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Research Article

Growth and dry matter production of hybrid maize through exogenous application of chitosan and thiourea under drought stress

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Abstract

Exogenous application of chitosan and thiourea could mitigate the water stress in maize plants due to their positive effects on maize growth and physiological processes. Therefore, application of chitosan and thiourea were studied in maize hybrids grown in plastic pots under water stress conditions during April, 2017. Maize hybrids (CML537/CML312 and Fakher-e-NARC) were sown in pots under 30 % pot soil moisture potential under greenhouse conditions. Three levels of Chitosan (125 mg L^{-1}, 250 mg L^{-1} and 500 mg L^{-1}) and thiourea (500 mg L^{-1}, 750 mg $L^{-1}$and 1000 mg L^{-1}) were exogenously applied at 20th, 30th and 40th days after germination of maize plants. Completely randomized design two factors factorial was used having three replication. Plant growth parameters i.e. shoot length, root length, shoot dry weight and root dry weight were significantly affected by bio- regulators. However significant interaction between maize hybrids and treatments were noted. Application of thiourea @1000 mg L^{-1} and hybrid CML537/CML312 significantly improved growth and dry matter production. Hence, it is recommended for better performance for growth and dry matter production of Hybrid maize.

Keywords: Chitosan; Maize; Thiourea; Water stress

Introduction

Maize (*Zea mays* L.) is the 3rd important cereal crop of Pakistan belongs to family Poaceae. Maize was cultivated in country on the area of 1191 thousands hectares with the production of 6.13 million tons during 2016-17 [1].

Water stress may occurred by change in temperature and rain-fall pattern which highly decreased in crops productivity [2]. Therefore observable agricultural losses happened due to drought sensitive crops failed to grown under such conditions [3].

Water stress at germination and early growth stages highly associated with overall success of crop, if stress indicates at first growth stages its effect on total production of the crop as a result. Therefore, efforts are required to understand how plants respond and adapt to water stress. The reduction in roots and plant growth is the earliest response of water stress. Whereas the reduction in transpiration of plants help in water conservation. [4].

Drought events may be counteracted through the use of anti-transparent [5]. These compounds are applied to foliage to limit the water loss they include both film-forming and stomata closing compounds, able to increase the leaf resistance to water vapor loss thus improving plant water use to assimilate carbon and in turn, the production of biomass or yield [6]. Whereas these compounds limiting the water loss deputed to evaporated leaf cooling [7].

Chitosan, a polysaccharide which is gaining by the deacetylation of chitin. Sources of chitin are shrimps, crabs and outer most layer crustaceans [8]. The most commonly used elicitors are yeast extracts, fungal carbohydrates and chitosan. It is second most abundant polysaccharide and very important source of cell wall of many fungi, insects and algae. Chitosan was first categorized as an elicitor in plant activating genes that underlie the biosynthesis pathway of secondary metabolites [9].

Chitosan is the anti-transparent compound has previously proved to be effective in pepper, chitosan is the natural come by deacetylation of chitin having less toxicity, biodegradable and no bad effects for the environment with many applications in agriculture. Chitosan was used in seed priming and also as bio fertilizers to increase crops production. In the crop cultivation, chitosan foliar application minimizes transpiration noted in pepper plant, which reduced water use up to 26-43% [6].

Application of the chitosan showed stimulated effect on plant growth attributed to increase in the availability and uptake of water. It also improve essential nutrients through adjusting cell osmotic pressure, and reducing the accumulation of harmful free radicals by increasing antioxidants and enzyme activities. So, different concentrations of chitosan enhancing shoot and root length, fresh and dry weights of shoots, roots and leaves area as well as the level of chlorophyll in leaves [10].

According to International Union of Pure and Applied Chemistry (IUPAC), Thio carbamide is the alternate name of Thiourea. With a molecular weight of 76.12 g/mol, thiourea molecule is made of four elements nitrogen (36.81%), carbon (15.77%), hydrogen (5.31%), and sulfur (42.11%). Oxygen in the urea molecule is replaced with sulfur to make thiourea molecule. This replacement makes the properties of thiourea different from urea. It is soluble in water with a solubility of 142 g/L at 25^{o}C. Two main functional groups in thiourea are thiol (–SH) and amino, which make it a physiologically more important molecule [12]. The thiol group represent the stabilization of chemistry of group proteins. The amino (–NH2) group acts as a base because it has a lone pair of electrons. It gives specific properties to the compounds possessing it [11].

