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THIOUREA APPLICATION PROTECTS MAIZE FROM DROUGHT STRESS BY REGULATING GROWTH AND PHYSIOLOGICAL TRAITS

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ABSTRACT: Drought stress at any stage of maize causes a marked reduction in growth and yield formation. Thiourea (TU), a well-known plant growth regulator, can ameliorate the abiotic stress by regulating the crop growth and development; however, little is known regarding its role in improving drought tolerance in maize. Therefore, this study was performed to examine the effectiveness of TU in reducing the negative effects of drought stress on maize crop. The treatments were consisted of three drought levels, control (normal irrigation), skip irrigation at the vegetative stage, and skip irrigation at the reproductive stage, and four TU concentrations, water spray, 400, 800, and 1200 mg L^{-1}. The findings showed that drought stress decreased plant height, cob diameter, grain per cob, chlorophyll content, relative water content (RWC), biological and grain yield, and harvest index. Nonetheless, TU application, particularly at 800 mg L^{-1}, significantly increased plant height, cob and stem diameter, grain per cob, chlorophyll (a and b) and RWC, biological and grain yield, and harvest index. The positive influence of TU application on the growth, yield and yield parameters was remained as 800 mg L^{-1} > 400 mg L^{-1} > 1200 mg L^{-1} > water spray (Figure 4d). In conclusion, TU application at 800 mg L^{-1} is recommended to mitigate effects of drought stress in maize to get the satisfactory production.

Keyword: Chlorophyll content, Drought stress, Maize, Thiourea application, Yield.

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INTRODUCTION

Maize (*Zea mays* L.) is cultivated under a diverse range of climatic and soil conditions for fodder, grain and bio-energy purposes all over the world (Ranum *et al.,* 2014; Zamir *et al.,* 2020; Dustgeer *et al.,* 2021). In 2019, maize was grown over an area of 183,507 thousand hectares which production of 354 million tonnes (FAO, 2020). In Pakistan, maize is the 3rd most imperative crop after wheat and rice that is cultivated on 1,318 thousand hectares with a production of 6.309 million tonnes (Govt. of Pakistan, 2019). However, under field conditions, various factors, including improper tillage practices and planting time, poor nutrient management, and the occurrence of abiotic stresses (Hassan *et al.,* 2020a; Hassan *et al.,* 2020b) reduced the maize growth, development, and economic yield (Liu *et al.,* 2016; Maqsood *et al.,* 2017).

Under changing climatic conditions, drought stress poses a devastating threat to global crop's productivity (Kogan *et al.,* 2019; Rasheed *et al.,* 2020), by affecting the plant's growth, and development (Tardieu e*t al.,* 2017; Bartlett *et al.,* 2019). Moreover, severe drought stress reduces the photosynthetic process by decreasing the activity of Ribulose-1,5-bisphosphate carboxylase-oxygenase (RUBISCO) enzyme and intake of CO_2 (Tezara *et al.,* 1999; Bota *et al.,* 2004). Furthermore, drought stress also reduced chlorophyll content (Rahbarian *et al.,* 2011), impaired cell elongation and division (Hussain *et al.,* 2008; 2009), reduced photosynthesis rate and relative water contents (Farooq *et al.,* 2009), and disturbed the balance between antioxidants and reactive oxygen species (ROS) (Ashraf, 2010). Drought stress leads to excessive production of ROS, which damages proteins, cell membranes, and nucleic acids (Sharma 2012; Kaur and Asthir, 2017). Drought stress also reported as a main yield-limiting factor for maize crop, and its occurrence during the grain filling stage reduced the grain yield by 79-81% (Monneveux *et al.,* 2005).

