ABSTRACT

In present study, we evaluated the influence of potassium (K) and chitosan (Ct) on sunflower (Helianthus annuus L.) under water limitation conditions. Plants of a drought tolerant sunflower hybrid viz. Hysun-33 were grown to flowering stage under normal conditions, and later subjected to drought stress by withholding irrigation at 50 days after sowing (DAS) for two weeks. Foliar spray of K (1%) and Ct (0.1%) were applied to both normal and water stressed plants, whereas the control plants were treated with water spray (control). The role of exogenous K and Ct application in improving drought tolerance of sunflower was determined by measuring various yield parameters. The obtained data was statistically analyzed following Fisher’s ANOVA technique and difference among the treatments was tested by Tukey test at probability level of 5%. Results of current study showed that the plants foliar sprayed with the combined Ct and K maintained the highest yield attributes (achenes weight, grain yield, biological yield) under water deficit conditions. Also, a marked increment in nitrogen (15%), phosphorous (16%) and K (20%) uptake was observed by Ct + K application in drought stressed plants. Our findings suggest that the application of Ct + K is an effective strategy to improve sunflower yield in arid areas.

Keywords: Sunflower, Drought, Potassium, Chitosan, Nutrients, Yield

1. INTRODUCTION

Water stress is a multidimensional abiotic stress that affects the plants at various growth stages (Majeed et al., 2020). It reduces the uptake of water and nutrients, which adversely affect the crop growth and yield (Shehzad et al., 2020). Plants have evolved various physiological and biochemical mechanisms to mitigate the drought stress. The important protecting mechanisms under the adverse drought situations are the activation of antioxidants and osmoprotectants (Nawaz et al., 2013). However, the ability to manage the adverse conditions varies with species and their genotype (Ashraf et al., 2006).

Sunflower is classified as a low to medium drought susceptible crop. The availability and distribution of water plays a major role in achene and oil production in sunflower (Hussain et al., 2016). Sunflower is considered more sensitive to drought at flowering stage, and it can tolerate the water stress at the beginning and end of growing period (Shehzad et al., 2020). It is reported that the worldwide reduction in sunflower production is linked with the drought (Cakmak, 2005). Drought is a predominant feature in summer which causes the 50 percent reduction in the yield of sunflower that have obtained in irrigated conditions (Ghabadi et al., 2013).

Attempts have been made to mitigate the effect of drought stress by various techniques like breeding of sunflower (Rauf, M. A., Nawaz, F., Aziz, M., Ahmad, W., 2021 Effect of supplemental potassium and chitosan on growth and yield of sunflower (Helianthus annuus L.) under drought stress. Agric. Sci. J. 3(1): 56-71.
2008), exogenous supply of different organic osmolytes, osmoprotectants, and growth regulators such as abscisic acid (Iqbal et al., 2009), proline (Ali et al., 2008), chitosan (Mondal et al., 2012), triazole (Robert et al., 2016) and potassium (Hussain et al., 2016).

Potassium (K) plays an important role for the survival of plans under drought conditions. The maintenance of proper water status is crucial for the survival of plant under drought conditions (White, 2013). Osmotic adjustment is a vital tool that is linked with maintaining high turgor potential and water retention in response to drought. The K⁺ is the most important inorganic osmotia in plants that plays prominent role in osmotic adjustment even under drought conditions (Soleimanzadeh et al., 2010). Exogenous supply of K have been reported to improve the tolerance of crops against various environmental stresses such as salinity (Cakmak, 2005), heavy metal stress in plants (Adrees et al., 2015) and drought (Xu et al., 2021).

