

ANALYSIS OF WATER STRESS CHARACTERISTICS IN *PHASEOLUS VULGARIS*ANIQA BATOOL^{1*}, AUDIL RASHID¹, IRFAN AZIZ² AND MOAZZMA AHMED¹¹Institute of Soil and Environmental Sciences, Pir Mehr Ali Shah-Arid Agriculture University Rawalpindi, Pakistan²Department of Agronomy, Pir Mehr Ali Shah-Arid Agriculture University Rawalpindi, Pakistan*Corresponding author's email: aniqabatoool@uuar.edu.pk

Abstract

Climatic extremes events triggered by global warming are having negative impacts on agricultural systems and economies of countries that are highly susceptible to drought. Some plant species are capable of developing responses to drought through stress tolerance or stress avoidance. The short life cycle and high nutritional value of the common bean *Phaseolus vulgaris* made it an ideal candidate to study the effects water stress. We tested three field capacity (FC) conditions for up to five weeks: 100% FC, 60% FC (moderate water stress), and 40% FC (severe water stress). Plant height, number of leaves, leaf area, biomass, and relative water content all showed a statistically significant decrease under water stress compared to control plants. Proline content accumulation and root length both increased significantly under water stress compared to control conditions. The highest accumulation of proline was observed under severe water stress (40% FC), demonstrating the tolerance level and capacity of *Phaseolus vulgaris* to survive short periods of drought.

Key words: *Phaseolus vulgaris*, Proline, Relative water content, Drought stress, Tolerance.

Introduction

Climate change driven by anthropogenic activities is the main cause for the increase in global temperatures that trigger extreme environmental events such as floods, droughts, and melting of glaciers. Among all natural disasters, drought causes the most drastic and adverse effects on the human population (Sankriti *et al.*, 2021). Trenberth *et al.*, (2014) reported that global warming has altered the global water cycle to the point where it is predicted that for centuries future generations will face more intense and longer periods of drought.

Climatic variations resulting from global warming due to greenhouse gas accumulation endanger crops growing on agricultural lands that are prone to soil moisture deficiency. Water availability is the basic requirement to regulate proper plant growth, cell function, and crop production. Increasing human population size is solely responsible for the exhaustion of water resources and land degradation for many agricultural sites experiencing drought. Water stress has become a great challenge for sustainable agricultural production by decreasing overall crop yield (Anderegg *et al.*, 2019).

Thirty-six percent of the earth is covered with arid & semiarid areas that contain approximately 40% of the global human population (Gaur & Squires, 2018). Crops grown in both arid and semiarid regions have been severely affected by restricted water supply due to increase in temperature and the resulting water scarcity concurrent with an increase transpiration rate. Pakistan, found within the arid and semiarid region, is a country whose agriculture-based economy has been adversely affected by drought conditions resulting from climate change.

Crop water use efficiency is an indicator of overall production in plants grown in arid and semiarid regions (Hatfield & Dold, 2019). There is a strong correlation between water use efficiency and plant water and CO₂ usage during photosynthesis (Cao *et al.*, 2021). It has

been reported that the combination of temperature increase and water stress have cumulative adverse effects on different crop yields compared to the effect of a single stressor (Sattar *et al.*, 2020; Jumrani & Bhatia, 2018).

Food insecurity due to natural hazards is predicted to negatively impact economies and overall population health and mortality in agricultural regions across the world. Another reason for food insecurity is a decrease in availability of agricultural land due to urbanization and industrialization. The lack of agricultural lands and resulting reduction in annual crop production negatively impact the agricultural supply chain. Small agricultural land holders are also facing problems due to a lack of sustainable agriculture caused by years of growing highly exhausted crops such as maize. This lack of crop rotation results in low soil fertility status and compaction of soil.

Compacted and nutrient deficit lands gradually reduced crop yield, necessitating the use of expensive fertilizers and tilling. A less expensive way to improve soil fertility and overcome soil compaction is to plant legume crops. Legumes hold water within the plant tissue and replenish the nutrient content of the soil since their roots contain nitrogen fixing bacteria. Planting legumes can reduce cost, improve soil organic matter levels, and increase soil fertility.

