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Full Length Article

Assessment of Bio-regulators to Improve the Performance of Maize Hybrids under Spring Conditions

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Abstract

Two maize hybrids (YH-1898 and 33M-15) contrasting in heat tolerance were evaluated for their performance under spring conditions and different levels of bio-regulators i.e. thiourea (7.5 m*M* and10 m*M*) and salicylic acid (0.10 m*M* and 0.20 m*M*) were applied. The responses were assessed in terms of growth, yield and some physiological attributes for two growing seasons. Results showed higher growth rate and yield of heat tolerant maize hybrid (YH-1898) than sensitive one (33 M-15) and exogenous applied bio-regulators significantly improved the growth attributes like leaf area index (LAI), leaf area duration (LAD), crop growth rate (CGR) and net assimilation rate (NAR) of these hybrids. Application of thiourea (10 m*M*) and salicylic acid (0.20 m*M*) reduced chlorophyll loss and increased cell membrane thermostability which significantly improved grain yield attributes including number of grains per cob, thousand grain weight and grain quality traits including grain protein and starch contents. Economically, highest net income and benefit cost ratio were calculated for thiourea @ 10 m*M* during both years. The present study findings suggest that the performance of spring planted maize hybrids particularly heat tolerant could be improved by exogenous application of bio-regulators particularly thiourea @ 10 m*M*. © 2018 Friends Science Publishers

Keywords: Cell membrane thermostability; Chlorophyll loss; Grain quality; Grain yield; High temperature; Maize growth

Introduction

In Pakistan maize is third most important cereal crop after wheat and rice, contributing 2.1% to value addition in agriculture and 0.5% to GDP (Govt. of Pakistan, 2015). The average maize yield of Pakistan (4.072 t ha^{-1}) is less than many other agricultural countries like U.S.A., China, Brazil, Mexico, India, Argentina, South Africa, Ukraine and Canada (Ihnen, 2010). Many factors count for low yield like low soil organic matter, water stress, lack of balanced nutrition, insects, weeds and temperature stress. In Pakistan, maize is grown in two seasons i.e. spring and autumn. Although, the yield of spring maize is higher than autumn but subjected to high temperature at reproductive stages (Afzal *et al*., 2008). High temperature has turned out to be the most considerable abiotic stress inhibiting the growth and altering physiological processes in plants (Noohi *et al*., 2009). According to Intergovernmental Panel on Climatic Change (IPCC) globally mean temperature will rise from 0.3°C to 0.7°C leading to global warming (IPCC, 2014). Diverse and often adverse changes in plant physiology, growth, development, and yield are associated with heat stress (Hasanuzzaman *et al*., 2012). Naveed *et al*. (2014) reported that for maize plant optimum day and night temperature ranges from 22 to 32°C and 16.7 to 23.3°C, respectively. But, under the climatic conditions of Pakistan, the temperature during reproductive stages of spring maize (May and June) is more than 35°C which adversely reduces pollination, grain setting and grain weight (Mitchell and Petolino, 1988; Cicchino *et al*., 2010). Early spring cultivation exposes the maize crop to low temperature (Afzal *et al*., 2008), while late sowing faces high temperature stress at reproductive stages (Tao *et al*., 2013). There is urgent need for improving the crop performance under both low and high temperature stress through genetic improvement and exogenous application of bio-regulators (Amin *et al*., 2013; Asthir *et al*., 2013). Among bio-regulators, thiourea is an effective bio-regulator imparting abiotic stress tolerance (Anjum *et al.,* 2011; Perveen *et al.*, 2013). It has critical role in enhancing plant growth rate under various stress and normal conditions (Anjum *et al.*, 2011; Srivastava *et al.,* 2009). As multiple roles for improving plant growth and yield, thiourea is water soluble and readily absorbable in living tissues (Anjum *et al.*, 2008; Mani *et al.*, 2013). An improvement in growth, relative leaf water contents, chlorophyll and oil contents, nitrate reductase activity and leaf nutrient status has been observed by 10 m*M* foliar spray of thiourea in sunflower which is associated with induction of non-enzymatic and enzymatic antioxidants (Akladious, 2013). Foliar applied thiourea (6.6 m*M*) decreased membrane injury and lipid peroxidation in wheat was associated with improved antioxidants activity and enhanced amino acids, the total soluble proteins and

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chlorophyll contents in all the tested genotypes. This improved performance was associated with increased plant height, peduncle weight, peduncle length and grain weight in wheat (Asthir *et al.*, 2013).