Similarly, Chitosan (Ct) has been observed to mitigate the drought stress in various crops like wheat (Zeng and Luo, 2012), Casterbean (Garg and Bhandari, 2016) and Maize (Lu and Fan, 2011). Ct is one of the anti-transparent substances that have been reported effective against drought and other abiotic stresses (Guan et al., 2009). Moreover, it also promotes the growth of plants (Farouk et al., 2011). Mondal et al. (2012) showed that Ct considerably increases the growth and development of basil plant under drought conditions. Ct may produce different metabolites, which reduce the transpiration losses and resulted more water is available to plant that ultimately improves the overall health of plants under water deficit conditions (Gornik et al., 2008). Zeng and Luo (2012) suggested that Ct is a compound that reduces the losses and mitigates the effects of drought stress.

However, reports regarding combined application of K and Ct are scant. Since these nutrients act as osmoprotectants, it would be interesting to evaluate their combined effects on sunflower under drought stress.

2. MATERIALS AND METHODS

2.1. Experimental Conditions

Experiment was performed at MNS University of Agriculture Multan, Pakistan during the year 2017. Seeds of sunflower cultivar “Hysun-33” were obtained from a private seed organization ICI sterilized with 5% sodium hypochlorite (NaOCl) solution and randomly selected healthy and pure seeds sown in the farm area of university. Soil samples were randomly collected from experimental site before and after sowing to determine the various physiochemical characteristics of soil (Table 1). NPK fertilizer dose at of 50:60:60 per acre were applied at the time of sowing incorporated in the soil and N fertilizer in three splits. Randomized complete block design (RCBD) was used for the experiment. The net plot size was 12.5 m² (5 m length and 2.5 m wide). After sowing irrigation was applied to seedlings with seven days interval until the seedlings reached the fourth leaf stage. Drought stress was induced by withholding irrigation for two weeks at flowering stage and applied the foliar spray of potassium and chitosan. The meteorological data of experimental site is given as Fig. 1.

| Texture          | Loamy |
|------------------|-------|
| pH               | 7.8   |
| Organic matter (%) | 0.63  |
| EC mScm⁻¹        | 4.23  |
Saturation (%) 36
Soil total Nitrogen (%) 0.342
Available Phosphorus (mg kg⁻¹) 9.50
Available Potassium (mg kg⁻¹) 210

Fig. 1. Meteorological data for the experimental site at university farm area Multan

2.2. Drought Stress and Nutrients (K and Ct) Application
The plants were exposed to two drought levels i.e. normal irrigations and water stress at flowering stage. The normal plants were grown by applying 05 irrigations of 3 acre inch, whereas water stress was imposed by withholding irrigation 50 days after sowing (DAS) for three weeks.

The K and Ct solutions of 1 and 0.1% were prepared by dissolving 10 g K₂SO₄ and 1 g Ct in 1000 ml double distilled and deionized water, respectively. Foliar spray was carried out 50 DAS early in the morning on a dry, sunny day using compression sprayer to ensure even distribution of nutrients on all the leaves. The plants were foliar applied with individual as well as combined K and Ct and were harvested at maturity to determine the effects of foliar application on yield components.

2.3. Nutrient analysis (N, P, K)
The seeds of normal and drought stress were collected and oven dried at 65°C for 72 hours and afterwards grounded for determination of NPK that accumulate in the grain as described by Hussain et al. (2016).

2.4. Oil contents and fatty acid
The method reported by Salgin et al. (2006) was used to determine achene oil content using Soxhlet apparatus.

2.5. Measurement of yield attributes
At physiological maturity, the plants were manually harvested to record various yield and yield attributes following standard procedures.

2.6. Statistical Analysis:
The obtained data was statistically analyzed following Fisher’s ANOVA technique and difference among the treatments will be tested by Tukey’s HSD test at 5% level of probability. using STATISTIX 9.1.

3. RESULTS
Drought stress considerably influenced (P<0.01) the head diameter, number of achenes per head, 1000 achenes weight, grain yield, biological yield and harvest
index of sunflower. It was observed that exposure to drought stress resulted in significant decrease in head diameter (25%), grain yield (15%), and 1000-achenes weight (10%) of sunflower (Fig. 2). However, foliar spray of K and Ct markedly increased the yield attributes. The maximum value (15.19 cm) was recorded in plants foliar applied with combined K + Ct, whereas the minimum value (9.92 cm) was noted in plants that received water spray (control). We found that exposure to drought stress at flowering stage resulted in 20% decrease in number of achenes of sunflower. Foliar spray of K and Ct significantly affected the grain yield compared to water spray. However, no significant difference was noted between K and Ct treatments. The maximum number of achenes (1448) was recorded in plants foliar applied with K + Ct, whereas the minimum value (848) was noted in plants that received Ct spray.