In recent years, various techniques have been proposed to reduce the adverse effects of drought, namely conservation tillage practices, cultivation of drought-tolerant varieties, and the use of osmo-protectants (synthetic compounds). The use of osmo-protectants is considered to be an easy-to-use and low cost method to mitigate the adverse effects different abiotic stresses

(Aamer *et al.,* 2018; Hassan *et al.,* 2019; Hassan *et al.,* 2020b). Thiourea is a nitrogen and sulfur-containing compound, which has been specifically proven to improve crop growth and productivity (Perween *et al.,* 2016; Wahid *et al.,* 2017; Zain *et al.,* 2017). It is reported that exogenously applied TU improved plant defense system and helped in translocation of photosynthates into phloem to enhance tolerance to abiotic stresses, particularly in pulses, oilseed and cereal crops (Srivastava *et al.,* 2009; Bhunia *et al.,* 2015; Singh and Singh, 2017). Also, foliar applied TU (10 mM) at critical growth stages of wheat effectively enhanced water productivity by maintaining higher leaf relative water content and modulating the stomatal opening under stress conditions (Pasala, 2017). Foliar application of TU (1,000 mg L^{-1}), at the vegetative stage, significantly lowered the lipid peroxidation and enhanced quantum efficiency of photosystem-II under drought stress (Vineeth *et al.,* 2016). At pre-flowering stage, the application of TU through seed priming and foliar treatments enhanced seed yield up to 24% (Mathur *et al.,* 2006). Thiourea amendments also up regulates the expression of different genes (thiourea responsive genes), which mitigates the oxidative stress through hormonal regulation (Srivastava *et al.,* 2009). Various studies reported the effectiveness of TU under abiotic stresses; however, little is known regarding its application under drought stress for maize crop. In this work, it was hypothesized that TU application effectively mitigates the adverse effects of drought stress on maize crop through maintaining its growth and productivity. For this experiment, our specific objectives were: (i) to examine the effects of TU application on maize growth, production and physiological attributes under drought stress, and (ii) to find out the suitable concentration of TU under drought stress.

MATERIAL AND METHODS

Experimental site: This field study was performed at Agronomic Research Area, University of Agriculture Faisalabad (UAF), Pakistan, during the autumn season 2018. The experimental soil was sandy loam with 48.5% sand, 31.8% silt, 21% clay, and contained organic matter (O.M) 0.70%, pH 7.9, electrical conductivity (EC) 1.85 dS m^{-1}, total nitrogen (N) 0.05%, available phosphorus (P) content 5.6 ppm, and exchangeable potassium (K) content 115 ppm. The soil physico-chemical properties and nutrient status were analyzed following the method of Homer and Pratt (1961). The climatic data during the experimentation was recorded at metrological observatory cell, which is located near the experimental site, and presented in Figure 1. Total rainfall during the growth period of autumn maize (from August to November) was 106 mm, of which maximum (81 mm) was concentrated during the first half of September. The average maximum (Tmax) and minimum (Tmin) temperature were 31.5°C and 18.5°C respectively. The average humidity throughout growing period was 73.0.

