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# MITIGATION OF DROUGHT STRESS IN WHEAT THROUGH EXOGENOUS APPLICATION OF PROLINE

Bushra[1], A. Kiran[2,*], M. Ahmad[3], T. Shahzad[4] and M. Sanaullah[1,*]

[1]Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad-Pakistan
[2]Department of Botany, University of Agriculture Faisalabad-Pakistan
[3]Department of Soil Science, University College of Agriculture and Environmental Sciences, the Islamia University of Bahawalpur-63100, Pakistan
[4] Department of Environmental Sciences, Government College University Faisalabad
[*]Corresponding Author's Email: sanasial@gmail.com, aysha.kiran@uaf.edu.pk

## ABSTRACT

Proline is an integral osmoprotectant produced in plants under stressed conditions to reduce the adverse effects of any abiotic stress. For the induction of drought stress tolerance exogenously applied proline could be an effective strategy. To evaluate this, a pot experiment was designed to mitigate drought stress on wheat through exogenous application of proline. Two water stress levels i.e., 70% and 30% (applied after 4 weeks) and 30mM proline was either applied in soil (after one week of water stress) or as foliar application (after 1 and 2 week of drought stress. In, another treatment double spray of proline was applied after 1 and 2 weeks of drought stress. When compared with control the proline application has a considerable share in improving plant growth attributes under drought stress. Maximum improvement in plant height (53.99 cm), count of tillers plant$^{-1}$ (3.33), root fresh weight (7.15 g), root dry weight (1.28 g), shoot fresh weight (32.75 g), shoot dry weight (3.03 g), chlorophyll contents (37.13 mg kg$^{-1}$), N (31%) and P concentration (0.23%) in plants was observed when proline was applied at 1st week and repeated after 2nd week of drought stress. In addition, 46% reduction in $Na^+$\$K^+$ ratio (0.28) in plant leaves was also observed in proline foliar application. The foliar application of proline showed better response than that of soil application. Proline application as foliar spray in two splits during drought stress sounds stronger effects to improve growth and physiology of wheat crop.

**Key words:** Drought stress; Osmoprotectants, Proline; Wheat; Water holding capacity.



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## INTRODUCTION

In recent past, crop yields in many countries have suffered from changes in temperature and precipitation. It is estimated that roughly 32–39% of observed yield reduction in major crops (rice, wheat, soybean and maize) is due to climate factors (Mancosu*et al*., 2015; FAO, 2020). Plant growth is significantly inhibited by various abiotic and biotic stresses, and soil physico-chemical and biological properties leading to huge economic losses (Shao *et al*., 2008). In the amidst of all abiotic stresses, osmotic stress that is pronounced due to salinity and drought is a major contributor in lowering plant growth and productivity(Shao *et al*., 2008; Liang*et al*., 2013).

Drought is a polygenic stress that inhibits the plant physiological functions and thereby productivity of crop plants. The hazardous effects induced by drought stress on plants include reduction in biomass production due to separate or combined interaction of several factors. Altered plant metabolism, inhibited cell expansion, reduced enzyme activity and impaired solute accumulation due to reduced uptake of ionic species are some recognizable factors (Khan *et al*., 2012).

Wheat (*Triticum aestivum* L.) is the most essential cereal crop over the globe with a target to feed over 9 billion people by 2050. Field crops production mainly decreased more by moisture stress than any other environmental stress (Zhu, 2002). Drought-tolerant plants are implementing some mechanisms to survive under water deficit regimes e.g., the processes that reduce the moisture loss by increasing resistance of stomata, increase in uptake of water by modifying root architecture, and storage of osmo-protectants (Nelson and Jensen, 1995). Amino acids are the most important osmoprotectants which plant stores, the noticeable of which are glutamate, proline and glycine-betaine. Similarly, some sugars including mannitol, sorbitol and trehalose also act as osmoprotectants. Proline is an integral osmo-protectant produced in plants under stressed conditions to reduce the adverse effects of any abiotic stress. It regulates the scavenging of free radicles in plants under stress thereby protecting water-stressed plants from toxic effects of reactive oxygen species (ROS). It has direct involvement in plant metabolism as a sink of energy, source of nitrogen and carbon compounds, scavenger of the hydroxyl radicals and protector of plasma membrane in plants from osmotic stress (Bartels and Sunkar 2005; Kishor *et al*., 2015; Chopra *et al*., 2018).

