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Research Article

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 on 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. Application of SA had improved the fruit dry weight as compared to the untreated plants.

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 & Shinozaki, 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 al*., 2010). Fenugreek (*Trigonella foenum-graecum*), belonging to the Fabaceae family, is an annual herb recognized for its aromatic leaves and seeds. It is being commercially grown in India, Pakistan, Afghanistan, Iran, Nepal, Egypt, France, Spain, Turkey, Morocco, North Africa, the Middle East, and Argentina (Flammang, *et al*., 2004; Altuntas, *et al*., 2005). It is commonly used as a spice as it has strong flavor and aroma and holds significance in traditional medicines (Rajagopalan, 2001). Fenugreek leaves and seeds are used by people of different countries of the world for various reasons, including medicinal uses. 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 & Einhellig, 1993). It plays an important role in ion uptake and transport, plant growth, and is also involved in endogenous signaling to trigger plant defense against pathogens. This study aims to explore the impact of foliar SA application on fenugreek's morphological growth and yield under drought conditions.

Material 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.

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Afterwards, drought stress was induced by withholding water, with treatments including well-watered control, 2 days of drought stress, and 4 days of drought stress. Solution of 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 (BFL) and before and after flowering (BAFL).

Plants were subjected to different irrigation schedules and salicylic acid treatments as follows:
Control: Plants were irrigated daily.
2D: Plants were irrigated every 2 days.
4D: Plants were irrigated every 4 days.

Salicylic Acid Treatments
For plants treated with salicylic acid, the following regimens were applied:
2SA1BFL, 2SA2BFL, 2SA3BFL: Plants were irrigated every 2 days and treated with respective concentrations of salicylic acid only once before flowering.
2SA1BAFL, 2SA2BAFL, 2SA3BAFL: Plants were irrigated every 2 days and treated with respective concentrations of salicylic acid once before and once after flowering.
4SA1BF, 4SA2BFL, 4SA3BFL: Plants were irrigated every 4 days and treated with respective concentrations of salicylic acid only once before flowering.
4SA1BAFL, 4SA2BAFL, 4SA3BAFL: Plants were irrigated every 4 days and treated with respective concentrations of salicylic acid once before and once after flowering.

These treatments were designed to evaluate the combined effects of irrigation frequency and salicylic acid application on plant growth and development.

Foliar spray of salicylic acid was applied till the solution dripped on the soil of the pot. Pots were arranged in six replications for each treatment. All plants were harvested at the fruit-bearing stage, and morphological parameters like fresh weight and dry weight of the whole plant, root length and shoot length of the plant, as well as yield-related traits like fresh weight and dry weight of the fruit, fruit length and number of seeds per fruit were recorded.

To study the root length, soil filled planted pots were dipped in a water basin and pots were slanted under the water. After the soil in the pots were loosen, slowly the soil was removed and the plants were slowly uprooted. Randomly 5 plants were selected for the morphological and yield parameters study. Primary root lengths of all plants were measured with the help of ruler. Similarly shoot lengths and fruit lengths were measured with the ruler. Fresh weight and dry weight were recorded by using electronic weighing balance.

Statistical Analysis: Mean and standard deviation was calculated by using Microsoft Excel. For defining the statistical significance of the results, analysis of variance (ANOVA) and Tukey's post-hoc test was applied ($p<0.05$) using R studio.

Results and Discussion

Morphological Parameters

Root Length and Shoot Length

The root length of all the plants from each group was measured. The highest root length was observed in the 4SA1BAFL treatment, while the lowest root length was recorded in almost all the 2D + SA treatment regimens (Fig.1) although the difference was not statistically significant. However, most of the SA treatments have resulted in the non-significant reduction in the root length. It was previously confirmed that SA has a profound effect on cell growth and division (Li, *et al*., 2022). Additionally, it was reported by earlier workers that the foliar application of salicylic acid maintained a better rooting system and thus helped in improving the drought tolerance of maize plants. Such results are consistent with earlier findings of Jadhav & Bhamburdekar, (2011), who observed a significant increase in root growth when groundnut plants were treated with an exogenous application of SA. The reduction in growth attributes of plants growing under drought stress conditions is a common phenomenon (Bhatt & Srinivasa Rao, 2005; Chegah, *et al*., 2013). Moreover, the work of

Waseem, *et al*., also showed that the root length character is insensitive to SA under drought stress (Ashraf, *et al*., 2006). In our work as well, we did not observe any significant alteration in the root length.