Likely salicylic acid (SA) is a phenolic compound and as an ordinary signaling molecule imparts resistance to biotic and abiotic stresses (Karlidag *et al.*, 2009). It takes part in the regulation of nutrient uptake, closure of stomata, transpiration, protein, chlorophyll production, photosynthesis and inhibition of ethylene biosynthesis in wheat (*Triticum aestivum* L.) seedlings (Shakirova *et al.*, 2003). Thus, it has been recognized as a plant hormone found to be a valuable heat stress bio-regulator. Foliar application with a 0.1 m*M* salicylic acid reduced relative electrolyte leakage and thiobarbituric acid reactive substances (TBARS) in heat stressed young grape leaves, representing that it can stimulate inherent heat tolerance in grapevines (Wang and Li 2006).

Many reports are available for the positive role of bio-regulators on crop performance under normal and stress conditions (Jagetiya and Kaur, 2006; Anjum *et al.*, 2008; Akladious, 2013; Asthir *et al.*, 2013; Mani *et al.*, 2013) but information on performance of spring maize hybrids contrasting in heat tolerance are not available. The present study therefore evaluated the potential of exogenously applied thiourea and salicylic acid in improving the performance of maize hybrids contrasting in resistance to high temperature grown under spring season with the possibility to be exposed to heat stress under natural field conditions.

Materials and Methods

Location and Crop Husbandry

The field experiments were conducted at the research Farm, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan for two spring seasons during 2015 and 2016. Experimental soil was sandy clay loam having ECe 1.30 dS m^{-1} and pH 7.96. The soil analysis indicated presence of 0.04% nitrogen, 5.7 mg kg^{-1} available phosphorus and 117.8 mg kg^{-1} extractable potassium. Two maize hybrids contrasting for heat stress tolerance [YH-1898 (heat tolerant) and 33 M-15 (heat sensitive)] were selected on the basis previous screening conducted at Maize and Millet Research Institute (MMRI) Sahiwal, Pakistan (Saleem-ur-Rahman *et al.*, 2013) and different doses of thiourea (7.5 m*M* and10 m*M*) and salicylic acid (0.10 m*M* and 0.20 m*M*) were selected from our preliminary screening (data not given). The experimental design was randomized complete block with factorial arrangements. The net plot size was of 6 m × 4.5 m and crop was planted with inter-row distance of 75 cm and 20 cm between plants using seed rate of 25 kg ha^{-1}. The crop was sown on 10 March during both years with the possibility to be exposed to the high temperature stress during the grain formation and development stages (Saleem-ur-Rahman *et al.*, 2013). Weeds were controlled manually. Furadan (3-G) was applied at four-leaf stage @ 20 kg ha^{-1} to protect the crop from maize borer and shoot fly attack. Before sowing seeds were treated with fungicide (Benlate® @ 2 g kg^{-1}) for the control of seed borne infection. Nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) were applied as Urea (N 46%), DAP (Diammonium phosphate; P 46%, N 18%) and SOP (Sulphate of potash; K 50%) @ 250-150-100 Kg ha^{-1} (NPK) respectively. Ten irrigations were applied keeping in view the weather conditions. The temperature details during both crop seasons is given in Fig. 1.