Growth regulators play the major role in the development and crop production. With the application of thiourea the crops of arid and semi-arid regions improved the crop growth and development [12]. The sulphahydral compound thiourea enhance the productivity of pulses by the amelioration of drought is well established, seed soaking foliar application increasing the yield of legumes under arid regions. In different stages virial concentrations of thiourea increased the crop growth and quality [13].

Materials and methods

The present research study was conducted during spring season of 2017 at Agricultural Research Farm University of Haripur, KPK. Research farm is situated in district Haripur with (34.00^0) North and longitude (72.93^0) East and it is situated at elevation 550 meters (1804 ft.) above the sea level. Rain fall and temperature data were collected and are summarized in (Table 1).

Experimentation

The experiment was laid in completely randomized design two factors factorial arrangement having three replications. The experiment was conducted under glass house in pots (height 22.5cm, base diameter 24cm and top diameter 28cm), Each pot contains 7.5 kg of soil, and supplied the nitrogen @ 6 g pot^{-1} by the source of urea, P_2O_5 @ 4.5g by source SSP and K_2O 3g pot^{-1} by source of potassium sulfate as recommended dose for maize NPK 120-90-60, respectively as recommended dose for maize crop. Two maize hybrids were used i.e. Fakher e NARC and CML 537/CML312. Five seeds were sown per pot, after germination seedlings was thinned to three plants in each pot, plants were allowed to grow for further 10 days so as to get them established well then placed in a glasshouse for giving further 50 days. Exogenous treatments of bio-regulators (chitosan and thiourea) were applied as a foliar treatments at 20, 30 and 40 days of germination. All pots were subjected to water stress treatments 30% pot soil moisture stress. Pots were weighed every two days, the loss in pots weight represents transpiration and evaporation. Cumulative water loss was added to each pot to compensate transpiration and evaporation. Accumulated water loss was calculated as the differences in pots weights between successive weights. Stress was gave from 10 days after germination of seedlings till uprooting plants. Chitosan and thiourea at 125, 250,500 mg L^{-1}, and 500, 750, 1000 mg L^{-1} were applied until dripping (until water drops dripped from leaves surface) using small pressure pump. After 60 days plants samples were uprooted and taken the data of shoot length (cm) with the help of measuring tape, root length (cm) with the help of measuring tape, shoot fresh weight (g) with the help of digital balance and root fresh weight (g) with the help of digital balance. The data were subjected to analysis of variance (ANOVA) using software of statistix 8.1. The differences among means were computed using Tukey's HSD test at 1 % probability level. Replicated mean values were used for computing standard error of mean using MS-excel Program.

Table 1. Average monthly metrological data during crop growth season

Month	Temperature (°C)			Relative humidity (%)	Rainfall (mm)
	Maximum	Minimum	Avg.		
March	23	10	16.5	35	124
April	28.0	14.0	29.9	36.80	104.0
May	34.0	18.0	33.0	31.44	74.0
June	39.0	24.0	23.5	50.55	80.0

Results and discussion

Shoot length (cm)

Shoot length were significantly affected by maize hybrids, treatments and its interaction under water stress condition (Table 2). Tall stature shoots (115.2 cm) were recorded in hybrid CML537/CML312 as compared to Fakher e NARC (107.5cm). It might be due to their genetic potential to utilized bio-regulators and nutrients properly in drought

stresses, which may be enhanced cell elongation and cell turgidity which enhanced shoot length [12].
The maximum shoot length 123 (cm) were recorded with application of thiourea @ 1000 mg L^{-1} followed by (120.2 cm) with thiourea @ 500 mg L^{-1}. However, minimum shoot length (95cm) was recorded in control (. Thiourea is basically growth regulator which might be enhanced nutrients uptake and reduces drought stress condition [17]. Exogenous application of thiourea was ready sources of nitrogen and also reduced oxidative damages in plant due to thiol and amino as functional groups which might improve plant growth and development [14].
Treatments and maize hybrids were significantly affected the shoot length under water stress condition. Treatment application from control to chitosan @ 500 mg L^{-1} with hybrid Fakher e NARC however, with application of thiourea shoot length increased from thiourea @ 500 mg L^{-1} to thiourae @ 750 mg L^{-1} while further increase shoot length decreased with Fakher e NARC. Whereas, CML537/CML312 shoot length remain constant with in control and chitosan @ 125 mg L^{-1} than increased were observed chitosan @ 250 mg L^{-1} and then shoot length decreased. While with application of thiourea increased with increasing thoiurea concentration (Figure 1). It might be due hybrid having their genetic potential to utilized more nitrogen. Thiourea increases N availability and crop uptake, metabolic processes very highly and hence increased growth and dry matter accumulation [15]. While chitosan having high nitrogen level which boost the growth by availability of nitrogen which helps in to promote protein level and reduce the stress effects [16].

