, 38.69 g plant^{-1}, and 4.573 g plant^{-1}, respectively.

Table 1. Showing water stress effect on plant height, root length and number of leaves after 30, 45 and 65 DAS respectively.

Number of days (DAS)	Water Stress Level	Plant Height (cm)	Root Length (cm)	Number of Leaves
30	Control	30.0	9.0	88
	Moderate	27.0	8.0	72
	Severe	31.0	6.9	44
45	Control	41.0	11.0	85
	Moderate	35.5	8.0	75
	Severe	33.0	7.5	40
65	Control	42.2	10.0	81
	Moderate	40.0	9.2	78
	Severe	39.0	8.0	70

ii. Fresh weight and Dry weight: Numerous investigations have demonstrated that the fresh and dry weights of plant tissues, such as leaves, roots, and aboveground biomass, can be considerably reduced by drought stress. For instance, when seedlings were stressed by water, their dry weight significantly dropped. Due to water scarcity and closed stomata, there is less carbon stored, less water is lost, and nutrient uptake is reduced, all of which contribute to this weight loss. Significant effects of water stress on plant growth include changes in biomass production, shoot and root growth, and leaf area (Table 2). Water stress can therefore negatively impact a plant's overall growth and development, resulting in a decrease in the fresh and dry weights of various plant parts. On weighing the fresh weights of the plant samples we again observed a conspicuous fluctuations in the results. With a decrease in the water availability, the fresh weight also decreased. Hence showing a directly proportional relationship. The results were same as above, that is showing direct proportional relation. Khalid (2006) reported that *Ocimum basilicum* L. and *Ocimum americanum* L. potted seedlings were treated with different levels of water stress significantly influenced the Fresh and dry weights of the herbs.

Table 2. Showing water stress effect on fresh and dry weight of plant after 30, 45 and 65 DAS respectively.

Number of days	Water Stress Level	Fresh Weight (g)	Dry weight (g)
30	Control	3.47	0.90
	Moderate	2.39	0.71
	Severe	1.63	0.57
45	Control	6.66	1.30
	Moderate	6.50	1.11
	Severe	3.51	0.84

65	Control	8.01	1.47
	Moderate	7.04	1.33
	Severe	5.49	1.16

iii. Estimation of chlorophyll content: Water stress has a significant impact on the concentration of chlorophyll a, b, and carotenoids in plants (Mibei *et al.* 2017, Syamsia *et al.* 2018, Yousaf *et al*. 2023). As the table 3 shown there is significantly decrease in the chlorophyll a, chlorophyll b and carotenoid as the water stress level has been increased. The harmful effects of water stress on photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotenoid contents, have been observed in various studies (Mibei *et al.* 2017, Syamsia *et al.* 2018, Yousaf *et al*. 2023). High chlorophyll and carotenoid contents are associated with plant tolerance to stress Syamsia *et.al* (2018), Mibei *et al.* (2017). The concentration of chlorophyll decreases under drought stress, and the impact of water stress on chlorophyll content is more deleterious in the pre-anthesis stage compared to during Yousaf *et al.* (2023) and Mibei *et al.* (2017). However, in low water stress, chlorophyll-a, chlorophyll-b, and total chlorophyll content increased by increasing stress pressure Syamsia *et al.* (2018). The concentration of some carotenoids and photosynthetic pigments decreases under water stress, while others increase progressively in control (Mibei *et al.* 2017). Nutrient management has been suggested as an approach to alleviate the adverse effects of water stress and maintain plant productivity (Shanthi *et al.* 2023).

Table 3. Showing water stress effect on chlorophyll a, chlorophyll b and on carotenoid of plant after 30, 45 and 65 DAS respectively.

Number of days	**Stress Level**	**Chl. a ($mg\ ml^{-1}$)**	**Chl. b ($mg\ ml^{-1}$)**	**Total Chl. ($mg\ ml^{-1}$)**	**Carote-Noid ($mg\ ml^{-1}$)**
30	Control	0.000233	0.000776	0.00058	0.00035
	Moderate	0.000172	0.000725	0.00056	0.00034
	Severe	0.000086	0.000714	0.00057	0.00028
45	Control	0.000424	0.000663	0.00066	0.00044
	Moderate	0.000386	0.000596	0.00056	0.00038
	Severe	0.000321	0.000529	0.00048	0.00032
65	Control	0.000473	0.000712	0.00069	0.00051
	Moderate	0.000464	0.000658	0.00061	0.00047
	Severe	0.000452	0.000593	0.00052	0.00045

iv. Estimation of Nitrogen: Water stress can have a negative impact on the nitrogen content of plants. Drought stress can reduce the nitrogen concentration in plants, and the concentration of nitrogen gradually decreases in all treatment periods when plants are exposed to drought (Shah *et al*. 2017). High nitrogen can also repress root elongation, limiting water foraging and preventing plants from developing stress avoidance mechanisms to cope with long-term drought stress (Sun *et al*. 2020). In addition, drought stress reduces leaf size, stem extension, and root proliferation, disturbs plant water relations, and reduces water-use efficiency, which can exacerbate water stress due to increasing transpiration area (Maki *et al*. 2023). Therefore, proper nutrition management, including nitrogen supply, is essential to alleviate the adverse effects of water stress and maintain plant productivity (Shah *et al*. 2017).

Table 4. Showing water stress effect on nitrogen content of plant after 30, 45 and 65 DAS respectively.

Number of days	**Water Stress Level**	**Nitrogen ($mg\ ml^{-1}$)**	**Protein ($mg\ ml^{-1}$)**
30	Control	0.337	2.106
	Moderate	0.332	2.075
	Severe	0.270	1.688
45	Control	0.419	2.611
	Moderate	0.356	2.225
	Severe	0.328	2.050
65	Control	0.462	2.888
	Moderate	0.422	2.638
	Severe	0.399	2.493

CONCLUSION

Plants are subjected to stresses wherever they grow, which tend to limit their development and survival. Moisture constraint can have an impact on nearly every plant process, from membrane conformation, chloroplast structure, and enzyme activity at the cellular level to development and decreased overall plant output, as well as increased vulnerability to extra stresses. Reduced photosynthetic activity and increased leaf withering are signs of water stress can have a negative impact on crop growth.

Another result of water stress is decreased nutrient uptake, decreased cell development and enlargement, leaf expansion, assimilation, translocation, and transpiration are all processes. Crop research aiming towards crop improvement and the development of high-yielding genotypes that can withstand unexpected climatic changes, particularly in places dominated by water deficits, has become an important subject in crop productivity research. As Kozlowski (1968) previously stated, there is a need to enhance crop production in the face of rising food shortages, and water conservation is a key aspect in overcoming food inadequacies. According to the results of the study, a variety of morphological, physiological, and biochemical responses have been linked with significant changes in drought stresses in *Ocimum sanctum* plants. However, there are significant gaps in our understanding of the causes and consequences of water stress in plants, and we need to step up our efforts to improve our understanding of the issue.

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