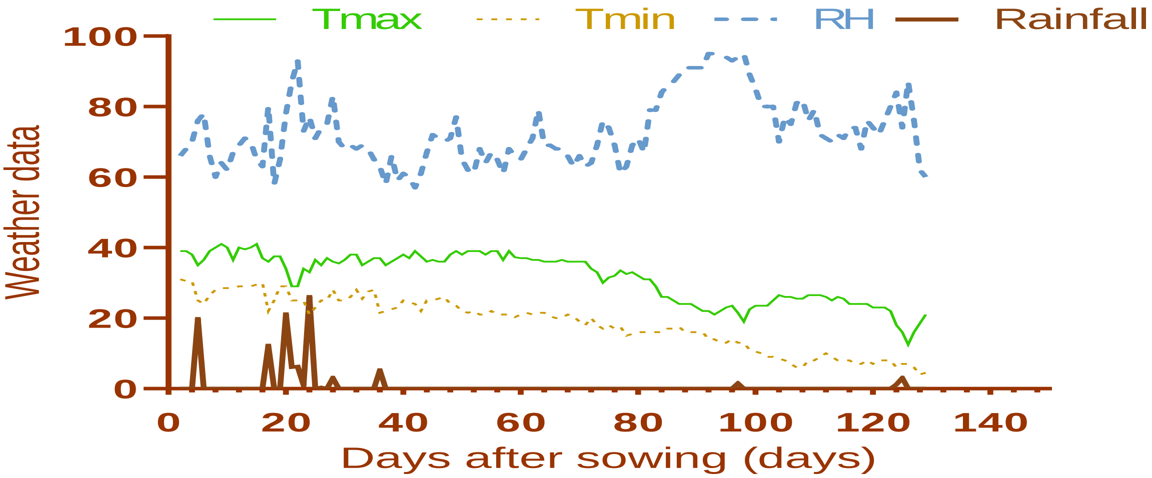

Figure 1: The weather conditions during crop growth period (0-140 days after sowing).

Crop husbandry: Seeds of maize hybrid Syngenta-6654 were procured from Syngenta Pakistan Limited, Faisalabad. The seeds were sown on August 11, 2018 with seed rate of 25 kg ha^{-1} into prepared soil (two harrowing along with planking) by maintaining the row space of 75 cm. Two seeds were sown on top of every hill by maintaining plant × plant distance of 20 cm and thinning was done ten days after sowing to maintain the equal number of plants. Seeds were treated with fungicide (Benlate @ 2 g kg^{-1} seed) to avoid the soil-borne diseases and to control the attack of insect pests. Based on soil analysis, the recommended dose of fertilizers NPK were applied at rate of 200, 165, 125 kg ha$^{-1,}$ using urea (N 46%), di-ammonium phosphate (P

46%, N 18%) and sulphate of potash (K_2O 50%). The full dosage of P and K and one half of N were applied at sowing and rest of N was applied after 20 and 45 days after sowing.

Experiment design and treatments: The study was executed in RCBD with split-plot arrangement and each treatment was replicated thrice. In this experiment, treatments were consisted on two factors, drought treatments and thiourea application. Drought was imposed by skip irrigation technique, and the treatments were as; (i) control (no skip-irrigation), ii) skip irrigation at vegetative stage, and iii) skip irrigation at reproductive stage. Thiourea was applied as foliar spray at silking (35 DAS) and grain filling (60 DAS) stages with following treatments; i) watery spray, ii) thiourea application at 400 mg L^{-1}, iii) thiourea application at 800 mg L^{-1}, and iv) thiourea application at 1200 mg L^{-1}. In control eight irrigations were applied whereas 7 irrigations were applied in skip irrigation at vegetative and reproductive stages.

Observations

Chlorophyll contents: The chlorophyll (chl) contents (chl. *a* and *b*) were determined by following the method of Peng and Liu (1992), with some modification. Firstly, 0.5 g fresh leaves was crushed in 10 ml of acetone (80%), and placed at 20°C for overnight. Next, after filtration (Whatman # 1 filter paper used), the absorbance was recorded at 645 and 663 nm wavelengths by using the spectrophotometer. A blank with 80% acetone was also run. Chlorophyll content was recorded twice at vegetative and reproductive growth stages with imposition of drought stress and after thio-urea application.

Leaf water contents: Relative leaf water content (RWC) was measured by using the protocol of Barr and Weatherley (1962). RWC was recorded twice at vegetative and reproductive growth stages with imposition of drought stress and after thio-urea application. For this purpose, 0.5 g fresh leaves were washed with water, and fresh weights were determined. Next, these samples were soaked in water for 24 h to determine the turgid weight. After oven drying at 80°C, the dry weights were recorded and RWC was calculated by following equation:

$$\text{LWC (\%)} = [(\text{fresh weight - dry weight}) / (\text{turgid weight – dry weight})] \times 100$$

Cell membrane permeability: The protocol described by Blum and Ebercon (1980) was used to determine the membrane stability, in terms of electrolyte leakage, both at the vegetative and reproductive stage. Cell membrane permeability was recorded twice at vegetative and reproductive growth stages with imposition of drought stress and after thio-urea application. Firstly, 0.3 g leaves were cut into small size pieces and placed in 30 mL distilled water for recording the initial electrical conductivity (EC) values (EC1). Next, the samples were transferred into a water bath for 2-hours at 90°C for recoding second EC value (EC2). The cell membrane permeability was determined by following formula:

$$EC = EC1 / EC2 \times 100$$

Growth and yield traits: Ten plants from each plot were randomly selected, and plant height was measured from base to top with a meter rod. The cob diameter, cob length, grains per cob was counted from ten randomly selected cobs. 1000-grain weight was calculated by using digital weighing balance, adjusting the moisture contents at 14%. At maturity stage, complete plots were harvested to determine the biological yield and grain yield. Harvest index (HI) was calculated as a ratio of grain yield to straw yield.

