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Morphological Responses of *Trigonella foenum-graecum* L. to Drought Stress and Salicylic Acid Treatment

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ABSTRACT:

Drought stress is a critical abiotic factor with profound implications for crop growth and productivity. This study aimed to investigate the morphological responses of Trigonella foenum-graecum (fenugreek) under varying levels of drought stress and foliar applications of salicylic acid (SA) at different stages of its life cycle. Morphological parameters, including shoot length, root length, fresh and dry weights of the whole plant, fruit length, number of seeds per fruit, and fresh and dry weights of fruits, were examined. Our findings revealed significant morphological changes in fenugreek in response to drought stress, leading to reduced shoot length, fruit length, number of seeds per fruit, and the fresh and dry weights of both the whole plant and fruits as water deficit severity increased.

Keywords: *Trigonella, drought stress, Salicylic acid, morphological parameters.*

INTRODUCTION:

Drought is a widespread issue that adversely affects agricultural land worldwide, primarily due to water deficit, extreme temperatures, and low atmospheric humidity (Szilgyi, 2003; Hirt & Shinkozaki, 2003). Drought is the major abiotic stress which affects every aspect of plant growth and can be responsible for reduction in crop production (Golbashy *et a*l., 2010). Fenugreek (*Trigonella foenum-graecum*), belonging to the Fabaceae family, is an annual herb recognized for its aromatic leaves and seeds. It is commonly used as a spice as it has strong flavor and aroma and holds significance in traditional medicines (Rajagoplan, M.S., 2009). Salicylic acid (SA) is a phenolic compound known for its role as a plant growth regulator and its potential to enhance plant responses to abiotic stresses, including drought (Farooq et al. 2009). Salicylic acid can also play a significant role in plant water relations (Barkosky and Einhelling, 1993). This study aims to explore the impact of foliar SA application on fenugreek's morphological criteria, growth, and yield under drought conditions.

Materials and Methods:

The experiment was conducted in the experimental field of the Post Graduate Teaching Department of Botany, RTMNU Nagpur University, Nagpur, in November 2022. Fenugreek seeds were procured from GPS Hybrid Seeds Pvt. Ltd., Nagpur. Seeds were sown in plastic pots containing a mixture of soil, cow-dung fertilizer, and sand in a 3:1:0.5 ratio. Germinated seeds were regularly irrigated for the first 15 days. Afterward, drought stress was induced by withholding water, with treatments including well-watered control, 2 days of drought stress, and 4 days of drought stress. Salicylic acid (SA) was applied as a foliar spray at concentrations of 0.5 mM, 1 mM, and 1.5 mM at two different stages: before flowering and before fruit formation. Salicylic acid concentrations were applied as foliar spray till the solution dripped on the soil of the pot. Pots were arranged in a complete randomized design with six replications for each treatment. All plants were harvested at the fruit-bearing stage, and various morphological parameters, as well as yield-related traits, were recorded.

The treatments of SA used for the present study were as follows:
Treatment solution SA1 – 0.5 mM SA: 70 mg of SA was dissolved in few ml of distilled water by adding 1 mL of ethanol and 200 µL of Tween 20 solution. Then the final volume made up to 1L with distilled water.
Treatment solution SA2 – 1 mM SA: 140 mg of SA was dissolved in few ml of distilled water by adding 1 mL of ethanol and 200 µL of Tween 20 solution. Then the final volume made up to 1L with distilled water.
Treatment solution SA3- 1.5 mM: 207 mg of SA was dissolved in few ml of distilled water by adding 1 mL of ethanol and 200 µL of Tween 20 solution. Then the final volume made up to 1L with distilled water.
(SA was added to 1ml ethanol to increase its solubility in distilled water and Tween 20 solution was added with spraying treatments as a surfactant at the time of application.)
The treatments were
1.Control (well -watered plants - irrigated daily)
2. 2D (2 days drought- watered plants after every 2 days)
3.4D (4 days drought- watered plants after every 4 days)
4. 2SA1FL (2 days drought + SA 0.5mM foliar treatment before flowering only)
5. 4SA1FL (4days drought + SA 0.5mM foliar treatment before flowering only)
6.2SA1FR (2 days drought+ SA 0.5mM foliar-applied- twice -first before flowering and second before fruiting)
7.4SA1FR (4days drought+ SA 0.5 mM foliar applied twice- first before flowering and second before fruiting)
8.2SA2FL (2 days drought+ SA 1mM foliar-applied only once before flowering)
9. 4SA2FL (2 days drought+ SA 1mM foliar-applied twice -first before flowering and second before fruiting)
10. 2SA2FR (2 days drought+ SA 1mM foliar-applied twice -first before flowering and second before fruiting)
11. 4SA2FR (days dr4ought+ SA 1mM foliar-applied twice -first before flowering and second before fruiting)
12. 2SA3FL (2 days drought+ SA 1.5mM foliar-applied only once before flowering)
13. 4SA3FL (4 days drought+ SA 1.5mM foliar treatment -applied only once before flowering)
14. 2SA3FR (2 days drought+ SA 1.5mM foliar-applied twice -first before flowering and second before fruiting)
15. 4SA3FR (4 days drought+ SA 1.5mM foliar-applied twice -first before flowering and second before fruiting)