A significant decrease in N, P and K content was also observed in water stressed sunflower plants. Drought stress considerably decreased the N (20%), P (18%), and K (30%) content in the achenes of sunflower. Foliar spray of K and Ct was found effective to alleviate the negative effects on achenes N, P and K content in flower. K and Ct improved N (15%), P (16%) and K (20%) content in achenes under drought stress conditions (Fig. 3).

The limited water supply markedly affected the achene oil content of sunflower. Exposure to drought stress markedly reduced (40%) the oil content of sunflower. Foliar spray of K and Ct significantly affected the grain oil content compared to water spray. The maximum grain oil content (24.75%) was noted in plants foliar applied with K and Ct in combination, whereas the minimum value (12.36 %) was recorded in plants that received water spray (Fig. 4). The maximum linoleic acid (44.60 %) was recorded in plant foliar applied with K and Ct in combination, whereas the minimum value (29.45%) was recorded in plants that received water spray (Fig. 4).

Analysis of variance for the data regarding oleic acid showed that drought stress significantly influences the oleic acid % of sunflower. It was observed that exposure to drought stress resulted in 15 % decrease in oleic acid % of sunflower. TP
Drought stress and Foliar spray treatments

No. of achenes head⁻¹

| Treatment | No. of Achenes | Normal Irrigations | Drought Stress |
|-----------|----------------|-------------------|----------------|
| K spray   | a              | 1400              | 1000           |
| Ct spray  | ab             | 1200              | 800            |
| K + Ct spray | abc       | 1600              | 1200           |

1000-achene weight (g)

| Treatment | 1000-achene Weight | Normal Irrigations | Drought Stress |
|-----------|---------------------|-------------------|----------------|
| K spray   | a                   | 90                | 60             |
| Ct spray  | ab                  | 80                | 50             |
| K + Ct spray | a         | 100               | 70             |

Drought stress and Foliar spray treatments
Drought stress and foliar spray treatments

Grain yield (tha\(^{-1}\))

| Treatment         | Normal Irrigations | Drought stress |
|-------------------|--------------------|----------------|
| WS                | 1.2 ± 0.1          | 1.0 ± 0.1      |
| K spray           | 1.4 ± 0.1          | 1.2 ± 0.1      |
| Ct spray          | 1.3 ± 0.1          | 1.1 ± 0.1      |
| K + Ct spray      | 1.6 ± 0.1          | 1.4 ± 0.1      |

Biological yield (tha\(^{-1}\))

| Treatment         | Normal Irrigations | Drought stress |
|-------------------|--------------------|----------------|
| WS                | 2.5 ± 0.2          | 2.3 ± 0.2      |
| K spray           | 2.7 ± 0.2          | 2.5 ± 0.2      |
| Ct spray          | 2.4 ± 0.2          | 2.2 ± 0.2      |
| K + Ct spray      | 2.8 ± 0.2          | 2.6 ± 0.2      |
Fig. 2. Effect of foliar applied K and Ct on yield components (head diameter, no. of achenes, 1000-achenes weight, biological yield) of sunflower under normal and drought stress. Values are mean ± SE.
Fig. 3. Effect foliar applied K and Ct on NPK contents in grain of sunflower foliar under normal and drought stress conditions. Values are mean ± SE.
Drought stress and foliar spray treatments

- **Oil content (%):**
  - WS: a
  - K spray: a
  - Ct spray: ab
  - K + Ct spray: a
  - Normal Irrigations: 20
  - Drought stress: 15