Statistical analysis: The data on collected traits were analyzed by Fisher's analysis of variance technique. Different letters were used to portray the significant difference among treatments by LSD at 95% of confidence interval (Steel *et al.*, 1997).

RESULTS

Plant height, Cob and stem diameter, and grains per cob: Results showed that drought stress significantly affected the growth parameters of autumn maize. Nonetheless, TU application significantly ameliorates the adverse effect of drought. There was a non-significant difference for plant height under no skip irrigation (control) and irrigation skipped at vegetative stages, however, skip irrigation at the reproductive stage led to a significant reduction in plant height. Foliar applied TU (400, 800 and 1200 mg L-1) caused a significant increment in plant height under drought stress. Thio-urea application (800 mg L-1) increased the plant height by 13.63% under control conditions compared no TU application (Figure 2a). In addition, the cob and stem diameters were significantly affected by drought treatments (Figure 2b,c). The plants under drought stress showed thinner stem and reduced cob diameters as compared normal (non-stressed) plants (Figure 2b,c). Moreover, TU application caused a significant improvement in stem and cob diameters, with maximum values under TU application at 800 mg L^{-1}, compared with water spray. Drought stress also had a significant impact on grains per cob (Figure 2d). Nevertheless, TU application significantly increased the number of grains cob^{-1}, with maximum increment under TU application at 800 mg L^{-1} (Figure 2d).

Chlorophyll contents, electric conductivity, and relative water contents: Chlorophyll content were significantly reduced under drought (Figure 3a,b). However, the foliar applied TU significantly increased the chlorophyll content under control as well as drought stress condition. The maximum chlorophyll content were recorded for TU application at 800 mg L^{-1}, followed by TU application at 400 and 1200 mg L^{-1} and water spray (Figure 3a,b). The electric conductivity showed a opposite trend to chlorophyll content (Figure 3c) where drought stress (skip irrigation at vegetative and reproductive stage) caused a marked increase in electric conductivity as compared with control plants. However, foliar applied TU significantly reduced the electric conductivity under control and drought stress condition, with decrease under TU application at 800 mg L^{-1} (Figure 3c). In addition, the relative water contents were significantly decreased under drought treatments (skip irrigation at vegetative and reproductive stage). However, foliar applied TU exhibited a marked increment in relative water content under drought stress (Figure 3d). The positive influence of TU application on relative water content was as; 800 mg L^{-1} > 400 mg L^{-1} > 1200 mg L^{-1} > water spray (Figure 3d).

Yield attributes: Drought stress, TU application and their interaction had significant impact on the yield attributes. Drought stress treatments (skip irrigation at vegetative and reproductive stage) caused a marked reduction in 1000-grain weight, biological yield, grain yield, and harvest index. However, foliar applied TU (400, 800 and 1200 mg L-1) significantly increased these traits in autumn maize. The increase in 1000-grain weight was ranged between 11-12%, depending on TU amount. Although, 1000-grain weight increased significantly under TU application, however, TU at 800 mg L-1 had maximum 1000-grain weight as compared to other concentrations (Figure 4a). The application of TU also increased the biological and grain yield under drought stress. The highest biological and grain yields were obtained for TU application at 800 mg L^{-1} (Figure 4c). The results also revealed that harvest index (%) was affected significantly by drought treatments and TU levels (Figure 4d). Under drought stress, the foliar application of TU significantly enhanced the HI, and the positive influence of TU application on HI was as; 800 mg L^{-1} > 400 mg L^{-1} > 1200 mg L^{-1} > water spray (Figure 4d).