On the other hand, the shoot length among the drought stress, the severe drought stress condition has resulted in the significant decrease in the shoot growth (Fig.2); however, the moderate 2D stress did not display any significant effect over this trait. Upon application of SA in both the moderate and severe stressed plants, we observed mixed responses in shoot length. Among the SA treatment regimens, the most drastic effect of SA was observed in 2SA2BFL and 4SA3BAFL treatments although it was not significant. It was previously reported that SA has the properties to increase the height of the plant exposed to salt stress (Hussain, *et al*., 2007; Farahbakhsh & Sajid, 2011). On the other hand, it was also shown that SA treatment only at lower concentrations has shoot-increasing effects, and above certain concentrations, its effect gets saturated (Larque-Saavedra & Martin-Mex, 2007). However, in our study and also in similar studies done by others (Waseem, *et al*., 2006), under drought stress, the SA application did not show its effects, suggesting that the mechanism of SA action and drought stress tolerance might work through different routes and consequently cause no shoot-improving effect.

Whole Plant Fresh Weight and Dry Weight

Control plants exhibited the highest fresh weight, while the 4D severe drought stress caused drastic loss in the fresh weight (Fig. 3), and the 2D stress caused no significant reduction in the fresh weight. In severe drought stress plants, SA treatment showed improvement in the fresh weight as compared to the untreated plants, whereas moderately drought-treated plants showed a decrease in the fresh weight, but the result was significant. The lone treatment of SA has been reported to improve the root and shoot dry weight (Larque-Saavedra & Martin-Mex, 2007).

Plant dry weight (DW) was also significantly found to be affected by the different treatments (Fig. 4). The ability of SA to increase the dry mass of the plant and to ameliorate the adverse effect of water stress may have significant implications in improving plant growth and overcoming the yield barrier resulting from limited water availability (Sayyari, *et al*., 2013). Under drought conditions, the 4D stress caused a significant reduction in the DW, and a similar reduction was observed in all SA-treated groups except 2SA1BAFL, which included the highest concentration of SA treatment in moderate drought conditions. These results suggest that lower concentrations of SA under drought conditions might be effective in maintaining the tissue biomass. Previous studies have also noted that SA regulates cell division and expansion (Li, *et al*., 2022), which might be the reason for the improvement in biomass at lower SA concentrations.

Yield Parameters

In the present study the yield parameters including fruit length, number of seeds per fruit, and fresh and dry weights of fruits, were assessed for all treatments.

Fresh Weight and Dry Weight of Fruit

The control group had the highest values, while the 2D and 4D drought had significantly affected the fresh weight of the plants (Fig. 5). Interestingly, most of the SA treatment, especially 0.5 and 1mM, resulted in the improvement of the fresh weight of fruits. The 1mM SA in the moderate 2D drought stress and double treatment of 0.5mM and 1mM SA in severe 4D drought stress condition displayed the highest fruit-fresh weight improvement capacity. A similar weight improvement effect was also observed by other workers at 0.25mM SA foliar application (Kazemi, 2013). In the case of the dry weight of the fruits, the 2D drought stress had a more prominent effect than the 4D group (Fig.6). However, the single SA treatment before anthesis has shown the fruit dry weight improvement capacity in both the moderate (2D) and severe (4D) drought conditions. Among the double treatment groups, the 1mM SA treatment has a better capacity, although not statistically significant, to maintain the dry tissue weight than the rest of the treatments. This property of SA again can be attributed to its regulatory action on cell division and expansion (Li, *et al*., 2022).