At the completion of its life cycle all the treated plants were harvested when fruit bears well developed seeds. For the present study whole plant fresh weight and dry weight, root length and shoot length as morphological parameters were studied. Fresh weight, dry weight For yield parameters fruit length and number of seeds per fruit recorded. Similarly fresh weight and dry weight of the fruits of all treated plants including control recorded. All readings were taken of 5 determinations.

Results and Discussion:

Morphological Parameters:

Root length and shoot length were measured for all treatments, including the control. Control plants displayed the longest shoot length, while the 4SA3FR treatment showed the shortest shoot length. The highest root length was observed in the 4SA1FR treatment, while the lowest root length was recorded in the 2D treatment. Foliar application of SA has significantly protected the root length of Trigonella plants from adverse effect of drought stress. The effect of SA on root length at the treatment of 4SA1FR was statistically at par (figure) . the adaptation mechanisms of plants to drought stress are closely related to exceptional modifications in root morphology particularly root length (Okon & Vanderleyden 1997). Foliar supplication of salicylic acid has maintained a better rooting system and thus helped in improving the drought tolerance of maize plants. Such results are confirmatory with earlier findings of Jadhav ad Bhamburedekar (2011) who observed the significant increase in root growth when groundnut plants were applied with exogenous application of SA. The reduction in growth attributes of plants growing under drought stress condition is a common phenomenon (Bhatt & Srinivasa Rao 2005; Chegah et al. 2013).

Sr. No.	Treatment	Root Length	Shoot Length
1	Control	**14.2±2.86**	**42±1.87**
2	2D	**9.2±4.08**	42±7.76
3	4D	13.2±3.34	30.8±5.54
4	2SAI FL	13.4±1.94	34.8±4.65
5	2SAI FR	10.2±2.48	39.2±5.44
6	2SA2FL	11±1.58	29.8±1.30
7	2SA2FR	10±2.12	35.6±3.13
8	2SA3 FL	10.6±2.70	34±4
9	2SA3FR	9.8±3.27	35±5.19
10	4SA1FL	11.6±1.34	31.6±4.39
11	4SA1FR	15.2±1.30	31.8±3.96
12	4SA2FL	12.2±2.86	33.6±3.04
13	4SA2FR	11±2	35.4±3.57
14	4SA3FL	10.8±1.48	33±4.06
15	4SA3FR	12±2.73	**28.6±2.30**

Table 1. Root and shoot length of the Trigonella.