Phaseolus vulgaris, also known as common bean and kidney bean, is grown worldwide. Due to its overall high nutrition value, *Phaseolus vulgaris* is a common food crop. It has the highest range of growth variations among different major and minor cereal food crops, including seed color, shape, size, and maturity time. It also has a remarkable diversity of species (> 41,000 varieties) and contains enzymes which promote human health as well as protect from disease. Common bean has a high protein and fiber content as well as high levels of carbohydrates, iron, vitamin A, vitamin B, and vitamin C (Celmeli *et al.*, 2018).

Phaseolus vulgaris is cultivated using various cropping systems in Latin America, Africa, the Middle East, China, Europe, the United States, and Canada. Latin America is the leading country for common dry bean production and consumer use. These beans are a traditional food in Brazil, Mexico, the Andean Zone, and America (Devi *et al.*, 2020). They are also grown in Asian countries, including Pakistan and India. In developing countries, where animal protein is costly, impoverished people consume common bean as a vegetable protein source. Because of its high protein and fiber content, it is known as “grain of hope” for poor citizens of developing countries. About 51% of bean production worldwide is cultivated on soil prone to water stress conditions due to lack of proper irrigation systems and poor agronomic practices (Khatun *et al.*, 2021). Sixty-five percent of crop annual yield losses occurred during extreme dry periods. Prolonged dry periods may increase the risk of crop loss up to 100%.

This study was designed to (1) determine the effect of water stress on morphological traits of common bean *Phaseolus vulgaris* and (2) to estimate proline synthesis in *Phaseolus vulgaris* under water stress conditions. Common bean was chosen for this study because it has a short life cycle and developing countries like Pakistan have the potential to grow it as an affordable choice of food with a higher ratio of dietary fiber value compared to other high food crops. Common bean is also affected by water stress conditions during its vegetative as well as reproductive stages.

Material and Methods

Experimental design: Twelve healthy *Phaseolus vulgaris* plants of approximately equal size and uniform growth were planted in a split plot arrangement as a randomized block with three stages of water supply; 100% F.C, 60% F.C, and 40% F.C.

Application of water stress treatments: Water stress treatments began 30 days after the establishment of seedlings. The initial soil moisture level for the three treatments were 100% of field capacity (F.C.). Water stress treatments were applied as described by Sankar *et al.*, (2008). Plants were retained at 100% F.C. up to 4 weeks. After four weeks, the plants of 60% F.C and 40% F.C. were continuously water stressed. To avoid the moisture loss due to evaporation from the soil surface and water loss through base hole of the pots, all of the pots (except control plants) were covered with white polythene bags by placing pots inside the bags. The bags were fixed to the plants stems until the termination of the experiment. Pots were weighed daily on a digital field balance to maintain field capacity levels.

Physical and chemical soil analyses: Soil pH, electrical conductivity, moisture content, texture, organic matter, and bulk density were analyzed by taking randomized samples from pots before and after experimental treatments. Soil was air-dried, ground and passed through a 2mm sieve. Soil pH and electrical conductivity were measured using a MM 40± multimeter. Soil moisture

content was analyzed using the gravimetric method reported by Varley (1972). Textural class for soil was determined using a modified Bouyoucos hydrometric method (Sheldrick & Wang, 1993). Organic matter of soil samples were measured by using the common Walkley-Black method. The bulk density of soil was also analyzed by using bulk density formula (Blake, 1965).

Plant height: Plants heights were individually determined on weekly basis using a meter stick.

Leaf area: Leaf area was determined from newly emerged leaves. Three leaves per treatment were taken for measurement. Leaf area (cm²) was calculated using a leaf area meter LICOR (Li. 3100 Area meter).

Number of leaves: The number of leaves was counted manually on a weekly basis.

Leaf relative water content: Fresh and fully expanded leaves were used for the determination of leaf relative water content using the Tahi *et al.*, (2007) method.

Root length: At the completion of the study, the plants were carefully uprooted after water application and the roots were washed thoroughly. Root length was measured with a ruler. Root weight was determined using a digital balance.

Biomass: After carefully harvesting the plants after the completion of the study, root and shoot fresh weight were determined separately. For dry weight, roots and shoots of plants were dried at 70°C for 72h and then weighed. Plant biomass was calculated as the ratio of root: shoot mass.