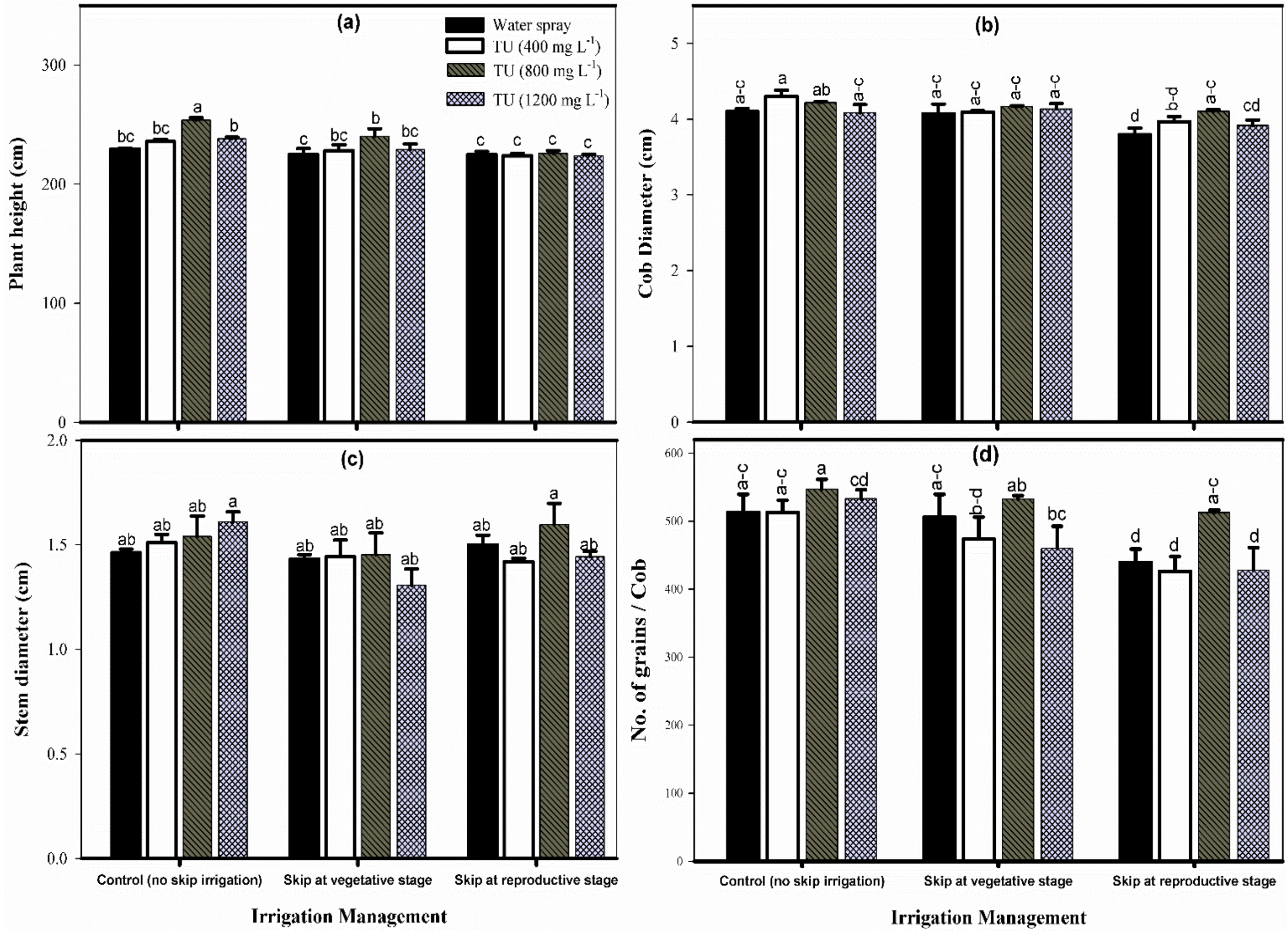

Figure 1: The influence of drought stress and thiourea (TU) application on (a) plant height, (b) cob diameter, (c) stem diameter and (d) number of grains per cob (No. of grains/cob) in maize. Data represents means ± SE (n=3). Different small letters on capped bar indicate the significant difference.