Root length (cm)

Root length were significantly affected by maize hybrids and treatments as well as its interaction under water stress condition (Table 2). Maize hybrids Fakher-e-NARC (38.7 cm) performed better as compare with CML537/CML312 (34.7 cm). Significantly difference between maize hybrids could be different nature of root structure and root growth. High root canopy helps more absorption of water and reduce water stress conditions. Prolific root system is important for stress tolerance and improved water uptake particularly under abiotic stress conditions in order to harvest better crop yield. Our results are in conformity with the findings [17, 18] who also found that prolific root system is important for stress tolerance and improved water uptake particularly under abiotic stress conditions in order to harvest better crop yield.
The maximum root length (42.6 cm) were observed with application of thiourea @ 1000 mg L^{-1} followed by thiourea @ 500 mg L^{-1} (39.9 cm) however, minimum root length (28.9 cm) were recorded in control pots. Normally sensitive genotypes have poor prolific roots than susceptible genotypes. Corresponding to this, a pronounced increment in root growth of sensitive wheat varieties to abiotic stress was reported with the application of thiourea and that strongly correlate to grain yield. It's also might be thiourea being water soluble, readily absorbable in the tissues that enhance the plant, it has ability to ameliorate stress. Our results are matching with [12] who also found that a pronounced increment in root growth of sensitive wheat varieties to abiotic stress was reported with the application of thiourea and that strongly correlate to grain yield.
Root length increasing with application of treatment from control to chitosan @ 250 mg L^{-1} in hybrid CML537/CML312 and hybrid Fakher-e-NARC however, root length decreased up to chitosan @ 500 mg L^{-1} in hybrid CML537/CML312 and increase in hybrid Fakher-e-NARC than increased with thiourae @ 500 mg L^{-1} in hybrid CML537/CML312 and slightly deceased were observed in hybrid Fakher-e-NARC.

Than root length decreased under thiourea @ 750 mg L^{-1} in hybrid CML537/CML312 and then linearly increased while increased in root length were noted in hybrid Fakher-e-NARC from thiourea @ 500 mg L^{-1} to thiourea @ 1000 mg L^{-1} showed in (Fig. 2). With regards to interaction of maize hybrids and treatment of chitosan and thiourea, bio regulator chitosan and thiourea both showed higher results. It might be stimulating effect of chitosan on plant growth which could be attributed to an increase in the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure, and reducing the accumulation of harmful free radicals (ORS) by increasing antioxidants and enzyme activities [19, 20].

Shoot dry weight (g)

Shoot dry weight were significantly affected by maize hybrids and treatments as well as its interaction under water stress condition (Table 2). Maize hybrid CML537/CML312 (35.7 g) performed better than hybrid Fakher e NARC (33.9 g). Its might be due to different growing habits and absorption capacity of bio-regulators by different hybrids of maize. Our results are in line with [14].

Exogenous application of chitosan and thiourea under drought stress also significantly effect on shoot dry weight of maize hybrids. Application of thiourea @1000 mg L^{-1} produced maximum shoot dry weight (35.7 g) as near to thiourea @ 750 mg L^{-1} application with the shoot dry weight of 38.3 (g) where minimum shoot dry weight was found in control (28.1 g). It may be due to imino and tiol Interaction between maize hybrids and treatments (H×T) had found significant variation for shoot dry weight mentioned in (Fig. 3). Shoot dry weight was increased in hybrid Fakher e NARC from control to chitosan @ 125 mg L^{-1}. While slightly decreased in hybrid CML537/CML312. With the application of chitosan @ 250 mg L^{-1} and chitosan @ 500 mg L^{-1} shoot dry weight increased in both hybrids. However, decrease in shoot dry weight was noted with application of thiourea @ 500 mg L^{-1} and again increase with Thiourea @ 750 mg L^{-1} and Thiourea @ 1000 mg L^{-1} treatments in both hybrids of maize. It might be due to increasing the availability of water by chitosan by improving the water uptake, essential nutrients and adjustment of cell osmotic pressure. However, it can also might be thiourea improved the photosynthetic efficiency of plant thereby overall improvement in growth and yield attributes. Similar type of findings have also been noted by [19].