Proline estimation: Samples of leaves were gathered at 90 days after sowing. Using the methods of Bates *et al.*, (1973) and Hamid *et al.*, (2003), 0.5 g of dried powdered leaves were homogenized in 10 ml 3% aqueous sulfosalicylic acid. The homogenate was separated in 2ml acid ninhydrin (prepared by warming 1.2 g ninhydrin in 30 ml glacial acetic acid) and afterward added to 2ml filtrate in a processing tube and put in hot water for 90 min. The reaction was ended in an ice shower. 4 ml toluene was added to the reaction mixture and was mixed for 30 min until the point when two stages were not isolated. The toluene was warmed to room temperature and was suctioned from the fluid stage. The absorbance was measured at 520nm on a spectrophotometer and the proline concentration was obtained from a standard curve using L-proline.

Statistical analysis

The experimental design was arranged in totally randomized blocks. Each block was subdivided into plots containing an alternate level of water stress level i.e., T1 (100% F.C), T2 (60% F.C) and T3 (40% F.C). The data regarding growth and morphological parameters were gathered and analyzed for analysis of variance (ANOVA) using SPSS software. The result means were compared using least significance difference (LSD) test at $p < 0.05$.

Results

Soil analysis: Bulk density of the soil was 1.19g/cm^3 . Soil particle distribution was 62% sand, 14% silt, and 26% clay. The soil was classified as sandy loam. Organic matter of soil was low, only 0.23%.

Soil pH, electrical conductivity, and moisture content, were tested before the start of experiment and again at its completion (up to five weeks later). Fig. 1 shows that the average soil pH for in the control pots was same throughout the experiment, 7.34 ± 0.06 (neutral). Both treatments T1 (100% F.C) and T2 (60% F.C), showed a slight increase by the end of the experiment, 7.37 ± 0.02 and 7.33 ± 0.01 , respectively. In contrast, T3 (40% F.C) showed a statistically significant decrease (7.25 ± 0.02) compared to other treatments ($p < 0.05$).

The soil electrical conductivity (EC) started at $3.02\pm 0.01\mu\text{S/cm}$ in the control and did not change over the course of the experiment (Fig. 2). EC slightly decreased in the T1 (100% F.C) soil. Electrical conductivity value significantly decreased in the T2 (60% F.C) pots to $3.0\pm 0.1\mu\text{S/cm}$ ($p < 0.05$). The greatest

reduction in EC was observed in T3 (40% F.C) pots, which dropped to $2.95\pm 0.1\mu\text{S/cm}$ ($p < 0.05$).

The decline in soil electrical conductivity correlated with a decrease in soil moisture content. At the beginning of the experiment the soil moisture content was 1.3%. T1 (100% F.C) had the same moisture content as control. T2 (60% F.C) showed a slight decrease in soil moisture content to 1.0%. Soil moisture content was greatly reduced in the T3 (40% F.C) condition to 0.01%.

Effect of water stress on plant height: Fig. 3 shows the effect of different water stress levels on plant height. In the control group, plant height significantly increased from $19.3\pm 1.15\text{cm}$ (week 1) to $45.3\pm 4.1\text{cm}$ (week 5; $p < 0.05$). T1 (100% F.C) plants also significantly increased in height, from $17\pm 0.1\text{cm}$ (week 1) to $42.3\pm 12.6\text{cm}$ (week 5; $p < 0.05$), although this increase in height was 0.9% less than the control group. A significant increase in height for T2 (60% F.C) was also observed, from $16.7\pm 1.3\text{cm}$ (week 1) to $39.3\pm 2.08\text{cm}$ (week 5; $p < 0.05$). The T3 (40% F.C.) plants did not significantly increase in plant height over the 5 week experiment.

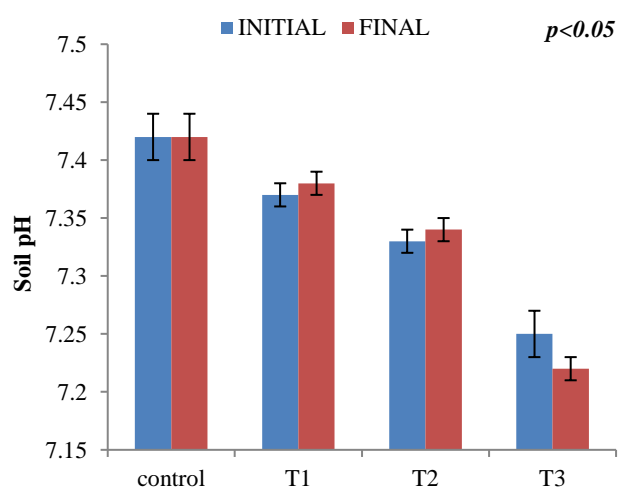


Fig. 1. Initial and final soil pH in control pots and under condition T1 (100% FC), moderate water stress (T2, 60% FC), and severe water stress (T3, 40% FC).