Keeping in view the integral role of proline in alleviating stress induced effects in plants the present study has been conducted to assess the efficacy of exogenously applied proline in inducing drought stress tolerance in wheat

## MATERIALS AND METHODS

A pot study was conducted in the wire-house of ISES, University of Agriculture Faisalabad. Soil was well prepared and analyzed for its physico-chemical properties which was sandy loam in texture, alkaline in reaction (pH; 7.5), non saline (EC; 1.24 dS $m^{-1}$), poor in organic matter (0.86%) while low in potassium (56.7 mg $kg^{-1}$) and phosphrous (6.9 mg $kg^{-1}$). Water holding capacity of soil was determined by pressure membrane apparatus (Cresswell *et al*., 2008). The plastic pots were filled with 8 kg of well-prepared soil for sowing of wheat seeds(Punjab 2011). There were 5 treatments and 15 number of experimental units with replication. The pots were arranged following the completely randomize design (CRD). Ten seeds per pot were sown that were thinned to 2 plant after successful germination. For first 4 weeks of plant growth, pots were irrigated by maintaining moisture at 70% of water holding capacity (WHC) of soil. Then the moisture was reduced in half pots up to 30% of WHC to induce drought stress. After 1 week of drought stress, 30mM proline was either applied in soil or as foliar application. In addition, foliar application of proline as a separate treatment was also made after 2 weeks of drought stress to optimize the application time. In, another treatment, double spray of proline was applied after 1 and 2 weeks of drought stress. For control treatment only distilled water was sprayed. Treatment plan is also given in following table for the clarity:

| Sr. No. | Treatments |
|---|---|
| T1 | Control |
| T2 | Soil application of proline after one week (Soil-Proline 7d) |
| T3 | Foliar application of proline after one week (Foliar-Proline 7d) |
| T4 | Foliar application of proline after two weeks (Foliar-Proline 14d) |
| T5 | Foliar application of proline after one week and repeated after second week (Foliar-Proline 7+14d) |

The chlorophyll contents (SPAD value) were determined using portable SPAD meter (SPAD-502) after55 days after sowing at 12a.m., the time of plant when maximum activities of photosynthesis in the leaves is there. For this, three healthy green leaves from each replication were selected and average of these was used as final reading. Crop was harvested after8 weeks of its growth. The plant samples were collected for analysis of proline (Bates *et al.,* 1973). Data regarding morphological and yield parameters were recorded by following standard protocols. After harvesting, plant shoot and root samples were separated, and oven dried for chemical analysis. For N and P contents root and shoot samples were examined by following the standard protocols (Ryan *et al*. 2001).

The data recorded for different parameters were subjected to statistical analysis through two-way analysis of variance using CRD-factorial design with three replicates and means were compared by least significant difference(LSD)test at $p \leq 0.05$ (Steel *et al*., 1997).

## RESULTS

***Growth parameters:*** Proline treatment, particularlty foliar application, signficantly improved wheat growth under drought stress. Data depicted that plant height of wheat significantly reduced due to drought stress (Table 1). The foliarly applied proline gave a significant improvement in plant height under drought stress. The results of soil apllication however, were non-significant when compared with control. Maximum plant height (58.10 ± 1.402 cm) was observed in the treatment T5, where foliar application of proline (30 mM) was carried out after one week and was repeated after second week under optimum irrigation conditions. Moreover, the maximum improvement in plant height (26.73%) under drought stress was also found in the same treatment. According to results the practiced drought regime significantly decreased the fresh weight of shoots (Table 1). The use of proline as mitigant under normal as well as drought stressed conditions significantly improved the shoot fresh weight.Under optimum moisture conditions, soil application increased, though not signficantly, the shoot fresh weight as compared to respective untreated control.All treatments involving foliar application of proline under optimum moisture conditions significantly increased shoot fresh weight. The maximum shoot fresh weight (35.49 ± 1.285 g) was observed in Foliar-proline 7+14d treatment although all the treatments involving foliar application induced similar increase in shoot fresh weight under optimum moisture conditions. Under drought stressed conditions, soil application (i.e. Soil-proline 7d treatment) signficantly increased shoot fresh weight when compared to respective control. Similarly, all treatments involving foliar spray of proline significantly elevated shoot fresh weight under drought condition. The maximum shoot fresh weight (32.75 ± 1.052 g) was observed in the treatment T5, where 85% rectification was observed in shoot fresh weight in proline applied treatments relative to control.

Data regarding number of tillers showed that there was non significant decrease in number of tillers plant$^{-1}$ under stress conditions. A non-significant improvement has been encountered in proline applied treatments (for soil and foliar application both) for number of tillers plant$^{-1}$ both under droughted and normal conditions.Maximum no of tillers plant$^{-1}$ (4 ± 0.33) was observed in the treatment T5 (Table 1).

Under drought stressed conditions, the minimum root fresh weight (2.69 ± 0.373 g) was observed in untreated control plants. A relative improvement of 166% has been observed under proline application for root fresh weight in foliar application of proline after one week and repeated after second week (Foliar-Proline) compared with control and showed a non-significant response when compared with soil application of proline after one week (Soil-Proline).The foliar application of proline have proved more pronounced in enhanceing root fresh weight as compared to soil application in normal regimes.The maximum root fresh weight (7.77 ± 0.562 g) was observed in foliar application of proline after one week (Foliar-Proline ) (Table 1).