Photosynthetic Pigment Determination

Chlorophyll *a*, *b* and total chlorophylls contents were determined by Arnon (1949). For which, 0.5 g of the fresh leaf collected from youngest one was homogenized with pestle and mortar in 80% acetone and made the volume up to 5 mL and filtered. The absorbance of the filtrate was read at 645 and 663 nm for chlorophylls *a* and *b* respectively using a UV-spectrophotometer (Hitachi-U-2001, Japan). Chlorophylls *a*, *b*, their total and ratio were calculated as described by Yoshida *et al.* (1976).

Chl. *a* (mg/g) = [12.7(OD663)-2.69(OD645)] x V/1000 x W
Chl. *b* (mg/g) = [22.9(OD645)-4.68(OD663)] x V/1000 x W
Total Chl. (mg/g) = [20.2(OD645) + 8.02 (OD663)] x V/1000 x W

Whereas V is the volume of the acetone used in extract (ml) and W as weight of fresh leaf tissue (g).

Growth Parameters

To determine leaf area, an area of (m^{-2}) from each plot was harvested 45 days after sowing and then four consecutive harvests with 15 days intervals. After harvesting, each sample was shifted in the lab. All leaves were removed from the stems of each sample and weighted separately. The sample of 5 gm leaves was used for leaf area using leaf area meter (Licor Model 3100, Nebraska, USA). The leaf area of 5 g leaves was multiplied to the total weight of each of each respective sample.

Leaf area index (LAI) was calculated by following formula given by (Watson, 1952), in which leaf area was divided by ground area.

The leaf area duration was calculated according to formula of Hunt (1978).

$$\text{LAD (days)} = (LAI_1 + LAI_2) \times (T_2 - T_1)/2$$

Where LAI_1 and LAI_2 are the leaf area indices at time T_1 and T_2 respectively.

The total dry matter accumulation during the cop growth at respective interval i.e. after 30 DAS and the after 15 days interval was used to calculate the crop growth rate (CGR) in (g m^{-2} d^{-1}) as described by Hunt (1978).

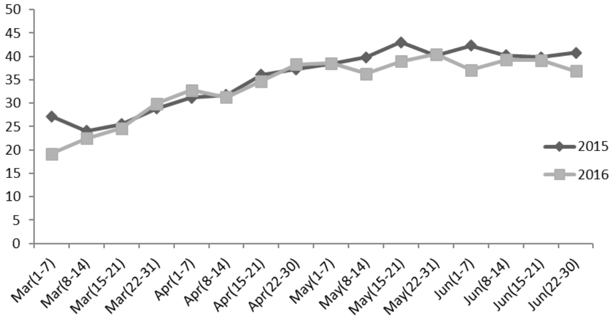

Fig. 1: Maximum temperature during growing season of crop in 2015 and 2016

$$CGR = (W_2-W_1)/(T_2- T_1)$$

Where; W_1 and W_2 are the total dry weight produced at first and second harvests at time of T_1 and T_2 of dry matter collected. Net assimilation rate (NAR) was estimated by using the formula proposed by Hunt (1978).

$$NAR\ (g\ m^{-2}\ d^{-1}) = \text{Total dry matter/Leaf area duration}$$

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Cell Membrane Thermostability (CMT)

The membrane permeability was expressed in terms of ion leakage from the leaves under stress. Fresh third leaf tissue (0.5 g) was taken, put in 10 mL distilled water, vortexed for 5 sec and measured for electrical conductivity at 0 h (EC_0). The test tubes containing leaves in distilled water were covered with aluminum foil and placed in refrigerator at 4ºC for 24 h and measured for electrical conductivity (EC_1). Then these test tubes were autoclaved at 120ºC and 0.10 MPa for 10 mints and electrical conductivity of dead tissues (EC_2) of the filtrate was measured. The relative membrane permeability (RMP) was determined by applying the following formula of Yang *et al*. (1996):

$$RMP\ (\%) = [EC_1- EC_0 / (EC_2 – EC_0)] \times 100.$$

Yield Traits

To determine yield traits, ten cobs were selected at random from each plot, the number of grain rows and grains per row of each cob were counted individually and averaged. The total number of grains per cob was calculated by multiplying number of grain rows per cob with number of grains per row. These cobs were sun-dried and 1000-grain was measured using electrical digital balance. Grain yield per plot was weighted and converted into hectare basis (t ha^{-1}).