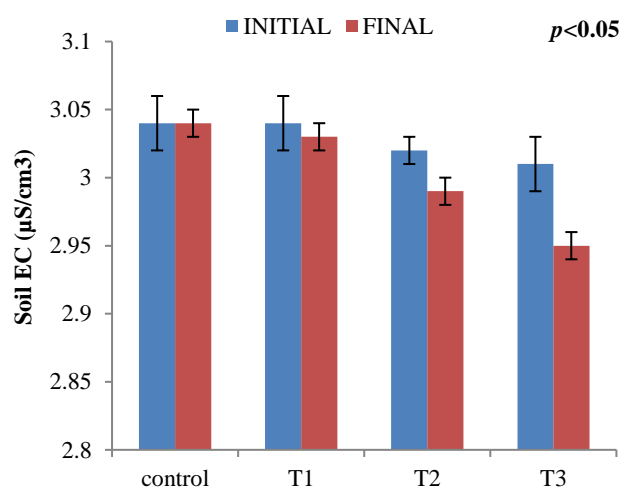


Fig. 2. Initial and final soil electrical conductivity under condition T1 (100% FC), moderate water stress (T2, 60% FC), and severe water stress (T3, 40% FC).

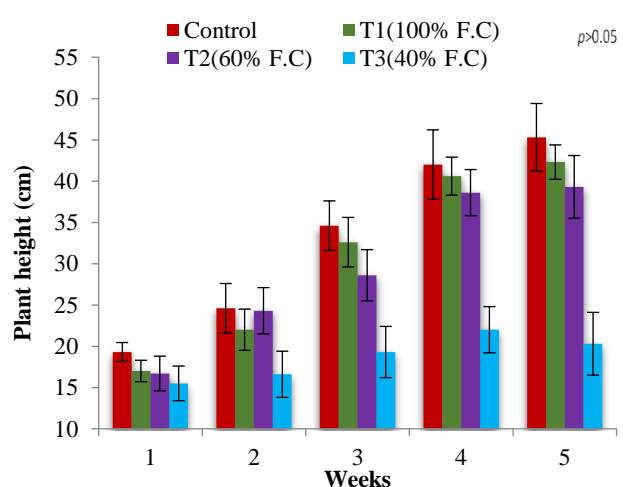


Fig. 3. Effect of water stress over time on plant height under condition T1 (100% FC), moderate water stress (T2, 60% FC), and severe water stress (T3, 40% FC).

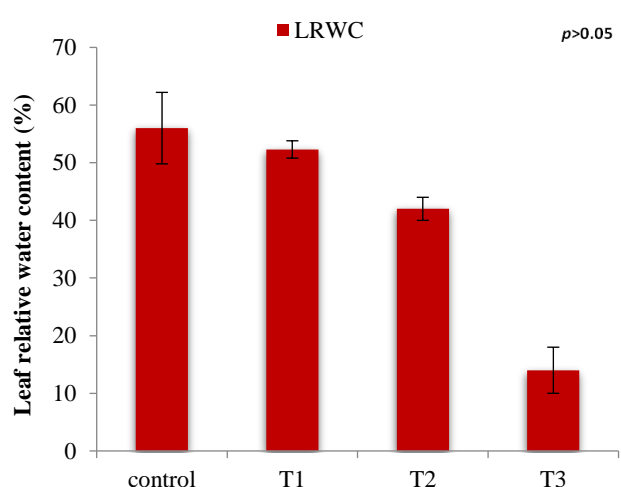


Fig. 4. Effect of water stress on leaf relative water content under condition T1 (100% FC), moderate water stress (T2, 60% FC), and severe water stress (T3, 40% FC).