Fruit Length and Number of Seeds Per Fruit

Water stress caused a decrease in yield. Yield is an important factor for yield improvement (Kumar, *et al*., 2001). Drought has a 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). Control plants had the longest fruit length, while the 4SA3FR-treated plants had the shortest fruit length (Fig. 7). The control group also exhibited the highest number of seeds per fruit, whereas the 2SA3BAFL-treated plants had the lowest seeds per fruit (Fig. 8). However, in both cases, the difference was not statistically significant. Previous studies have demonstrated that SA application has a positive role in fruit production (Larque-Saavedra & Martin-Mex, 2007; Vlot, *et al*., 2009), fruit quality and antioxidant defence in the fruits (Slaymaker, *et al*., 2002; Durner & Klessig, 1995), and it also influences seed production (Abreu & Bosch, 2009).

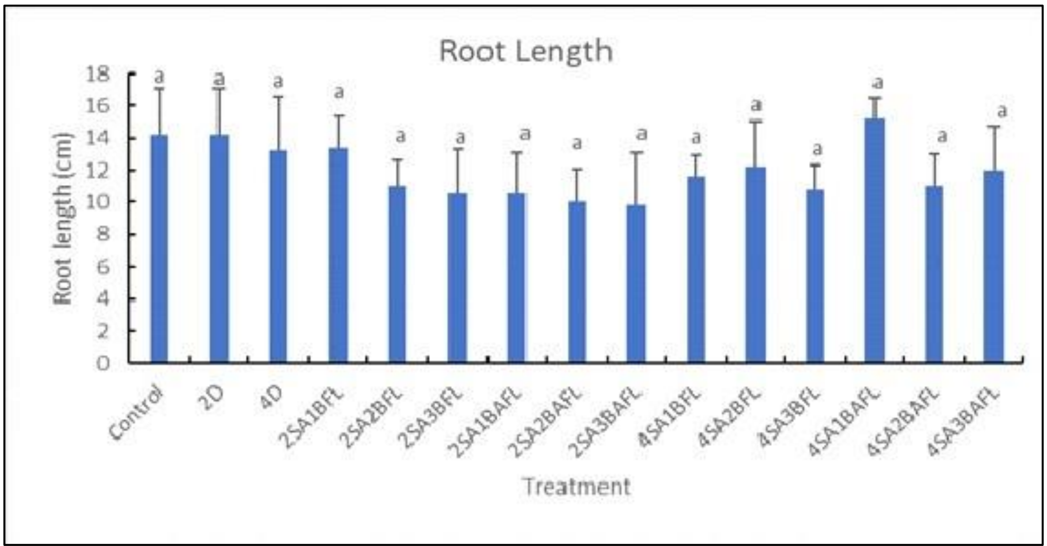

Fig. 1: Effect of 3 different concentrations of foliar application of Salicylic acid on root length of *Trigonella* at different drought conditions. Means with same letter did not differ significantly according to Tukey's multiple comparison test (P<0.05) via ANOVA.

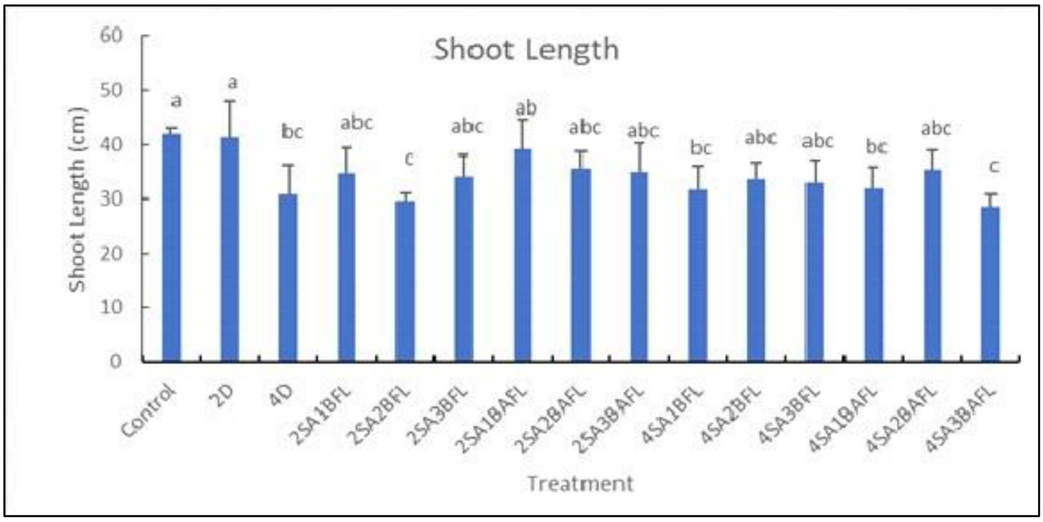

Fig. 2: Effect of 3 different concentrations of foliar application of Salicylic acid on shoot length of *Trigonella* at different drought conditions. Means with same letter did not differ significantly according to Tukey's multiple comparison test (P<0.05) via ANOVA.