- **Oleic acid (%):**
  - WS: abc
  - K spray: ab
  - Ct spray: ab
  - K + Ct spray: a
  - Normal Irrigations: 2.5
  - Drought stress: 1.5

Fig. 4. Effect foliar applied K and Ct on oil content, oleic acid, linoleic acid and palmitic acid in grain of sunflower under normal and drought stress conditions.
4. DISCUSSION

Water stress is a major threat to agricultural productivity (Elsalahy et al., 2020). Drought stress imparts significant losses in crop yield due to decreased photosynthetic activity under water stress conditions (Guan et al., 2009). The results suggest that imposition of drought stress at flowering stage negatively influences the yield attributes of sunflower. Similar results were reported by Hasina et al. (2011) in wheat who found that water deficiency at flowering stage results in significant loss in grain yield.

Foliar spray of K and Ct was found effective to ameliorate the drastic effects of water stress in sunflower. Fan et al. (1999) observed that foliar application of nutrients significantly increased the crop yields. K is a key element that positively influences photosynthetic machinery and protein synthesis and regulates ionic balance and enzymatic activity to improve plant growth and yield (Salami and Saadat, 2013). The fertilization of plants significantly improve their tolerance to various environmental stresses. However, K uptake and translocation under stress conditions is influenced by various factors such as soil physio-chemical characteristics, duration of stress as well as type of species (Silla and Escudero, 2006). Exogenous K supply helps to maintain ionic homeostasis and charge balance in membranes to support the changes at cellular and whole plant levels (Hussain et al. 2016). Foliar spray of K is critical in plants exposed to drought stress because water stress limits plants ability to access soil N. However, the identification of best combination of K with other nutrients is also essential to obtain optimum crop yields. Both in vivo and in vitro application of chitosan (Ct) has been reported to upregulate the accumulation of bioactive secondary metabolites (Yin et al., 2011). Foliar spray of Ct on aerial plant parts of Thymus daenensis was reported to increase dry matter accumulation and essential oil contents under drought stress conditions (Bistgani et al., 2017). Similar results were found in present study that foliar spray of Ct is an effective approach to increase yield attributes of sunflower under water deficit conditions. The increased grain yield in Ct or K applied sunflower plants under drought stress conditions might be attributed to the ability of these nutrients to stimulate drought tolerance responses such as maintenance of photosynthetic apparatus and activation of antioxidant machinery (Shabbir et al., 2016).

Dzung et al. (2011) reported that foliar spray of Ct caused 26–43% reduction in water use of pepper plants exposed to drought stress conditions. The reduced transpiration by exogenous Ct supply suggest that it may be utilized as an effective antitranspirant to reduce irrigation water consumption in agriculture.

Our results indicated that combined application of K and Ct significantly affects the yield and yield attributes as compared to other treatments. Interaction between K and other nutrients has been found effective to improve drought tolerance in plants. The reports of Hussain et al. (2016) showed that foliar spray of K in combination with N markedly enhanced the yield and nutrients uptake in sunflower under drought stress. They were of the view that K positively interacts with other nutrients to regulate photosynthesis and enzymatic activity in plants exposed to various environmental stresses. Similar results were published by Shabbir et al. (2016) in wheat who reported that combined application of K with N and P was more effective than their individual applications.

The agricultural productivity is badly affected by drought stress which reduces the uptake of water and nutrients (Fulda et al., 2011). Plant roots cannot absorbs few major nutrients from soil that are essential for plant
growth and better yield under water stress (Heidarian et al., 2011), however foliar spray of K and Ct found more effective. Results of that study indicated that N, P and K content was significantly decreased under drought stress condition as compared to normal plants. Iqbal et al. (2009) observed similar results in sunflower and reported that drought stress influences the uptake and accumulation of nutrients in grains. Limited water availability restricts transpiration rate and impairs active transport and membrane permeability, consequently leading to reduced N uptake by roots and its translocation from root to the shoots (Anjum et al., 2011). A significant increase in achene N content was noted in plants supplemented with K + Ct (1% and 0.1% respectively) providing evidence that K interacts with other molecules to regulate water status in plants exposed to drought stress. It is believed to be involved in activation of more than 60 enzymes responsible for plant growth and development under water limited environment (Hu and Schmidhalter, 2005). El-Zieny et al. (1990) suggested that K and water stress affect N uptake in different plant parts. Achene N content is correlated with K supplementation since K supply influences N metabolism by regulating nitrate reductase (NRA) activity (Hussain et al., 2016).