Root dry weight (g)

Root dry weight significantly affected by maize hybrids and treatments as well as its interaction under water stress condition (Table 2). Maize hybrid CML537/CML312 (12.4 g) performed better than Fakher e NARC (10.8 g). The difference between roots dry weight might be due to different water absorption capacity of their roots and growing habits [6].

Significantly difference were found between treatments applied under water stress condition (Figure 4). Root dry weight with the application of thiourea @ 1000 mg L^{-1} best results were noted (15.0 g) followed by chitosan @ 500 mg L^{-1} application (12.7 g) as compared to other treatments applied. Alternatively minimum root dry weight were found in control (9.0 g). A great number of researches provide evidence regarding efficacious role of foliar applied thiourea in enhancing physiological mechanisms and improving final yield. In pearl millet, foliar application of thiourea @ 1000 mg L^{-1} at pre-flowering stage significantly enhance the crop growth and dry matter as compared to water application under control plants [21].

Root dry weight significantly increased from control to chitosan @ 500 mg L^{-1} and then decreased with thiourea @ 500 mg L^{-1} and increased with thiourea 750 mg L^{-1} and thiourea @ 1000 mg L^{-1} treatments. Fig. 4. A study showed that out of a range of thiourea used was the most effective in the induction of callus, origination of shoot and root from the hybrid maize explant (Sanaullah *et al*., 2016). Chitosan also significantly affected it might be due to reviving effect of chitosan might be distinction to enhance the availability of water uptake, cell osmotic pressure and essential nutrients. Our results match with [22]. Group's thiourea provided more nitrogen and reduce oxidative damage [14].

Table 2. Shoot length, root length, shoot dry weight and root dry weight as affected by bio-regulators and maize hybrids

Bio-regulators	**Shoot length (cm)**	**Root length (cm)**	**Shoot dry weight (gram)**	**Root dry weight (gram)**
Control	95.0±4.20 c	28.9±0.87 e	28.1±1.17 d	9.2±0.20 d
Chitosan @ 125 mg/L^{-1}	99.1±3.61 c	33.8±1.04 d	30.9±0.71 c	11.3±0.43 c
Chitosan @ 250 mg/L^{-1}	113.9±5.38ab	34.6±0.99 cd	36.5±1.55 b	12.3±0.47 b
Chitosan @ 500 mg/L^{-1}	117.6±4.50ab	38.3±3.54 b	37.1±1.20 b	12.7±0.55 b
Thiourea @ 500 mg/L^{-1}	110.8±3.57 b	38.8±1.52 bc	32.3±1.50 c	9.7±0.26 d
Thiourea @ 750 mg/L^{-1}	120.2±5.91ab	39.9±1.33 b	38.3±0.70 ab	11.2±0.49 c
Thiourea @ 1000 mg/L^{-1}	123.0±6.29 a	42.6±1.57 a	40.4±0.41 a	15.0±0.73 a
LSD (0.05)	9.90	3.3	2.92	1.32
Hybrids				
Fakher e NARC	107.5±2.75 b	38.7±1.41 a	33.9±1.02 b	10.8±0.35 b
CML537/CML312	115.2±3.54 a	34.7±1.11 b	35.7±1.09 a	12.4±0.51 a
Significance	0.0048	0.0001	0.0035	0.0000
Interactions				
T x H	**Figure 1**	**Figure 2**	**Figure 3**	**Figure 4**

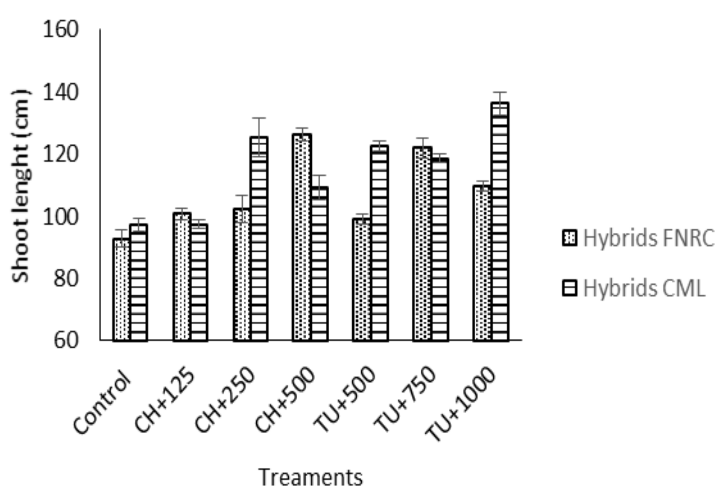

Figure 1. Shoot length (cm) as affected by interaction of bio-regulators and hybrids under water stress condition