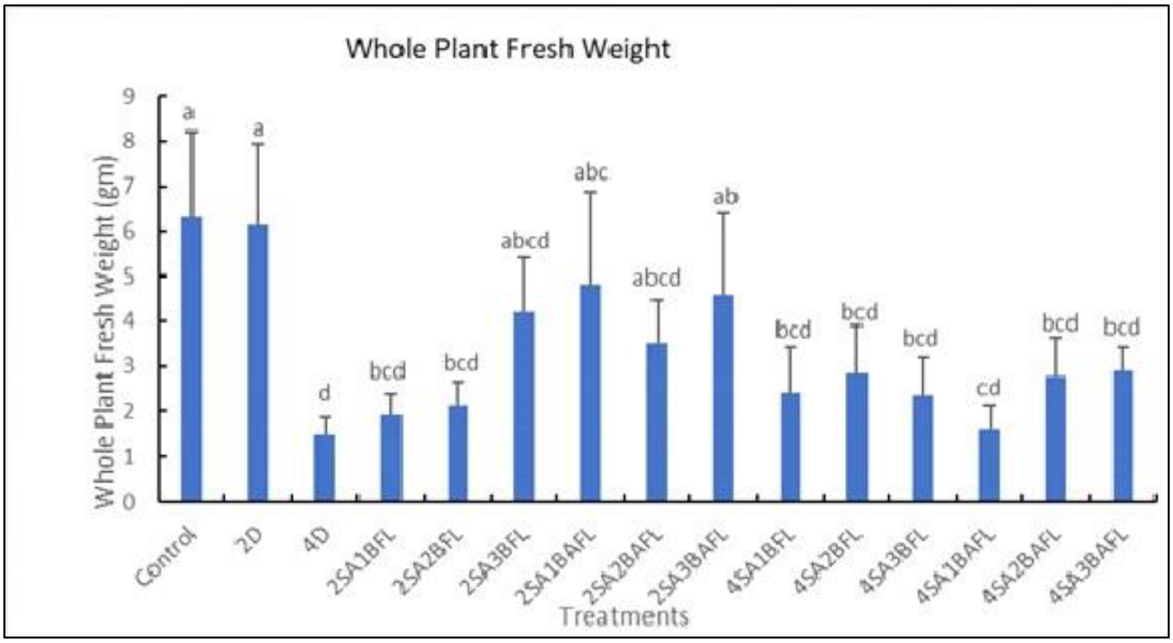

Fig. 3: Effect of 3 different concentrations of foliar application of Salicylic acid on whole plant fresh weight of *Trigonella* at different drought conditions. Means with same letter did not differ significantly according to Tukey's multiple comparison test (P<0.05) via ANOVA.