A significant reduction in achene P content was recorded in plants exposed to water deficit conditions. These findings are in conformity with the reports of Ashraf et al. (1998) in wheat and rice crops that water stress reduces P uptake and accumulation in crops. The low diffusion rate and mass flow of nutrient in the soil pores reduced P uptake in grain (Chapin, 1991). Improved P content in achenes of plants supplemented with K + Ct is in agreement with the findings of Sangakkara et al. (2000) who stated that foliar spray of K can be considered as an effective strategy to mitigate drought stress in plants, also reported by Hussain et al. (2013) in barley who found increased P content in plants foliar applied with K.

Contrarily, a marked increment in achene K content was recorded in water stressed plants. The highest K concentration was noted in plants treated with K + Ct under normal and stress conditions. Literature indicated negative effects of abiotic stresses on K uptake and translocations within plants (Khondakar et al., 1983; Ashraf et al., 1998). A marked increase in K content of water stressed plants has already been reported in wheat (Nawaz et al., 2015), eggplant (Kirnak et al., 2001) and broccoli (Yildirim et al., 2007). The increased K concentration might be attributed to enhanced stomatal conductance (Kant and Kafkafi, 2006). Foliar spray of nutrients is considered an environment friendly approach to increase nutrient use efficiency in plants (Fageria et al., 2009). Increased K content were also recorded in barley seeds supplemented with K (Ouda et al., 2005).

Currently, plant breeders are paying more attention to enhance sunflower oil and protein contents by identifying the new character. Oil is a major end product of sunflower, which are synthesized and deposit in the seed during pod filling (Gatto et al., 2015). Drought induced reduction in oil contents is in accordance with the reports of Bistgani et al. (2017) who found marked reduction in essential oil content of Thymus daenensis exposed to water deficit conditions. Contrarily, Simon et al. (1992) found significant increase in oil content of sweet basil plants subjected to drought stress. They were of the view that drought stress reduced leaf area but increased the surface area of oil glands resulting in increased oil content. The difference in oil content might be attributed to difference in species, severity of drought stress as well as
other growth or environmental conditions. Our results are line with Pattigrew (2008) who found that fatty acid profile is highly affected under drought stress. A significant decrease in oleic acid content was noted in sunflower under drought stress however, K + Ct application was found effective to increase oleic acid content under water deficit conditions compared to water spray. The results of our study are in conformity with the findings of Baligar et al. (2001) who observed that use of different micronutrients increase oleic acid content under drought stress. The maximum linoleiac acid was noted in plant foliar applied with K and Ct in combination, whereas minimum value (32.43 %) was recorded in plants that received water spray. The results are line with Benloch et al. (2015) who found that fatty acid profile is highly affected under drought stress. Foliar spray of K and Ct significantly affect palmitic acid content compared to water spray. However, significant difference was noted between K and Ct treatments. The maximum palmitic acid content were noted in plant foliar applied with K and Ct in combination, whereas minimum value was recorded in plants that received water spray. The results of our study are in accordance with Ramachandra et al. (2001) who found that fatty acid content of oilseed crops is affected by the water availability under drought stress.

5. CONCLUSION

In conclusion, our results showed that drought stress considerably reduces the yield of sunflower. However, the foliar spray of K + Ct was found effective to mitigate the drought induced damages in sunflower. The protective effects of K + Ct were found related to the increase in the uptake of N, P and K that markedly increased the yield and oil quality attributes of sunflower under dry conditions.

6. CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflict of interest.

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