A significant reduction in shoot and root dry weights have been encounterd under drought stress. Intuitively, similar to fresh weights, root and shoot dry weights increased in response to proline application. The foliar application of proline induced higher improvement in dry weight as compared to soil application under normal conditions. The maximum shoot dry weight (3.66 ± 0.248 g) was observed in T5. Data regarding root dry weight showed that under drought stressed conditions, the minimum root dry weight (0.78 ± 0.095 g) was observed in untreated control plants. The proline application caused a significant amelioration in root dry weight under drought stressed conditons.Maximum root dry weight (1.28 ± 0.081 g) was observed in T5 where imrovement was 65% when compared with respective untreated control. These results however, were non-significant when compared with T2 and T3. The results of T3 and T4 under drought stress were non-siginificant with respective untreated control plants.

***Physiological parameters:*** Although drought stress decreased the chlorophyll content, this decrease was non significant. Overall, the drought did not induce signficant decrease in chlorophyll content across treatments (Figure 1). The soil and foliarly applied proline significantly improved the chlaorphyll contents, when compared to respective controls, under normal as well as drought stressed conditions. Under normal irrigation conditions, the maximum chlorophyll contents (37.80 ± 0.379 mg g$^{-1}$ fresh weight) were observed in T3, where 10% higher chlorophyll contents were observed than untreated control.A significant improvement in chlorophyll contents under drought stressed conditions have been observed when proline was foliarly sprayed.Maximum improvement in chlorophyll contents (17% ) was observed in the treatment T5under optimum irrigation conditions.

Drought stress significantly increased the proline content in wheat plants (Figure 2). The minimum proline contents was observed in control plants that were provided with optimum moisture. Under drought stress, single foliar application after 7d and double foliar application after 7 and 14d significantly increased the proline content comapred to drought stressed controls. The results of soil apllication and those of foliar application after two weeks (T4) however, were non-significant when compared with control. Maximum proline contents (19.43 ± 0.177) were observed in T5 under drought stressed conditions. This treatment gave the maximum improvement in proline contents (26%) when compared with respective untreated control plants.

With the induction of drought stress, in leaves the $Na^+/K^+$ ratio decreases prominantly (Figure 3).The exogenously applied proline caused a significant decrease in $Na^+/K^+$ ratio in leavesunder normal irrigation as well as drought stressed conditions. Under drought stressed conditions, the maximum reduction in $Na^+/K^+$ ratio in leaves (41%) was observed in T5 and it was statistically significantly less than all other treatments and untreated control plants.

***N & P uptake:*** Drought stress significantly decreased the concentration of phosphorus and nitrogen in shoot but it was significantly improved due to proline application both under control and stressed conditions (Figure 4A and 5A). The significantly higher concentration of phosphorus in shoot (0.357 ± 0.009 %) was observed in T5 under optimum irrigation conditions. It was significantly (67%) higher than respective untreated control plants. Under drought stressed conditions, the highest phosphorus (0.29 % ± 0.012) and nitrogen concentration (1.42 %± 0.026) in shoot was observed in T5. The results provided that drought stress decreased the concentration of phosphorus and nitrogen in roots but this reductionwas non-significant (Figure 4B and 5B). Application of proline on foliagetriggered a significant improvement in concentration of phosphorus and nitrogenin roots under drought stress.

The results of soil proline application however, were non-significant in case of phosphorus uptake when compared with control. Foliar application of proline (7+14d) significantly increased the phosphorus and nitrogen concentration in roots under normal as well as drought stressed conditions and was maximum among all treatments.

**Table 1: Effect of soil and foliar applied proline on growth parameters of wheat under drought stress in pot trial (Average of 3 replicates).**

| Treatments | Plant height (cm) | | Shoot fresh weight( g plant$^{-1}$) | | Shoot dry weight (g plant$^{-1}$) | |
|---|---|---|---|---|---|---|
| Drought stress | 70% | 30% | 70% | 30% | 70% | 30% |
| Control | 51.92$^{bc}$ | 42.60$^{e}$ | 24.31$^{d}$ | 17.74 $^{e}$ | 1.96$^{de}$ | 1.25$^{e}$ |
| Soil application of proline after one week (soil-proline 7d) | 53.80$^{ab}$ | 47.46$^{de}$ | 27.96$^{cd}$ | 26.91 $^{cd}$ | 2.86$^{a-c}$ | 2.31$^{cd}$ |
| Foliar application of proline after one week(foliar-proline 7d) | 57.73$^{ab}$ | 52.70$^{ab}$ | 34.20$^{ab}$ | 27.20 $^{cd}$ | 3.53$^{ab}$ | 2.99$^{ab}$ |
| Foliar application of proline after two weeks(foliar-proline 14d) | 53.61$^{ab}$ | 51.63$^{cd}$ | 32.78$^{ab}$ | 30.68 $^{bc}$ | 3.29$^{ab}$ | 2.75$^{bc}$ |
| Foliar application of proline after one week and repeated after second week (foliar-proline 7+14d) | 58.10$^{a}$ | 53.99$^{ab}$ | 35.49$^{a}$ | 32.75 $^{ab}$ | 3.66$^{a}$ | 3.03$^{ab}$ |
| | **Number of tillers plant$^{-1}$** | | **Root fresh weight (g plant$^{-1}$)** | | **Root dry weight (g plant$^{-1}$)** | |
| Control | 3.33$^{ab}$ | 2.67$^{b}$ | 3.99$^{d}$ | 2.69 $^{d}$ | 0.91$^{cd}$ | 0.78$^{d}$ |
| Soil application of proline after one week (soil-proline 7d) | 3.67$^{a}$ | 3.33$^{ab}$ | 6.18$^{bc}$ | 6.38 $^{a-c}$ | 1.35$^{ab}$ | 1.26$^{ab}$ |
| Foliar application of proline after one week (foliar-proline 7d) | 3.67$^{a}$ | 3.33$^{ab}$ | 7.77$^{a}$ | 6.07 $^{c}$ | 1.26$^{ab}$ | 1.14$^{ab}$ |
| Foliar application of proline after two weeks (foliar-proline 14d) | 3.33$^{ab}$ | 3.33$^{ab}$ | 6.29$^{bc}$ | 5.84 $^{c}$ | 1.14$^{ab}$ | 1.03$^{bc}$ |
| Foliar application of proline after one week and repeated after second week (foliar-proline 7+14d) | 3.67$^{a}$ | 3.33$^{ab}$ | 7.61$^{ab}$ | 7.15 $^{a-c}$ | 1.47$^{a}$ | 1.28$^{c}$ |