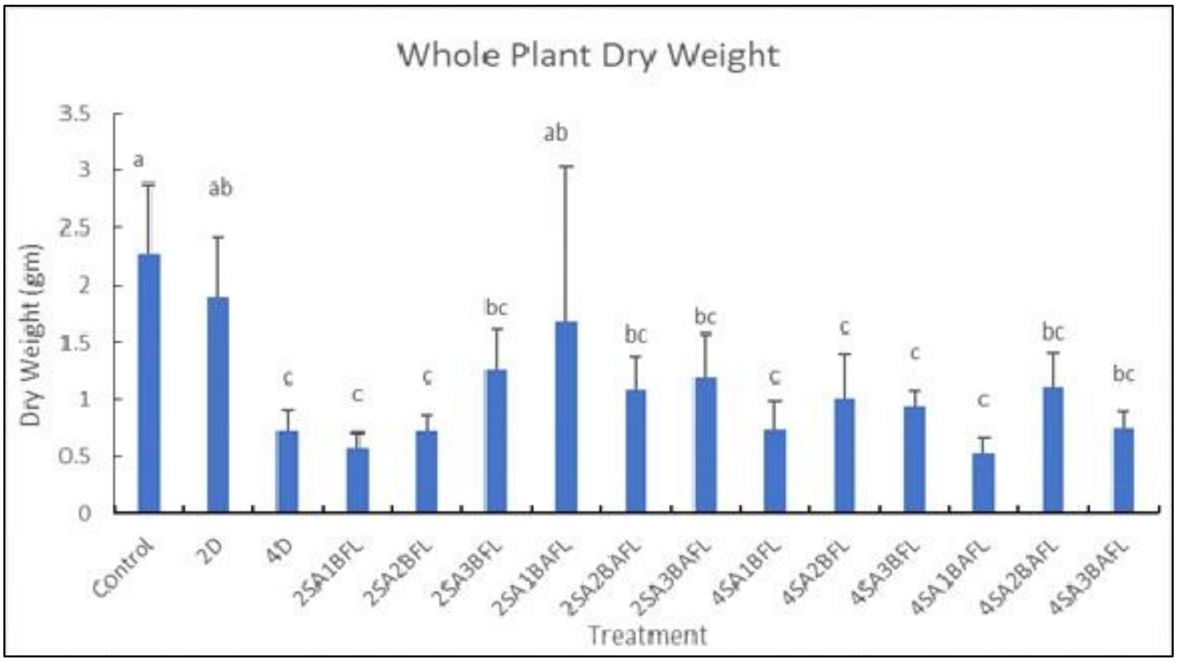

Fig. 4: Effect of 3 different concentrations of foliar application of Salicylic acid on whole plant dry weight of *Trigonella* at different drought conditions. Means with same letter did not differ significantly according to Tukey's multiple comparison test (P<0.05) via ANOVA.

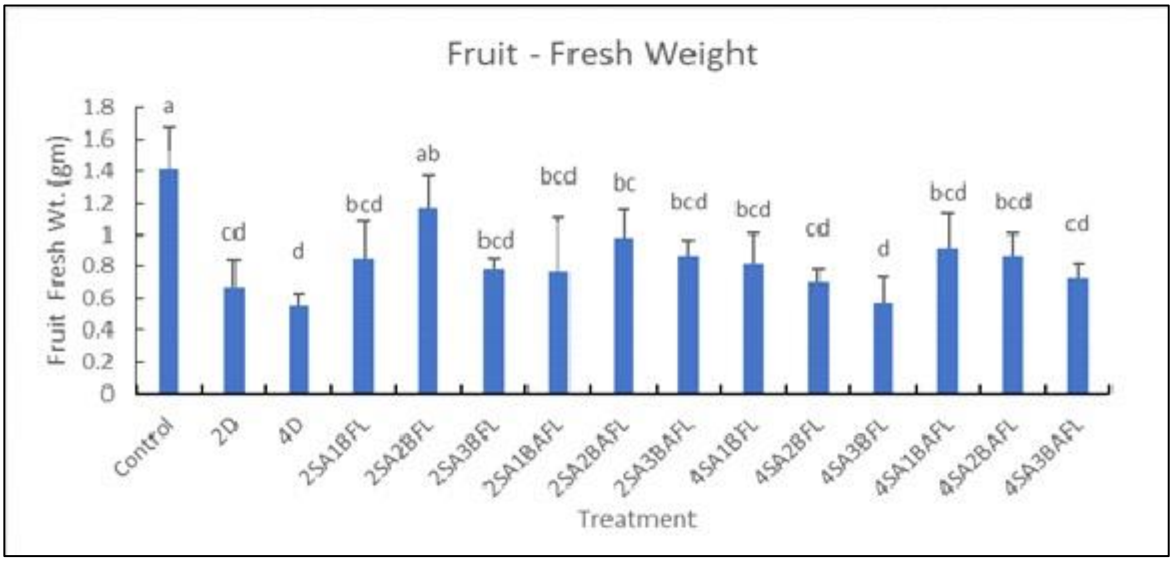

Fig. 5: Effect of 3 different concentrations of foliar application of Salicylic acid on fresh weight of fruit of *Trigonella* at different drought conditions. Means with same letter did not differ significantly according to Tukey's multiple comparison test (P<0.05) via ANOVA.