Economic Analysis

The partial budget of the both experiments was made using the inputs and outputs prices of the local market of Faisalabad, Pakistan following the procedures as described in the chapter 3 of CIMMYT training manual (CIMMYT, 1988).

The gross income and total expenditure was calculated in local currency (rupees; Rs.). Net income was calculated by subtracting gross income from total cost while benefit cost ratio (BCR) was calculated by dividing gross income to total cost.

Statistical Analysis

The data was analyzed statistically using Fisher's analysis of variance technique (Steel *et al.*, 1997) and treatment means showing F-values significant were compared using LSD value at 5% probability level using Statistix software version 8.1.

Results

Impact on Crop Growth

Hybrids had significantly ($P<0.01$) different leaf area index (LAI) (Fig. 2a & b) and during both years more LAI was recorded in YH-1898 than 33M-15. LAI of both hybrids was similar till 65 days after sowing and after which heat tolerant hybrid YH-1898 maintained higher LAI. Among bio-regulators, maximum LAI was recorded in maize plants treated with TU @ 10 m*M* at 70 days after sowing during both years and minimum LAI for control. Likely hybrids also differed significantly ($P<0.01$) for leaf area duration (LAD) with maximum for YH-1898 during both years of study (Fig. 3a & b). Higher LAD was found for YH-1898 at later stages of crop growth. Bio-regulators responded varied for years and highest LAD was found for plants sprayed with TU @ 10 m*M* and for SA @ 0.10 m*M* during 2016. While minimum LAD for recorded for control without any spray during both years (Fig. 3c & d).

Crop growth rate (CGR) was also significantly ($P<0.01$) different for hybrids and bio-regulators during both years. At initial stages of crop growth i.e. up to 70 days after sowing hybrid 33M-15 had more CGR than tolerant hybrids during both years (Fig. 4a & b), which drastically reduced later. Thereafter, heat tolerant YH-1898 hybrid relatively maintained a higher CGR during both years. Among the bio-regulators, TU @ 10 m*M* produced highest CGR during 2015 and for TU @ 7.5 m*M* during 2016 (Fig. 4c & d). Similar trend was observed for net assimilation rates (NAR) for hybrids, bio-regulators and years of experimentation (Fig. 5a & b).

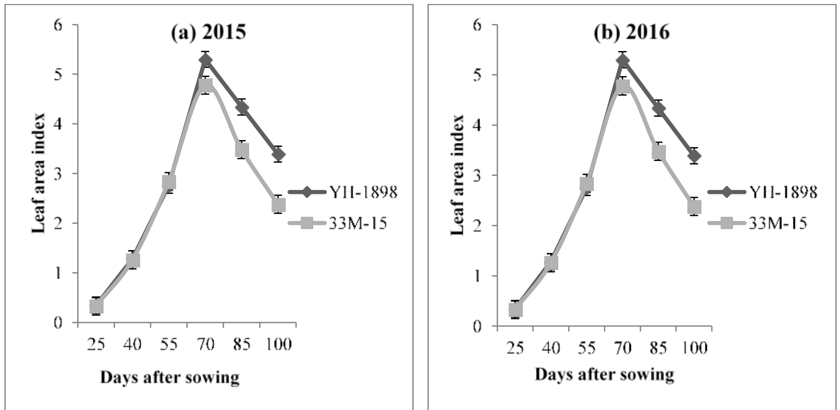

Fig. 2: Effect of hybrids on leaf area index of maize during 2015 (a) and 2016 (b)