Means sharing the same letter(s) are statistically similar to each other at 5% level of probability under least significant difference test.

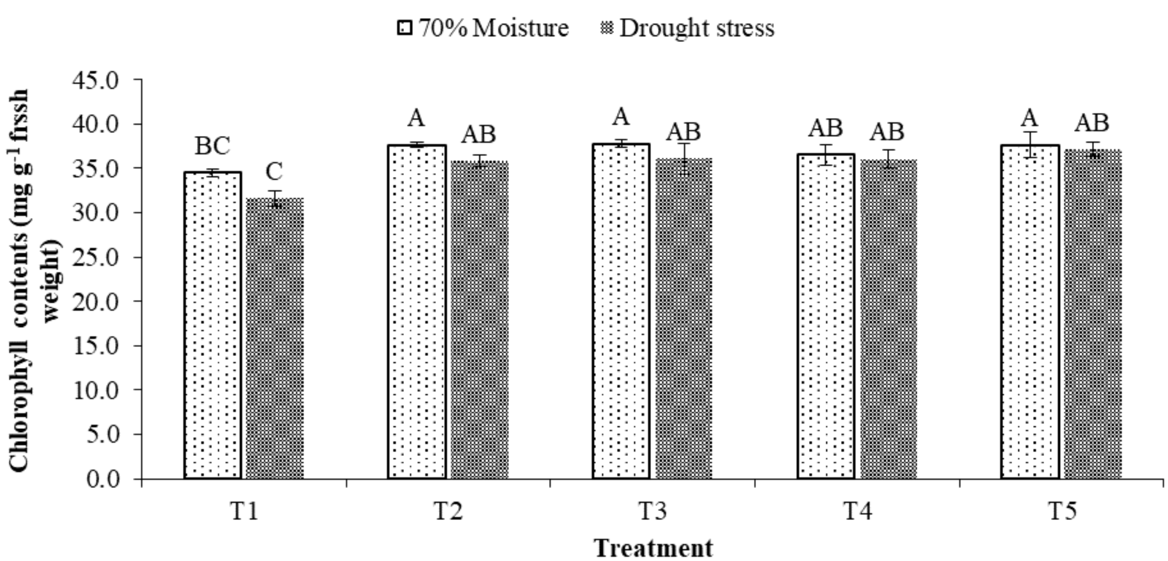


**Fig. 1. Effect of soil and foliar applied proline on chlorophyll contents in leaves of wheat under drought stress.** Bars sharing same letters are statistically non-significant at 5% level of probability ($p \leq 0.05$). The data are means ± SE of three replicates.T1 = control; T2 = soil application of proline @ 30 mM after one week; T3 = foliar application of proline @ 30 mM after one week; T4 = foliar application of proline @ 30 mM after two weeks; T5; foliar application of proline @ 30 mM after one week and repeated after second week