Effect of water stress on shoot length: Shoot length increased in all of the plants over the course of the experiment. In the T2 (60% F.C) condition, the shoot length increased 13.2% less than was observed in the control condition. However, T3(40% F.C) increased in length from $15.5\pm 2.1\text{cm}$ to $22.2\pm 2.8\text{cm}$ up to the fourth week but declined in length $20.2\pm 3.8\text{cm}$ by the fifth week. In contrast with the control group, a 55.4% decrease in shoot length was observed.

Effect on leaf relative water content: A significant decline in leaf relative water content due to the soil water deficit. The relative water content in the leaves of the control group was $56\pm 6.2\%$. T1 (100% F.C) showed $52\pm 1.5\%$ leaf relative water content (7% decrease compared to control). T2 (60% F.C) had $42\pm 2\%$ leaf relative water content with 25% significant decrease compared to control ($p<0.05$). T3 (40% F.C) had $14\pm 4\%$ of leaf relative water content, a 75% decrease compared to the control group ($p<0.05$) (Fig. 4).

Effect of water stress on number of leaves: Fig. 5 shows a significant difference in the number of leaves in plants grown under various water stress conditions. In the control group, there were 19 ± 3.06 leaves in the first week, which significantly increased by the fifth week to 42 ± 5.03 leaves ($p<0.05$).

In condition T1 (100% FC), the number of leaves significantly increased from 12 ± 3.06 (week 1) to 33 ± 6.43 (week 4; $p<0.05$). In the fifth week, there was a slight 0.01% decrease to 32 ± 6.01 leaves, which was 31% lower than the control at week 5.

In condition T2 (60% FC), the number of leaves significantly increased from 14 ± 2.0 (week 1) to 34 ± 5.02 (week 4; $p<0.05$). In the fifth week, there was a decrease to 28 ± 6.11 leaves, which was 33% lower than the control at week 5.

In condition T3 (40% FC), there was an overall decrease in the number of leaves compared with the rest of the treatments and the control group. There was a decrease

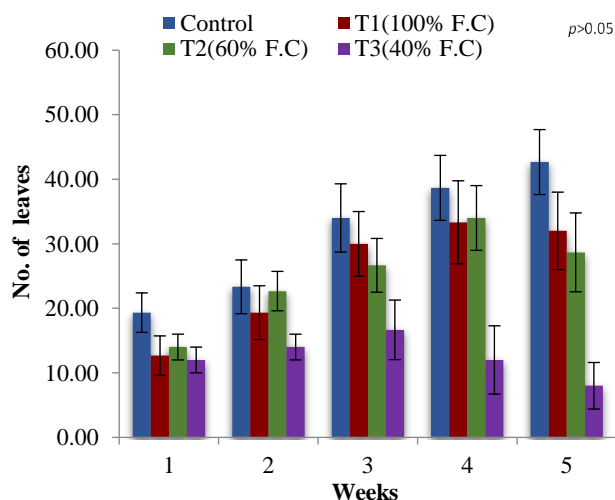


Fig. 5. Effect of water stress on number of leaves over time under condition T1 (100% FC), moderate water stress (T2, 60% FC), and severe water stress (T3, 40% FC).

to 12 ± 2.0 leaves in week 1 to 8 ± 3.61 leaves in week 5, which was 81% lower than the control at week 5 ($p<0.05$).

Effect of water stress on leaf area: The effect of different water deficit levels on leaf area are shown in Fig. 6. The control group showed a significant increase in leaf area from $25.97\pm 2.58\text{cm}^2$ (week 1) to $63.20\pm 6.62\text{cm}^2$ (week 5; $p<0.05$).

T1 (100% F.C.) also showed a significant increase in leaf area from $23.83\pm 2.15\text{cm}^2$ (week 1) to $60.40\pm 7.26\text{cm}^2$ (week 5; $p<0.05$). After 5 weeks, the T1 (100% F.C.) plants had 4% less leaf area than the control plants.

T2 (60% F.C.) also showed a significant increase in leaf area from $22.83\pm 1.37\text{cm}^2$ (week 1) to $32.93\pm 2.42\text{cm}^2$ (week 3; $p<0.05$). By week 5, leaf area decreased 23.3% to $25.23\pm 2.78\text{cm}^2$ ($p<0.05$). After 5 weeks, the T2 (60% F.C.) plants had 60% less leaf area than the control plants.