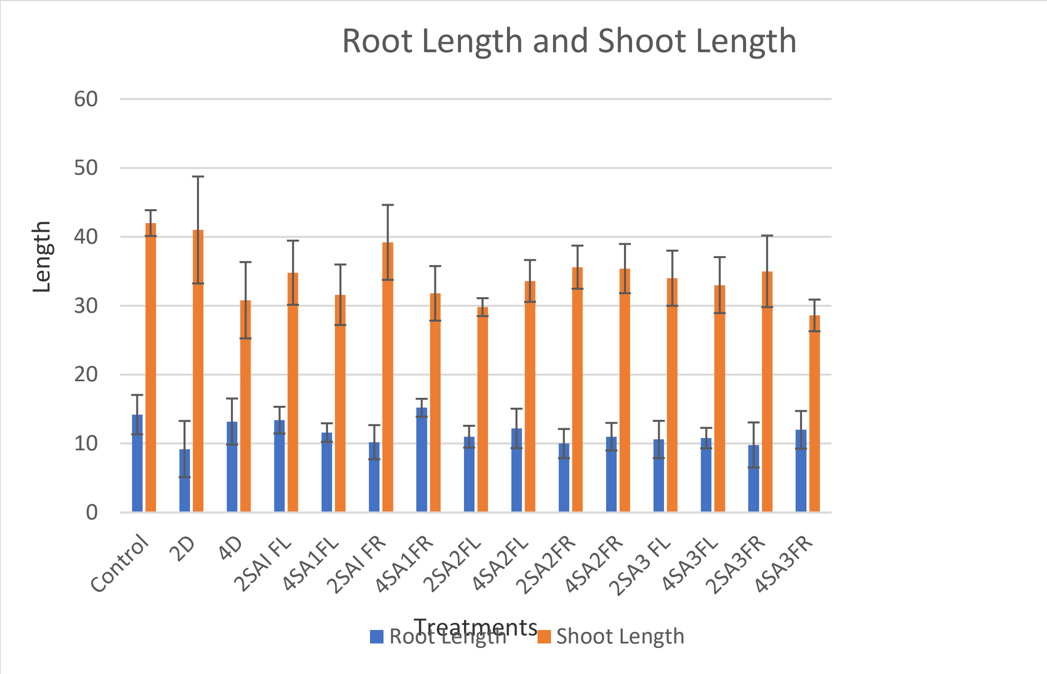

Fig. 1. Root and shoot length of the *Trigonella*

Fresh and dry weights of the whole plants were determined for all treatments. Control plants exhibited the highest fresh weight, with the 4D-treated plants displaying the lowest fresh weight. In terms of dry weight, control plants also had the highest values, while the 4SA1FR-treated plants had the lowest dry weight. This study showed shoot fresh weight and dry weight were significantly reduced by drought stress as compared to control (Figure and). Foliar application of salicylic acid has effectively protected the plants of Trigonella from the adverse effect of drought conditions.

Sr. No.	Treatment	Fresh Weight (Whole Plant)	Dry Weight (Whole Plant)
1	Control	**6.29±2.22**	**2.27±0.61**
2	2D	6.12±1.87	1.89±0.53
3	4D	**1.52±0.36**	0.71±0.19
4	2SAI FL	1.91±0.54	0.57±0.12
5	2SAI FR	4.17±2.34	2.12±1.35
6	2SA2FL	2.14±0.54	0.72±0.14
7	2SA2FR	3.91±0.89	1.08±0.28
8	2SA3 FL	4.19±0.88	1.25±0.35
9	2SA3FR	4.60±1.88	1.18±0.39
10	4SA1FL	2.42±1.04	0.72±0.26
11	4SA1FR	1.63±0.45	**0.53±0.14**
12	4SA2FL	2.83±1.20	1.00±0.39

13	4SA2FR	2.78±0.99	1.11±0.29
14	4SA3FL	2.38±0.82	0.93±0.14
15	4SA3FR	2.88±0.50	0.73±0.15

Table 2. Fresh weight and dry weight of the whole plant.

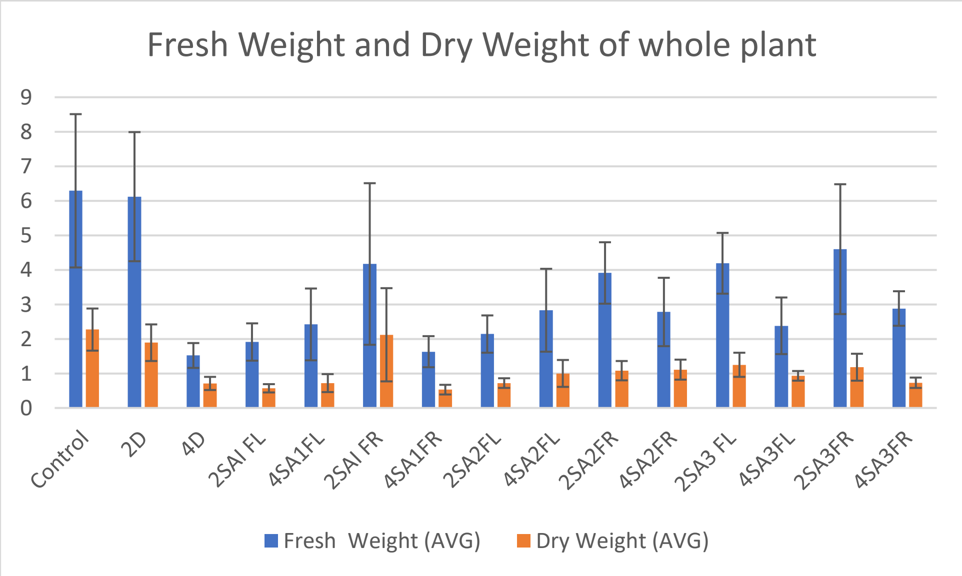

Fig. 2. Fresh weight and dry weight of the *Trigonella*

The foliar application of SA on plants helped them to increase the tolerance to drought stress. The ability of SA to increase the dry mass of plant, to ameliorate the adverse effect of water stress, may have significant implications in improving the plant growth and overcoming the yield barrier resulted from limited water availability (Sayyari et al. 2013).