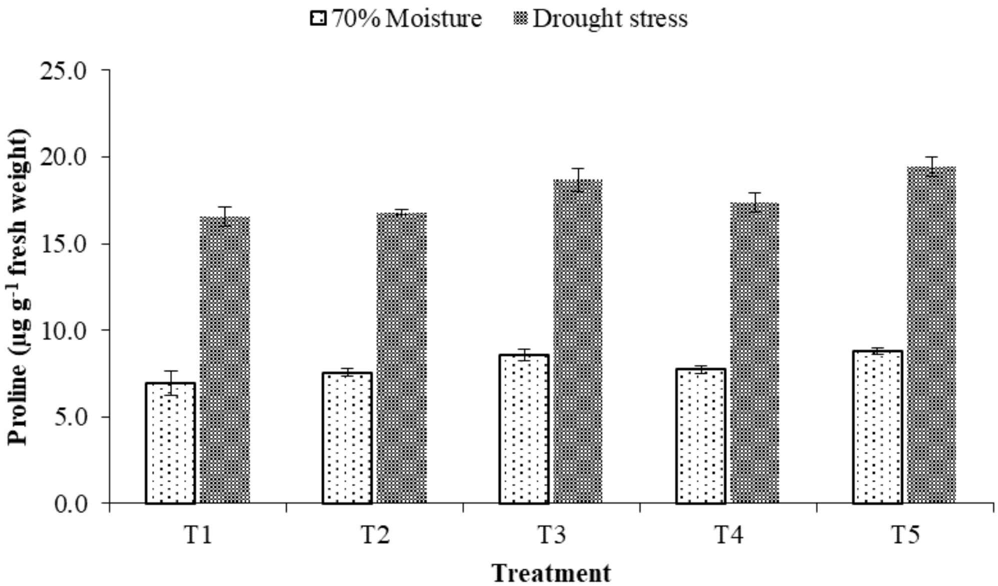


**Fig. 2. Effect of soil and foliar applied proline on proline contents in leaves of wheat under drought stress**Bars sharing same letters are statistically non-significant at 5% level of probability ($p \leq 0.05$). The data are means ± SE of three replicates. T1 = control; T2 = soil application of proline @ 30 mM after one week; T3 = foliar application of proline @ 30 mM after one week; T4 = foliar application of proline @ 30 mM after two weeks; T5; foliar application of proline @ 30 mM after one week and repeated after second week

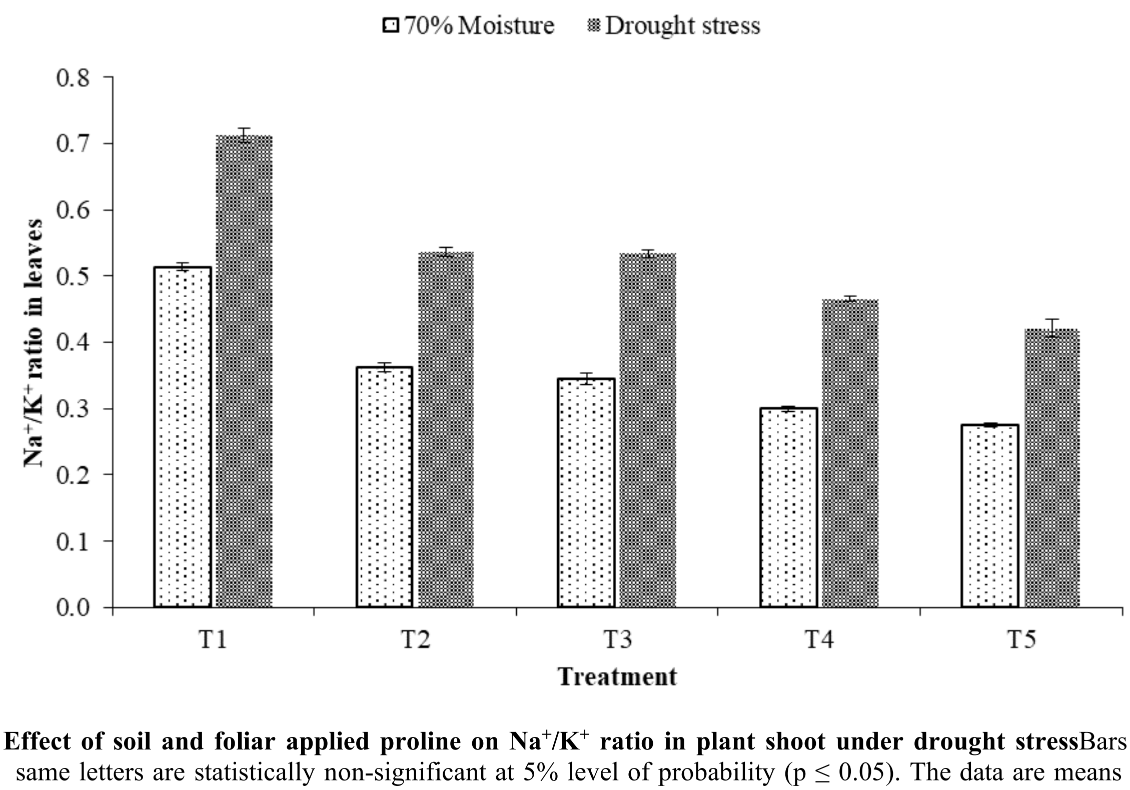


**Fig. 3. Effect of soil and foliar applied proline on $Na^+/K^+$ ratio in plant shoot under drought stress**Bars sharing same letters are statistically non-significant at 5% level of probability ($p \leq 0.05$). The data are means ± SE of three replicates. T1 = control; T2 = soil application of proline @ 30 mM after one week; T3 = foliar application of proline @ 30 mM after one week; T4 = foliar application of proline @ 30 mM after two weeks; T5; foliar application of proline @ 30 mM after one week and repeated after second week