Leaf area in T3 (40% F.C) decreased throughout the experiment. At week 1 it was $23.13\pm 1.10\text{cm}^2$ and the value decreased to $11.50\pm 1.87\text{cm}^2$ at week 5, which was an 82% decrease in leaf area compared to the control group.

Effect of water stress on root length: Overall, plants grown in drier soils achieved longer root length compared to those grown with high water availability. T3 (40% F.C) had the longest root length ($26\pm 2\text{cm}$). The control group root length ($15\pm 3\text{cm}$) was 42% less than T3 (40% F.C; $p<0.05$). T1 (100% F.C) root length measured $15.6\pm 2.5\text{cm}$, which was 40% less than T3 (40% F.C; $p<0.05$). T2 (60% F.C) root length measured $23\pm 3\text{cm}$ (12% decrease compared to T3 (40% F.C) (Fig. 7).

Effect of Water Stress on Biomass: Fig. 8 depicts the effect on biomass under different water deficit levels at the end of experiment. The control group exhibited the highest biomass value, $0.3\pm 0.03\text{g}$. T1 (100% F.C) had a biomass of $0.24\pm 0.03\text{g}$ (20% decrease from control). T2 (60% F.C) had a biomass of $0.19\pm 0.03\text{g}$ (37% decrease from control). T3 (40% F.C) had a biomass of $0.13\pm 0.01\text{g}$ (57% decrease from control; $p<0.05$).

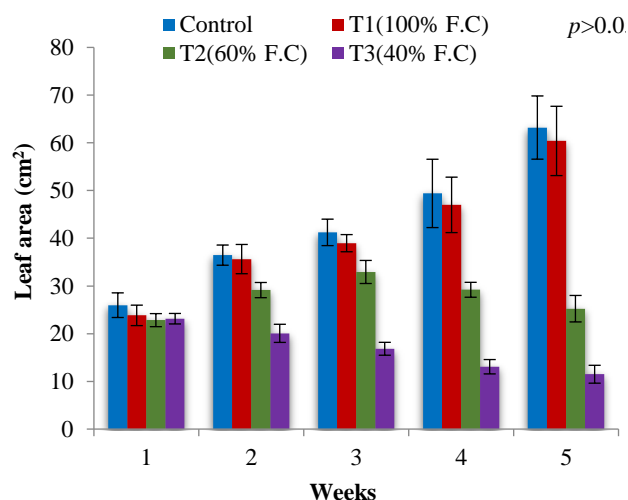


Fig. 6. Effect of water stress on leaf area over time under condition T1 (100% FC), moderate water stress (T2, 60% FC), and severe water stress (T3, 40% FC).

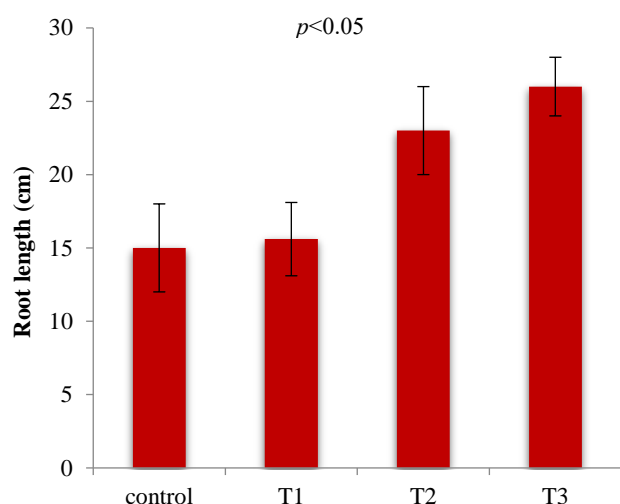


Fig. 7. Effect of water stress on root length under condition T1 (100% FC), moderate water stress (T2, 60% FC), and severe water stress (T3, 40% FC).