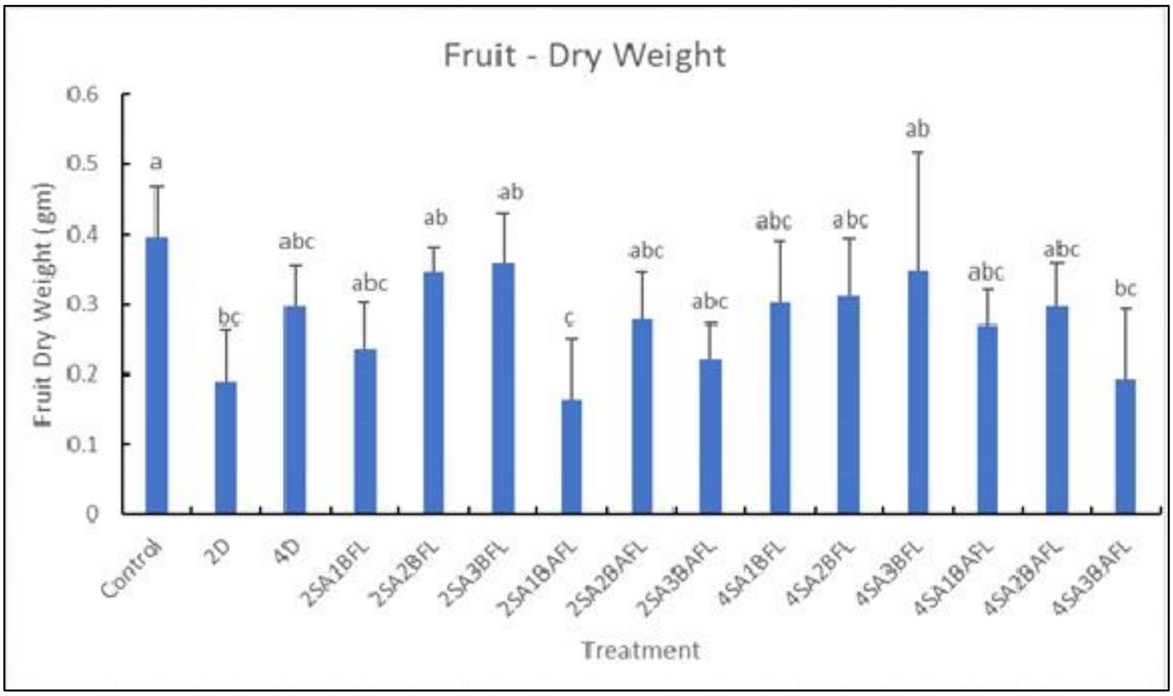

Fig. 6: Effect of 3 different concentrations of foliar application of Salicylic acid on dry weight of fruit of *Trigonella* at different drought conditions. Means with same letter did not differ significantly according to Tukey's multiple comparison test (P<0.05) via ANOVA.