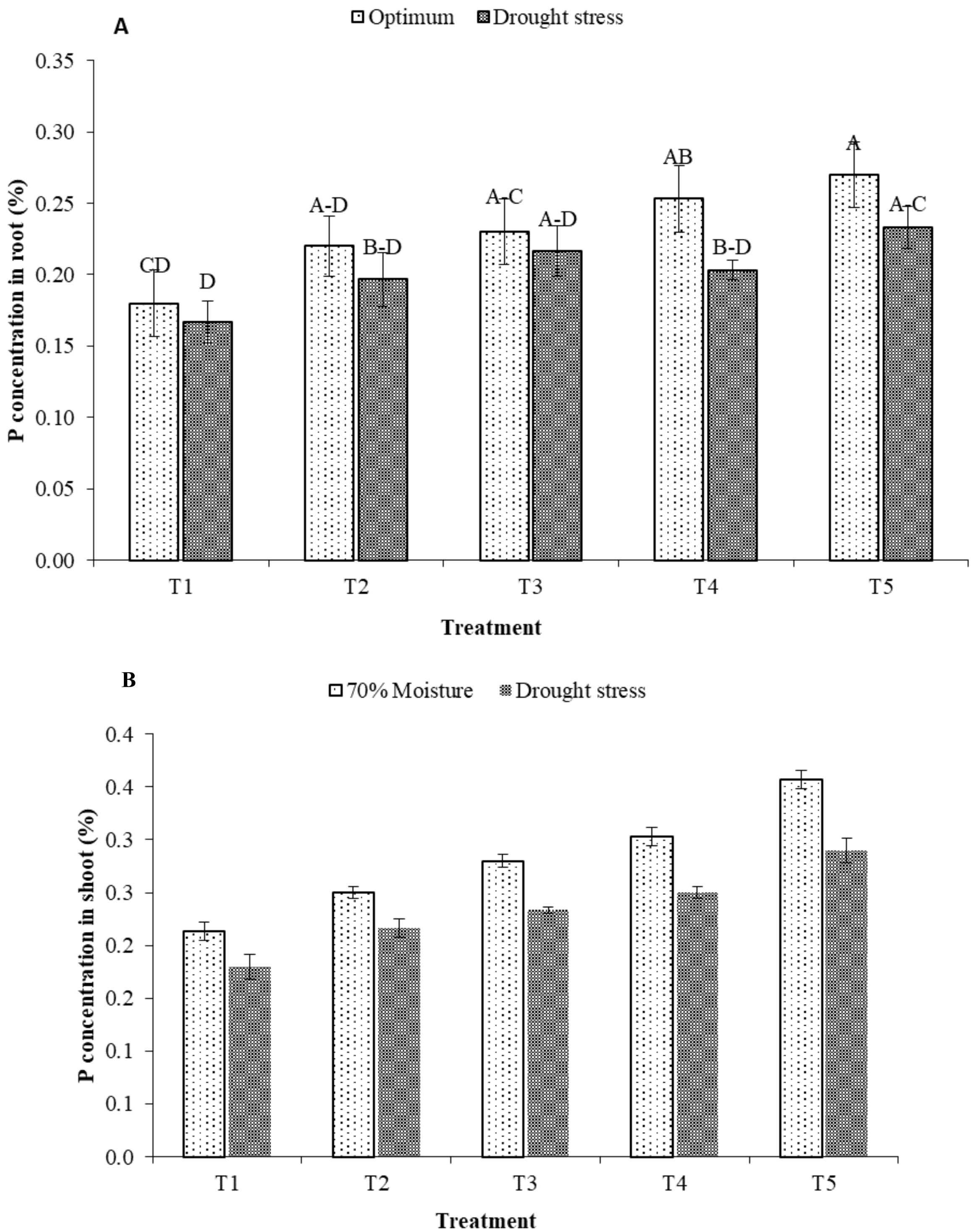


**Fig. 4. Effect of soil and foliar applied proline on phosphorus concentration (%) in shoot [A]and root [B] of wheat under drought stress.** Bars sharing same letters are statistically non-significant at 5% level of probability ($p \leq 0.05$). The data are means ± SE of three replicates. T1 = control; T2 = soil application of proline @ 30 mM after one week; T3 = foliar application of proline @ 30 mM after one week; T4 = foliar application of proline @ 30 mM after two weeks; T5; foliar application of proline @ 30 mM after one week and repeated after second week