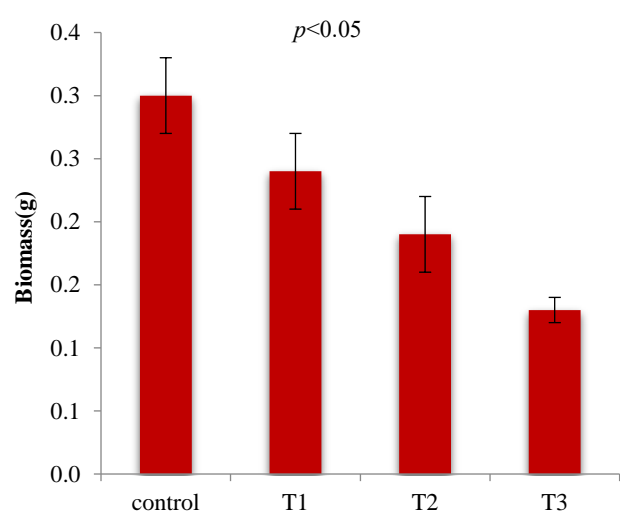


Fig. 8. Effect of water stress on biomass under condition T1 (100% FC), moderate water stress (T2, 60% FC), and severe water stress (T3, 40% FC).

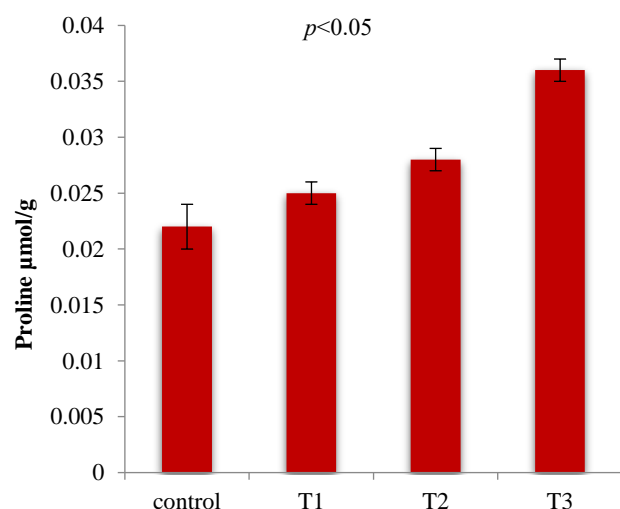


Fig. 9. Effect of water stress on proline content under condition T1 (100% FC), moderate water stress (T2, 60% FC), and severe water stress (T3, 40% FC).

Effect of Water Stress on Proline Content: The control group had a proline content of $0.022 \pm 0.002 \mu\text{mol/g}$. T1 (100% F.C) plants had $0.025 \pm 0.001 \mu\text{mol/g}$ proline, a 12% increase compared to the control group ($p < 0.05$). T2 (60% F.C) plants had $0.028 \pm 0.001 \mu\text{mol/g}$ proline content, a significant difference from control group ($p < 0.05$). T3 (40% F.C) plants had the highest proline content ($0.036 \pm 0.001 \mu\text{mol/g}$), a 40% increase compared to control (Fig. 9).

Discussion

Water is a major limiting factor on which overall crop growth and development depends, making climatic change one of the hardest research challenges for plant researchers and the agricultural sector. Water stress has become a major constraint for the agricultural crop production as the plant life cycle is mainly dependent on proper irrigation.

During periods of drought, some plants are capable of adjusting for water loss during dry periods and maintaining their leaf relative water content. There are number of plant species that naturally undergo water stress during their life cycle and can survive under severe stress conditions. Many have the ability to cope with periods of drought by adopting tolerance mechanisms such as protein accumulation, osmotic adjustment, and by closing their stomata to slow transpiration rate.

This study investigated the morphological parameters of *Phaseolus vulgaris* under three different field capacity (FC) conditions for up to five weeks: 100% FC, 60% FC (moderate water stress), and 40% FC (severe water stress). Along with morphological characteristics, accumulation of proline content in leaves of *Phaseolus vulgaris* was also estimated. We observed different responses of *Phaseolus vulgaris* under water stress after 5 weeks.

In the 40% F. C. water stress condition, the soil pH became slightly more acidic, by 0.1 pH unit, and soil moisture content dropped from 1.3% to 0.01%. The soil electrical conductivity (EC) started at $3.02 \pm 0.01 \mu\text{S/cm}$ in the control and dropped to $2.95 \pm 0.1 \mu\text{S/cm}$ ($p < 0.05$). These results align with those reported by Gao *et al.*, (2022), who demonstrated that plant responses are affected when soil moisture drops below certain levels and that soil chemical properties change under prolonged drought stress.