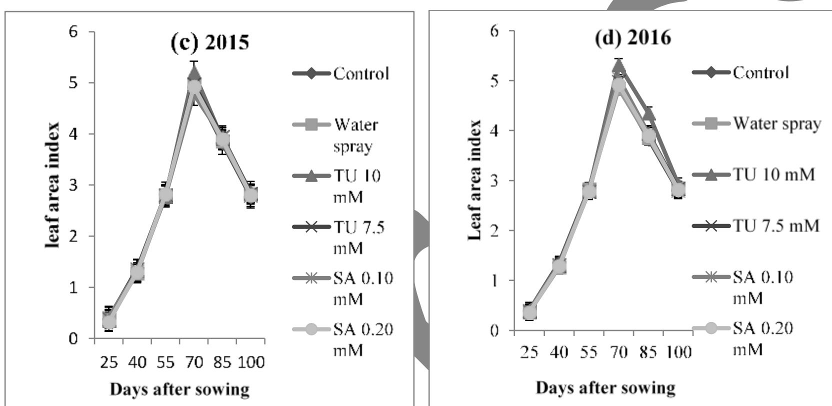

Fig. 2: Effect of bio-regulators on leaf area index of maize during 2015 (c) and 2016 (d)
TU= Thiourea; SA= Salicylic acid

Leaf Chlorophyll Contents

Maize chlorophyll *a* contents were significantly ($P<0.01$) affected by hybrids and bio-regulators in both the years (Table 1). All possible interactions of hybrid and bio-regulator were non-significant ($P>0.05$) for maize chlorophyll *a* contents. During both the years, significantly maximum chlorophyll *a* contents were recorded for heat tolerant maize hybrid. During first year, significantly highest chlorophyll *a* contents (2.41 mg g^{-1}) were recorded for SA @ 0.20 m*M* followed by TU @ 7.5 m*M* and SA @ 0.10 m*M* while during second year, SA @ 0.10 m*M* expressed highest chlorophyll *a* contents followed by TU @ 7.5 m*M*. During both years, minimum chlorophyll *a* contents were recorded for control. Chlorophyll *b* contents differed significantly ($P<0.01$) by hybrids in both years and bio-regulators in 2015 (Table 1). All possible interactions of hybrids and bio-regulators were non-significant. During both years, significantly ($P<0.01$) maximum chlorophyll *b* contents were recorded for heat tolerant hybrid. During first year, SA (0.10 m*M*) was best bio-regulator in maintaining highest chlorophyll *b* contents. But during second year, effect of bio-regulators was non-significant ($P>0.05$) for maize chlorophyll *b* contents. Total chlorophyll contents was significantly ($P<0.01$) different for years, hybrids and bio-regulators treatments on total chlorophyll of maize plants during both years of study (Table 1). All possible interactions of hybrids and bio-regulators were non-significant ($P>0.05$) for both years. Significantly ($P<0.01$) highest total chlorophylls contents were recorded for heat tolerant hybrid during 2015 and 2016. Chlorophyll *a*/*b* ratio was also significantly ($P<0.01$) different for hybrids and bio-regulators (Table 1) while interaction was non-significant ($P<0.05$). Significantly ($P<0.01$) highest chlorophyll *a/b* ratio was observed in maize plants of heat tolerant hybrid and among bio-regulators no significant difference was observed as compared to control.

Cell Membrane Thermostability (%)

Impact of hybrids and bio-regulators was significant ($P<0.01$) on cell membrane thermostability (CMT) (Table 1) and interactions were non-significant ($P>0.05$) during both years. Heat tolerant hybrid in both years had significantly ($P<0.01$) more CMT during 2015 (12.44%) than 2016 (13.34%).