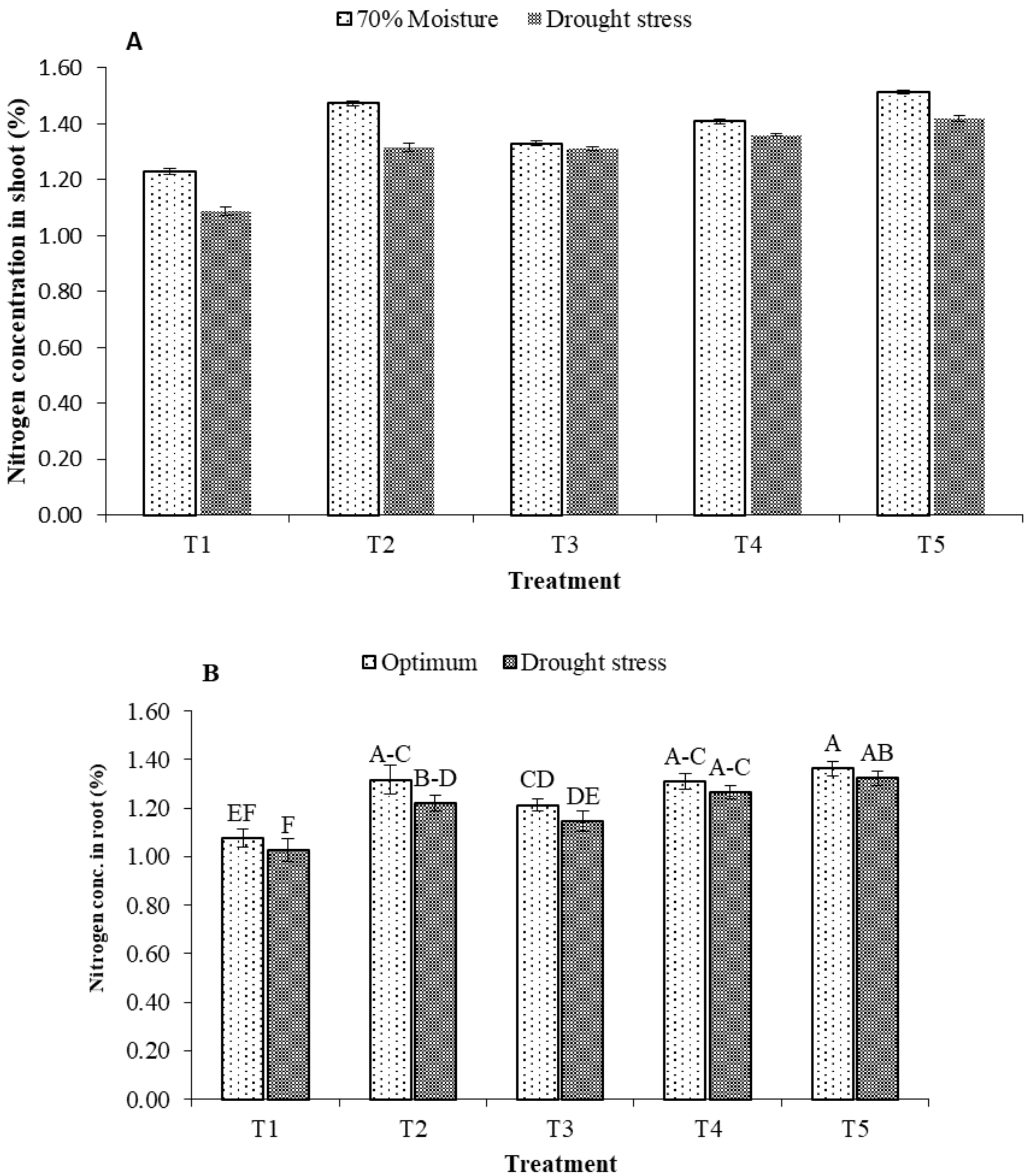


**Fig. 5. Effect of soil and foliar applied proline on nitrogen concentration (%) in shoot [A] and root [B] of wheat under drought stress**Bars sharing same letters are statistically non-significant at 5% level of probability ($p \leq 0.05$). The data are means ± SE of three replicates. T1 = control; T2 = soil application of proline @ 30 mM after one week; T3 = foliar application of proline @ 30 mM after one week; T4 = foliar application of proline @ 30 mM after two weeks; T5; foliar application of proline @ 30 mM after one week and repeated after second week

## DISCUSSION

Increased accumulation of proline contents for implying some levels of osmotic adjustment in wheat under stress exposure has been reported previously. Positive correlation of wheat grain yield was found with proline content under drought stressed conditions (Mwadzingeni *et al*., 2016) while Qayyum *et al*., (2016) suggested high proline accumulation in wheat is a good indicator of drought tolerance which could be useful during genotype selection.

Proline is among the low molecular organic compounds produced in the cytosol of plants that alleviates the osmotic potential hence stabilizes the cell turgor of stressed plants (Rhodes and Samaras, 1994). The maintenance of cell turgidity by producing compatible solutes is termed as osmotic adjustment (OA) (Blum, 2017). Osmotic adjustment is the major protective mechanism in plants that helps them to maximize water uptake under drought stressed conditions. There exists a significant positive correlation of OA with crop yield that indicates the importance of osmolytes production for sustaining agricultural productivity under drought stress (Blum, 2017). Proline is a potential osmolytes that plants biosynthesize under stress (Tefera *et al*., 2021; Ashraf and Foolad, 2007, Khalid *et al.,* 2013). Proline is considered as an osmo-protectant that regulates the scavenging of free radicles in plants under stress. The exogenous application of proline results in the induction of stress tolerance in plants. It is the most widely studied organic solute in crop plants due to its involvement in stress tolerance induction (Delauney and Verma, 1993). It has been well documented that exogenous application of proline increases its endogenous levels in water-stressed plant tissues (Ali *et al*., 2007; Ashraf and Foolad, 2007). Thus, exogenous application of proline might help to ameliorate the negative effects of water stress on crop plants.