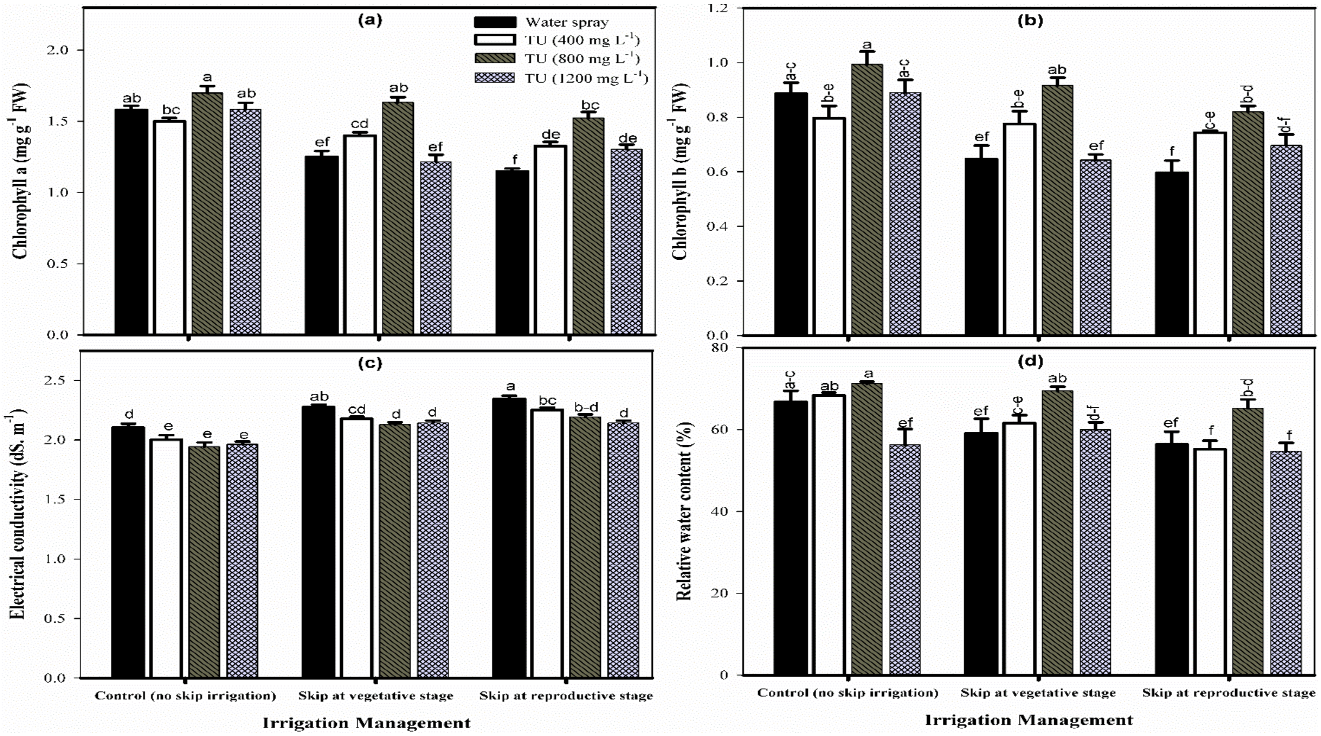

Figure 2: The influence of drought stress and thiourea (TU) application on (a) chlorophyll a, (b) chlorophyll b, (c) electrical conductivity and (d) relative water content in maize. Data represents means ± SE (n = 3). Different small letters on capped bar indicate the significant difference.