Experiment done by Yildirim and Dursun (2009) found highest yield of tomato at 0.5 mM Salicylic acid foliar treatment in greenhouse conditions. It was reported that foliar application of low concentration of SA increased the photosynthetic activity in basil and marjoram which resulted inn enhanced their plant height, number of internodes, number of branches and leaves as well as leaf area, fresh and dry weights. Similarly, Gutierrez-Coronado *et al.* (1998) reported that low concentrations of salicylic acid has enhanced the growth of soybean, maize (Shehata *et al*. 2001, El- Mergawi & Abdel-Wahed 2007), and wheat plants (shakirova et al. 2003, Iqbal *et al*. 2006), whereas high concentrations of salicylic acid caused an inhibitory effect on growth of tomato, lupine, wheat and maize plants (Kord & Hathout 1992, Haroun et al. 1998, Singh & Usha 2003, Abel-Wahed *et al*. (2006).

Yield Parameters:

Yield parameters, including fruit length, number of seeds per fruit, and fresh and dry weights of fruits, were assessed for all treatments. Control plants had the longest fruit length, while the 4SA3FR-treated plants had the shortest fruit length. The control group also exhibited the highest number of seeds per fruit, whereas the 2SA3FR-treated plants had the lowest seeds per fruit. Regarding fresh weight of fruits, control plants had the highest values, while the 4D-treated plants had the lowest fresh weight. For dry weight of fruits, control plants once again displayed the highest values, whereas the 2SA1FR-treated plants had the lowest dry weight.

Water stress caused decrease in biological yield. Biological yield is an important factor for the yield improvement (Kumar et al. 2001). Drought has pronounced effect on fruit development and yield. The yield loss caused by drought stress was mainly due to an increased rate of floral and pod abortion (Liu *et al* 2003).

Sr. No.	Treatment	Fresh weight of fruit	Dry weight of Fruit
1	Control	**1.40±0.26**	**0.39±0.07**
2	2D	0.66±0.17	0.19±0.07
3	4D	**0.55±0.07**	0.29±0.05
4	2SAI FL	0.84±0.23	0.23±0.06
5	2SAI FR	0.76±0.34	**0.16±0.08**
6	2SA2FL	1.17±0.19	0.34±0.03
7	2SA2FR	0.98±0.17	0.27±0.06
8	2SA3 FL	0.78±0.05	0.35±0.06
9	2SA3FR	0.86±0.09	0.22±0.05
10	4SA1FL	0.81±0.19	0.30±0.08
11	4SA1FR	0.92±0.21	0.27±0.04
12	4SA2FL	0.70±0.07	0.31±0.08
13	4SA2FR	0.87±0.13	0.29±0.06
14	4SA3FL	0.57±0.16	0.40±0.18
15	4SA3FR	0.72±0.10	0.19±0.10