***Effect of exogenous application of proline on growth attributes of wheat:*** In current study, the effect of proline was evaluated on wheat under drought stress. Reduced soil moisture content significantly reduced growth traits included plant height, shoot and root fresh weight and root length and all these parameters were well remediated by the proline application.

Talat *et al*. (2013) reported that all growth attributes were significantly decreased when plants were undergone to high water stress but the exogenous application of osmolytes significantly improved these attributes of plants. The improvement in growth parameters might be due to the ablation of osmotic stress consequences by direct involvement in plant metabolism as sink of energy, supply of nitrogen and carbon compounds, scavenging the hydroxyl radicals and protecting plasma membrane in plants from osmotic stress (Szabados and Savoure, 2010; Kishor *et al*., 2015). Similarly, Khalid *et al*. (2013) have proved that the exogenous application of proline significantly improved the vegetative growth under stress. The proline application has been reported to increase the seedling growth by increasing stem height, secondary branching and leaf area of *Cordia myxa* under salinity stress (Mayahi and Fayadh, 2015). Similarly, exogenously applied proline improved plant growth of *Oryza sativa* under salinity stress by enhancing plant height and root length (Teh*et al*., 2015).

***Improvement in physiological attributes of wheat due to exogenous application of proline:*** The exogenous application of osmolytes results in the regulation of osmotic potential that leads to the amelioration of toxic effects of ions and plant can grow normally. Osmolytes mainly retards the activity of $Na^+$ and $Cl^-$ ions in cytoplasm which may be the potent cause of plant height rectification.

The exogenous application of osmolytes results in the improvement of ion balance and the regulation of water relations within plant's cell. Previously reported that the exogenous application of proline resulted in improved regulation of osmotic potential under stress-induced osmotic stress as proline regulates the ionic concentration in cell solution and manages the osmotic potential in vacuole (Hoque *et al*., 2007; Deivanai *et al*., 2011; Chen and Murata, 2008). The present findings were supported by the work of (Kafi, 2009) and Hoque *et al*. (2007), who also states the positive effects of proline application on height of drought stressed plants. It has been reported that drought decreases the chlorophyll content may be due to the degradation of chloroplasts and photosynthetic pigments under drought stress (Kafi, 2009). The decrease in chlorophyll contents can lower the plant photosynthetic activity by reducing stomatal conductance, water use efficiency, photosynthetic pigments synthesis, and translocation of carbohydrates (Baker *et al*., 2007; Sultana *et al*., 1999). The findings of this study reveal no significant effect of drought on chlorophyll content which are not in accordance with Kahlaoui*et al.* (2013) and Talat *et al.* (2013) may due to insufficient period of stress because the decreasing trend of chlorophyll content may lead to significant difference if the drought persist for further few weeks.

Talat *et al.* (2013) who reported the improvement chlorophyll contents and other plant physiological attributes due to use of proline and L-tryptophan. According to our results a decrease in leave $Na^+/K^+$ ratio and increment in leave proline content have been observed. The improvement in physiological attributes is considered due to the regulation and alteration in the uptake of $Na^+$ and $K^+$ ions due to the enhanced levels of osmolytes into plant cells (Deivanai *et al*., 2011). It's a well-documented phenomenon that application of proline as drought mitigant maintains $Na^+/K^+$ and increases proline accumulation in plants under stress. For example, Wu *et al*. (2017) reported the decrease in $Na^+/K^+$ ratio and as increase in proline concentration of *Onobrychisviciae folia* Scop plants under salinity stress.

***Effect of exogenous application of proline on nutrient concentration in wheat plants:*** In the present study, decrease in nitrogen and phosphorus in plant roots and shoot was observed under drought stress which may be attributed to the decrease in stomatal conductance and transpiration rate of plants (Pessarakli, 1999). The exogenously applied proline improved the nutrients uptake in water-stressed plants. The use of 30 mM proline as mitigant proved effective in increasing nitrogen and phosphorous concentration in wheat plants.

The same results have also been reported by Ali *et al*. (2007). In grasses, it has been observed that increase in accumulation of proline under water-stressed conditions enhanced the uptake of shoot nitrogen that might be attributed to improvement in stomatal conductance and water transpiration rate (Singh *et al*., 1973; Tanguiling, 1987). Proline application can be considered as an efficient approach for the amelioration of negative effects of water stress as observed in the present study.

**Conclusion:** The variable response of soil and foliar application of proline was observed in growth, physiological and chemical parameters of wheat under drought stress. The foliar application of proline effect was more significant on growth attributes as compared to soil application. Maximum improvement in growth and yield attributes was observed when proline is applied after first week and repeated after second week of drought stress. It is concluded that proline application as foliar spray in two splits during drought stress could be an effective strategy to improve growth and physiology of wheat crop. So, the application of proline may extensively be studied at field level and can be recommended to the farmers.

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