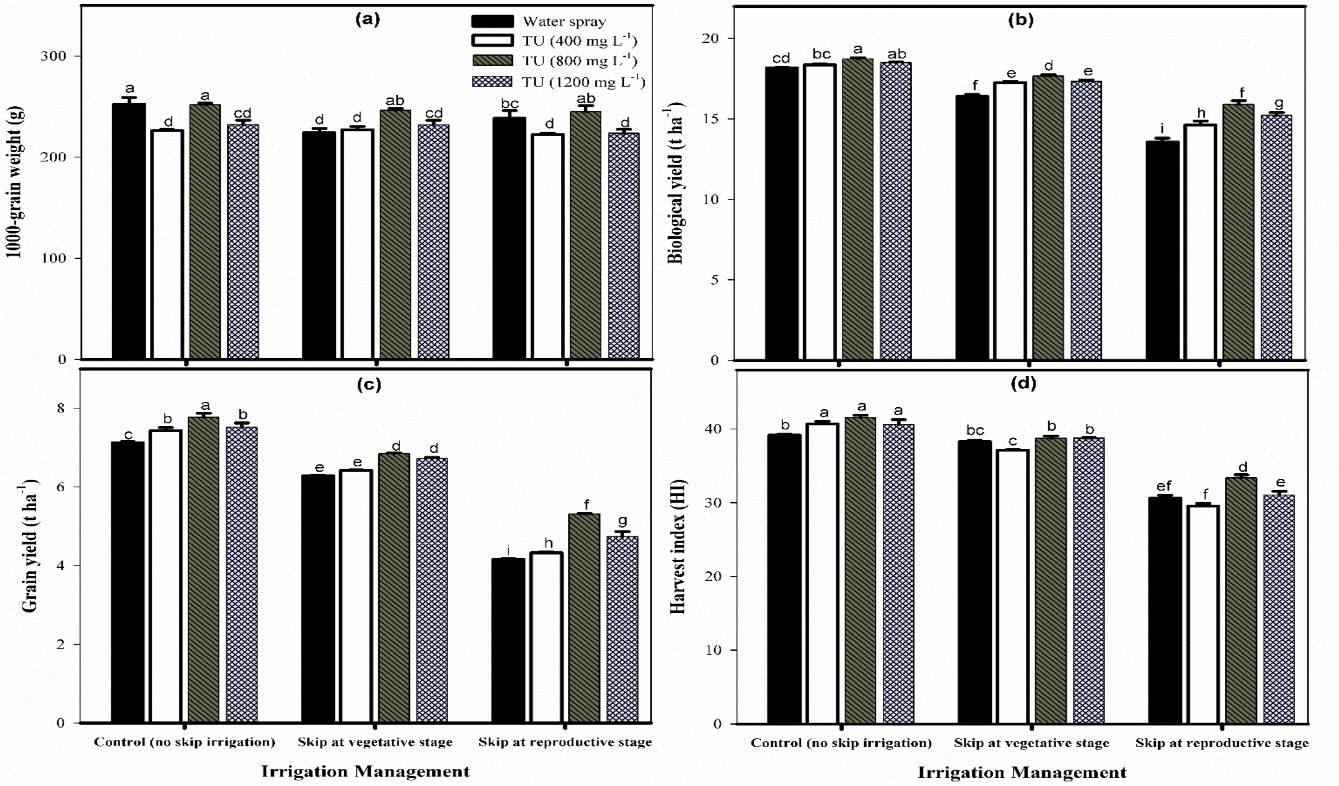

Figure 3: The influence of drought stress and thiourea (TU) application on (a) 1000-grain weight, (b) biological yield, (c) grain yield and (d) harvest index (HI) in maize. Data represents means ± SE (n = 3). Different small letters on capped bar indicate the significant difference.