Table 3. Fresh weight and dry weight of fruit

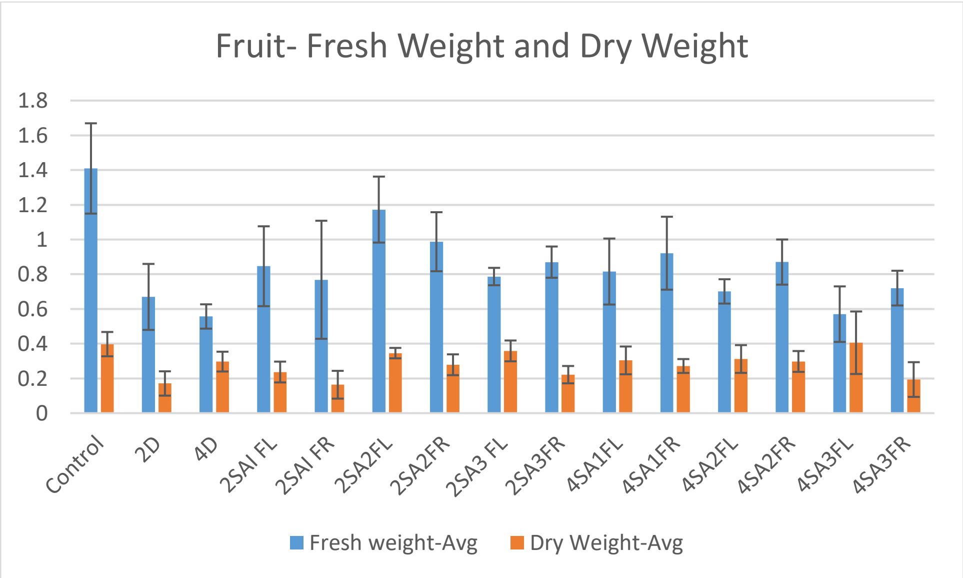

Fig. 3 Fresh and dry weight of fruit

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Sr. No.	Treatment	Fruit Length	Number of seeds per fruit
1	Control	12.60±1.08	13.8±2.68
2	2D	12±1.0	14±4.63
3	4D	11.6±1.81	11.2±1.30
4	2SAI FL	11.1±1.51	11.4±3.20
5	2SAI FR	11.6±1.34	15±2.34
6	2SA2FL	12.7±1.09	14.2±2.86
7	2SA2FR	11.2±1.09	12.2±1.78
8	2SA3 FL	12.7±0.97	12±3.53
9	2SA3FR	10.9±1.24	10.6±2.40
10	4SA1FL	10±1	13.2±2.16
11	4SA1FR	11.6±0.89	12.6±2.50
12	4SA2FL	11.1±1.88	10.8±1.92
13	4SA2FR	11.4±0.54	12±2
14	4SA3FL	12.2±0.83	12.6±4.72
15	4SA3FR	9.5±1.06	11.4±2.96

Table 4. Fruit length and number of seeds per fruit

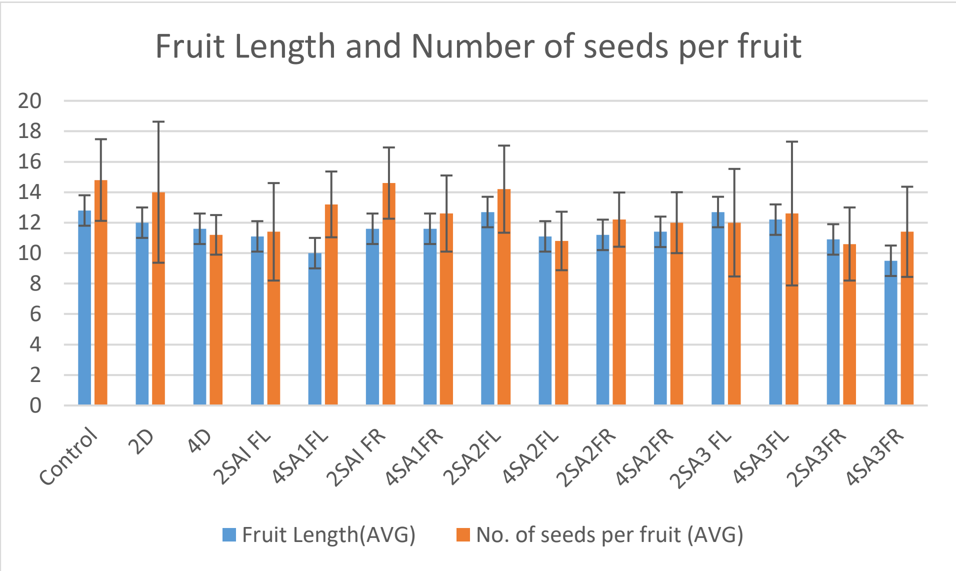

Fig. 4 Fruit length and number of seeds per fruit

CONCLUSION:

This study demonstrates that drought stress significantly affects the morphological characteristics and yield parameters of fenugreek plants. As drought severity increases, there is a notable reduction in shoot length, fruit length, number of seeds per fruit, and the fresh and dry weights of both the whole plant and fruits. Salicylic acid

(SA) application, particularly at higher concentrations, mitigates some of the adverse effects of drought stress on root length and yield-related traits.

Significance of the Work:

Drought stress poses a substantial challenge to agriculture and food security. Understanding how fenugreek responds to drought stress and the potential benefits of SA application can inform strategies to enhance crop resilience in water-deficient conditions. Fenugreek, with its culinary and medicinal significance, serves as an important model for this study.

Future Prospects of this Work:

Future research in this area can delve into the molecular and physiological mechanisms underlying fenugreek's responses to drought stress and SA treatment. Investigating gene expression patterns, biochemical pathways, and stress-related signaling mechanisms can provide a deeper understanding. Additionally, long-term studies assessing the stability and adaptability of fenugreek and other crops to varying environmental conditions can contribute to practical strategies for improving crop resilience and food security in drought-prone regions.

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