It has been reported that the presence of adequate water is an important key factor that cause variation in leaf area expansion (Wang *et al.*, 2019; Hatfield & Dold, 2019). Other authors have shown that leaf area is an important indicator of water stress in plants (Yang *et al.*, 2021; Casadebaig *et al.*, 2008). Leaf area of control and T1 (100 % F.C) showed an increasing trend throughout the experiment. T2 (60% F.C) plants showed an increase in leaf area until the third week. T3 (40% F. C) plants exhibited a drop of 86% in leaf area when compared with control.

Plant height in both the control and stressed groups responded in the same way as for leaf expansion. Plant height of control group and T1 (100 % F.C) showed an increasing trend throughout the experimental period while T2 (60% F.C) declined in the final week. The T3(40%

F.C) plants exhibited a 55% decrease compared to the control group. This decline in stem elongation under water stress condition may be responsible for the reduction in leaf area due to less branching. A reduction in plant length under water stress may be due to reduced cell turgidity and its impacts on leaf cell development (Kapoor *et al.*, 2020). Similar to our findings, dos Santos *et al.*, (2022) demonstrated that terminal water stress increases shoot length during initial time period of imposed stress, but under severe water stress conditions, the plant height is reduced.

Number of leaves per area is another important water stress indicator in many plants. We observed that number of leaves decreased in stressed plants compared to the control group. A reason for the decline in the number of leaves is an increase in severity of water stress that inhibits the growth of leaf cells, leading to leaf senescence (Le Roux *et al.*, 2020). This decrease in number of leaves under water stress is one of the water tolerance and conservation mechanisms of plants to cope water stress under the availability of low soil moisture (Seleiman *et al.*, 2021; Basu *et al.*, 2016).

Relative water content is an important relevant plant physiological parameter while studying water stress in plants. We observed an increase in relative water content in the control group but a 75% decrease in relative water content under severe water stress condition, T3 (40% F.C), compared with control. These findings are similar to the findings reported by others (Guillermo *et al.*, 2021). It has been reported that the mechanism of relative water content decline under stressful conditions is due to the passive process of leaf dehydration (Trueba *et al.*, 2019).

When plants respond to drought conditions with a reduction in root length and structure, they have a reduced ability to survive periods of drought (Kang *et al.*, 2022). We observed a remarkable increase in root length of T3 (40% F.C) by 46% compared to control during the water stress period. This indicates that *Phaseolus vulgaris* can adapt to water stress by increasing root length and volume to explore more soil moisture during dry period, which has been observed in other studies (Polania *et al.*, 2022; Tapia *et al.*, 2022).

It has been reported that heat and drought stress due to early leaf senescence and damage in different plant species led to a decrease in biomass accumulation (Alhaithloul *et al.*, 2019; Seleiman *et al.*, 2021; Ahluwalia *et al.*, 2021). Plant dry as compared to fresh weight is relatively low under water stress thereby dry mass/fresh mass ratio is considered as plant stress indicator (Zhang & Zhou, 2019). We observed a 57% decrease in the biomass of the T3 (40% F.C) condition plants compared to the control condition plants.

Proline is an amino acid with increased production when plants are stressed. Plants accumulate proline to prevent water loss in stress conditions (Hosseinifard *et al.*, 2022). Findings of Vujanovic *et al.*, (2022) showed that plants can accumulate 100- fold higher proline content under stressed conditions and the amount of proline is higher in leaves and roots. Results from the present study showed that water stressed plants had high proline values compared to control. Among treatments, the T3 (40% F.C) condition showed highest proline value.

Conclusion

Due to increasing global warming, the world is facing extreme climate events, which directly affect the global agricultural system. The economies of countries in arid and semi-arid regions, such as Pakistan, are highly susceptible to short term water stress. Some plants are capable of developing different responses to escape periods of drought through stress tolerance or stress avoidance. Due to its short life cycle and high nutritional value, the common bean *Phaseolus vulgaris* was selected to study the effects of different water stress on morphological traits and proline content accumulation. As expected, our results indicate that water plays a significant role in the growth and development of *Phaseolus vulgaris*. Results regarding all parameters showed significant difference at ($p < 0.05$) by the end of five weeks. It is interesting that in the most water stressed plants, there was the longest root length and the highest proline accumulation, indicating the tolerance level and capacity of *Phaseolus vulgaris* to survive under a short duration of drought.

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