DISCUSSION

Under field condition, plants are exposed to different abiotic stresses that limit their growth, development, and productivity. The plant growth and development are usually improved due to the improvement of plant resistance to the environmental stresses, including drought stress (Bartlett *et al.*, 2019), and there is a strong evidence that TU plays an essential role in plant growth, development and yield formation (Pasala, 2017; Zain *et al.*, 2017; Naz and Perveen, 2021). The shortage of water in rhizosphere inhibits early plant growth, disturbs chlorophyll biosynthesis, photosynthetic rates, and leaves relative water content (Dias *et al.*, 2018) and resultantly decreases crop productivity (Chattha *et al.*, 2017; Hassan *et al.*, 2017; Hassan *et al.*, 2020a). Drought stress limits photosynthesis and imposes oxidative stress to plants (Hussain *et al.*, 2019). Plants have evolved several mechanisms, including adjustment of water balance in cell, and reduction in light interception, to cope with drought stress. However, the ability of the plants to withstand drought stress varied with severity of drought (Hussain *et al.*, 2019), plant genotypes (Saud *et al.*, 2016), and the stage of crop at which drought occur (Anjum *et al.*, 2016). In recent studies, crop growth and physiological traits have been widely used to estimate the drought tolerance in crops (Anjum *et al.*, 2016; Singh and Singh, 2017). In this study, we observed a marked reduction in plant height, stem and cob diameter under drought conditions (skip irrigation at vegetative and reproductive stage), however, TU application attained the significantly higher plant height even under drought stress. Moreover, the reduction in number of grains cob^{-1} was also reported under drought conditions however, we observed that TU application markedly increased the number of grains cob^{-1} under drought conditions. It suggests that TU application reduced the adverse effects of drought stress by maintaining growth parameters (Shanu *et al.*, 2013). In response to drought stress, TU improves the plant morphology and growth pattern, and thereby enhanced the drought tolerance in crops (Singh and Singh, 2017). Therefore, our findings confirmed that TU application triggers the drought stress by improving the growth parameters.

Photosynthesis pigments, including chlorophyll, are important for photon harvest (Caffarri *et al.*, 2014). Abiotic stresses reduced the chlorophyll content (Zafar *et al.* 2017; Abdel-Latef *et al.*, 2019), thereby caused a marked reduction in photosynthetic rates in crop plants. In this work, our results had showed that drought stress significantly reduced the chlorophyll content however, TU application led to a considerable increase under drought stress. Thiourea application induced an increase in photosynthesis efficiency, which is highly associated with the up-regulation of Rubisco large subunit genes that protect the plant photosynthesis factory under water deficit conditions (Vineeth *et al.*, 2016). Also, drought stress had a significant effect on cell membrane permeability. These findings are consistent with Mozdzen *et al.* (2021) where the authors reported higher electrolyte leakage in maize under drought stress. The increase in electrolyte leakage under drought stress occurs mainly due to the membrane damage under drought induced oxidative stress (Assaha *et al.*, 2016). Moreover, TU application showed a significant increase in membrane stability, and resultantly decreased the electrolyte leakage under drought stress. Similar findings were also reported by Kaya *et al.*, (2019), where TU application decreased the electrolyte leakage under stress conditions. Leaf relative water content (RWC), widely used as an index of tissue water status was significantly decreased under water stress conditions (Dias *et al.*, 2018; Waqas *et al.*, 2019). TU application significantly improved the RWC and enhanced crop water use under drought stress conditions (Pasala, 2017).

Furthermore, drought stress decreased the 1000-grain weight, grain and biological yield, and HI. The reduction in grain yield was due to reduction in plant height and number of grains per cob (Figure 1a, d). However, TU application significantly increased the 1000-grain weight, yield, and HI. Similar to our findings, Zain *et al.* (2017) demonstrated that TU application improved the seed yield and yield traits by approving the photosynthesis efficiency of plants under drought conditions. Similarly, Pandey *et al.* (2013) reported that under drought stress, increased seed yield under TU application was mainly attributed to the higher photosynthetic rates.

Conclusion: Drought stress caused adverse effects on the growth, physiological- and yield-traits in autumn maize. Nevertheless, TU application effectively ameliorated the negative effects of drought stress, mainly through improvement in growth, chlorophyll and RWC, yield and yield attributes. Moreover, TU application at 800 mg L^{-1} resulted in maximum increase in growth, and physiological traits and yield of maize. In sum, TU application at 800 mg L^{-1} is recommended to mitigate adverse effects of drought to obtain satisfactory productivity.

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