Volume 13, Issue 09 (2024) pp.6514-6522

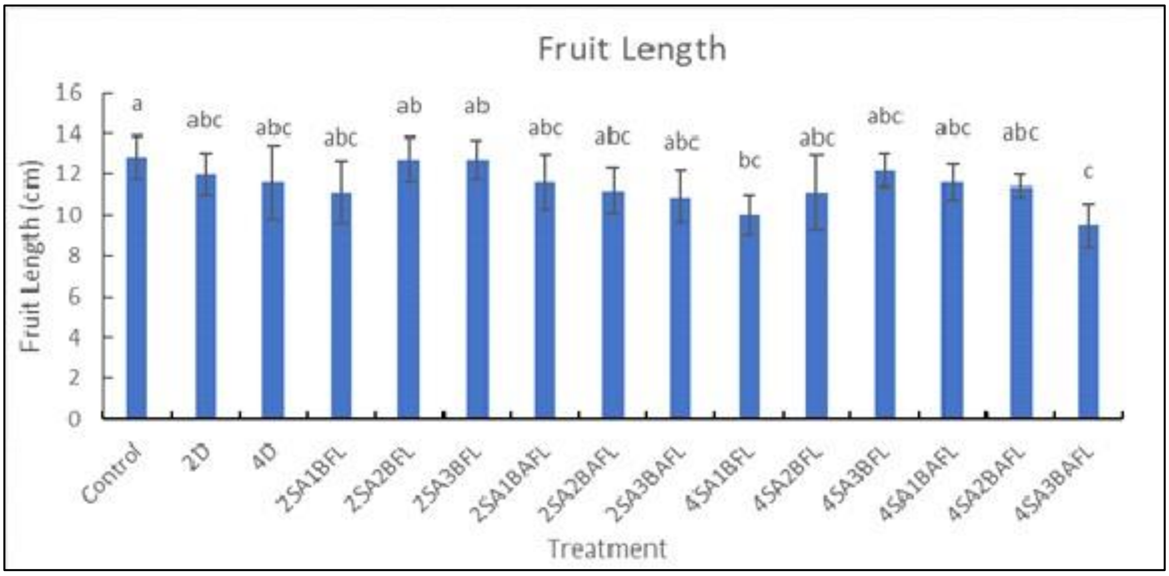

Fig. 7: Effect of 3 different concentrations of foliar application of Salicylic acid on fruit length of *Trigonella* at different drought conditions. Means with same letter did not differ significantly according to Tukey's multiple comparison test (P<0.05) via ANOVA.

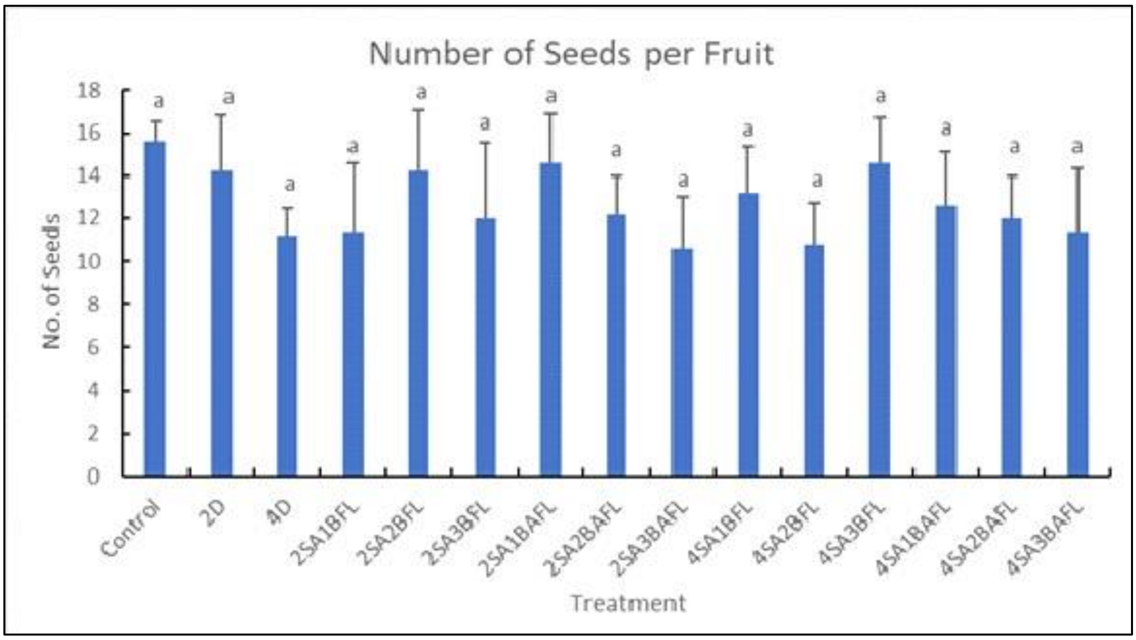

Fig. 8: Effect of 3 different concentrations of foliar application of Salicylic acid on number of seeds per fruit of *Trigonella* at different drought conditions. Means with same letter did not differ significantly according to Tukey's multiple comparison test (P<0.05) via ANOVA.

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.

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