Table 1: Effect of thiourea and salicylic acid on chl *a*, chl *b*, total chl, chl *a/b* ratio and cell membrane thermostability of maize

Treatments	Chl. *a* (mg g^{-1})		Chl. *b* (mg g^{-1})		Total Chl. (mg g^{-1})		Chl. *a/b* ratio (mg g^{-1})		Cell membrane thermostability (%)	
Year	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Hybrids (H)										
YH1898	2.54 a	2.74 a	1.2a	1.71a	4.45 a	3.11 a	1.71 b	2.04 a	12.44 a	13.34 a
33M-15	2.11 b	2.13 b	1.08b	1.15b	3.29 b	2.67 b	1.85 a	1.96 b	9.39 b	10.21 b
LSD ($p \leq 0.05$)	0.27	0.31	0.12	0.21	0.02	0.01	0.05	0.03	1.12	1.04
Treatments (T)										
Control	2.21c	2. 31e	1.07 b	1.32	3.63 d	2.76 e	1.85 a	2.05 a	10.75 d	11.66 b
Water spray	2.24 c	2.36 d	1.10 b	1.36	3.72 c	2.80 d	1.81ab	2.03 b	10.80 d	11.70 b
TU 10 mM	2.37 ab	2.47 bc	1.19 a	1.48	4.02 a	2.98 a	1.78bc	1.98 c	11.06 a	11.86 a
TU 7.5 mM	2.38 ab	2.50 ab	1.19 a	1.47	3.97 b	2.94 b	1.77bc	1.99bc	11.02 ab	11.82 a
SA 0.10 mM	2.37 ab	2.53 a	1.21 a	1.48	3.96 b	2.93bc	1.74cd	1.97 c	10.95 bc	11.82 a
SA 0.20 mM	2.41a	2.45 c	1.19 a	1.49	3.94 b	2.91 c	1.72 d	1.97 c	10.92 c	11.80 a
LSD ($p \leq 0.05$)	0.05	0.04	0.03	Ns	0.03	0.02	0.04	0.04	0.03	0.07
Interaction (H×T)										
LSD ($p \leq 0.05$)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Means sharing the same case letter for a parameter do not differ significantly at $p \leq 0.05$; Chl= Chlorophyll; TU= Thiourea; SA= Salicylic acid; NS= non-significant

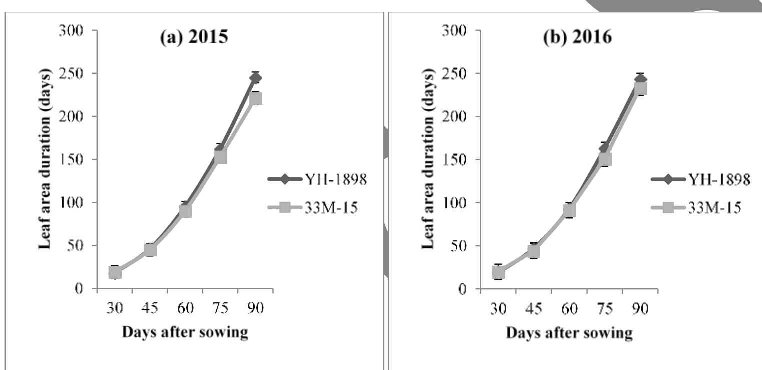

Fig. 3: Effect of hybrids on leaf area duration of maize during 2015 (a) and 2016 (b)

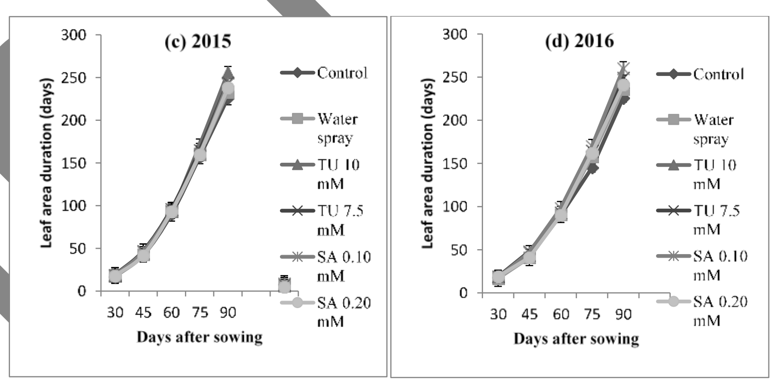

Fig. 3: Effect of bio-regulators on leaf area duration of maize during 2015 (c) and 2016 (d)
TU= Thiourea; SA= Salicylic acid

Among bio-regulators, application of TU @ 10 m*M* was most effective treatment with highest CMT (11.06%) that was significantly similar with TU @ 7.5 m*M* during first year.