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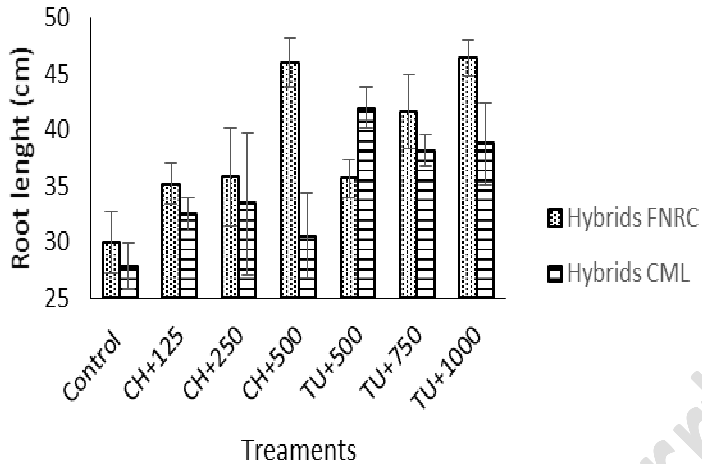

Figure 2. Root length (cm) as affected by interaction of bio-regulators and hybrids under water stress condition

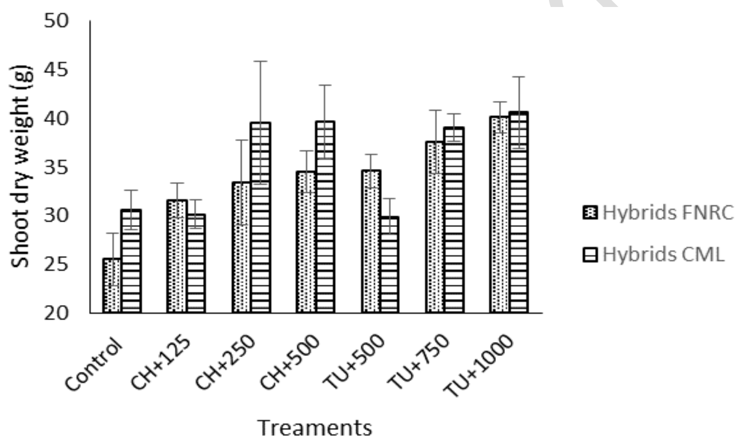

Figure 3. Shoot dry weight 0.5 (gram/leaf) as affected by interaction of bio-regulators and hybrids under water stress condition

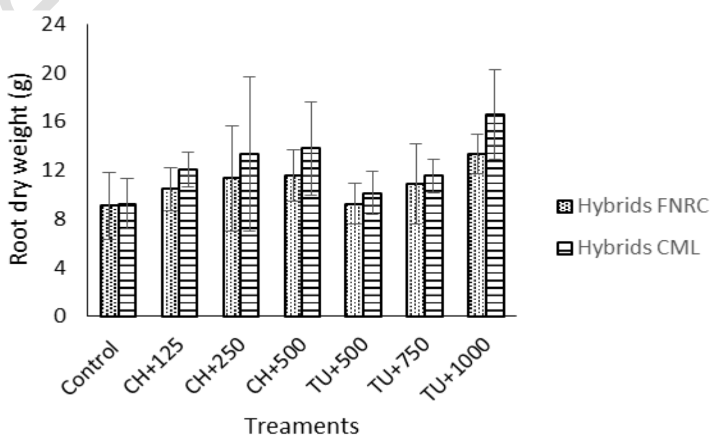

Figure 4. Root dry weight 0.5 (gram/leaf) as affected by interaction of bio-regulators and hybrids under water stress condition

Conclusion

Application of thiourea @1000 mg L^{-1} and hybrid CML537/CML312 significantly improved growth and dry matter production. Hence, it is recommended for better performance for growth and dry matter production of Hybrid maize.

Authors' contributions

Conceived and designed the experiments: A Shakoor, A Khan & SU Khan, Performed the experiments: A Shakoor & S.N Malik, Analyzed the data: K Azeem & F Naz, Wrote the paper: A Shakoor, K Naveed, SM Khan & M Saeed.

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