Ear Growth and Yield

Heat tolerant hybrid had longer cobs and cob diameter than 33 M-15 during both years. Maize plants treated with bio-regulator TU @ 7.5 m*M* had significantly ($P<0.01$) maximum cob length and cob diameter during both years and was significantly similar with SA @ 0.10 m*M* and 0.20 m*M* during first and 2nd years (Table 2).

Number of grain rows and grains per row per cob were significant ($P<0.01$) for hybrids and bio-regulators

Table 2: Effect of thiourea and salicylic acid on cob length, cob diameter, number of grain rows per cob, number of grains per row and number of grains per cob of maize

Treatments	Cob length (cm)		Cob diameter (cm)		No. of grain rows per cob		No. of grains per row		No. of grains per cob	
Year	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Hybrids (H)										
YH1898	19.11 a	18.90 a	3.97 a	3.80 a	14.70 a	14.18 a	28.35 a	31.07 a	417.6 a	441.33 a
33M-15	14.86 b	16.00 b	3.09 b	3.18 b	11.43 b	12.08 b	18.20 b	20.92 b	208.5 b	253.41 b
LSD ($p \leq 0.05$)	0.37	0.47	0.07	0.13	0.28	7.65	2.71	2.57	18.35	24.29
Treatments (T)										
Control	16.43 c	16.83 b	3.41 c	3.29 b	12.64 c	12.46 b	21.45 e	23.97 c	278.89 e	303.91c
Water spray	16.66 bc	16.79 b	3.46bc	3.32 b	12.81bc	12.57 b	21.96de	24.43 c	288.74 de	312.53c
TU 10 mM	17.06 abc	17.88 a	3.54abc	3.60 a	13.57 a	13.68 a	25.52 a	26.78 b	355.41 a	386.66 a
TU 7.5 mM	17.64 a	17.99 a	3.66 a	3.65 a	13.12abc	13.52 a	24.53 ab	26.59 b	330.56 b	367.74 ab
SA 0.10 mM	17.28 ab	17.73 a	3.59 ab	3.54 a	13.29 ab	13.29 a	23.32 bc	27.83 a	319.77 bc	358.91 b
SA 0.20 mM	16.87 bc	17.49ab	3.51bc	3.53 a	12.98bc	13.25 a	22.87 cd	26.35 b	304.97cd	354.46 b
LSD ($p \leq 0.05$)	0.64	0.81	0.13	0.15	0.49	0.53	0.59	0.99	21.40	21.33
Interaction (H×T)										
LSD ($p \leq 0.05$)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Means sharing the same case letter for a parameter do not differ significantly at $p \leq 0.05$; TU= Thiourea; SA= Salicylic acid; NS= non-significant

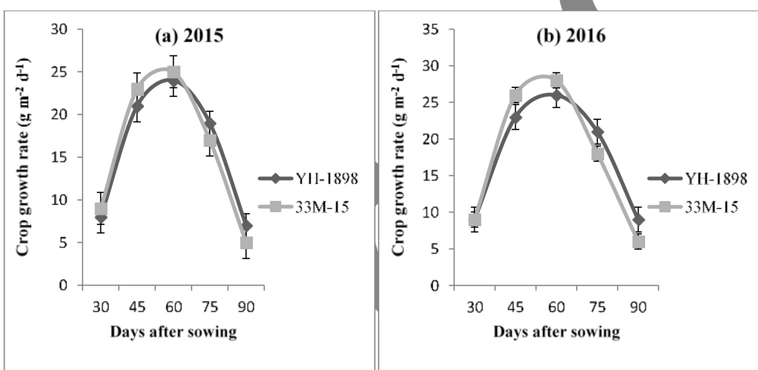

Fig. 4: Effect of hybrids on crop growth rate of maize during 2015 (a) and 2016 (b)

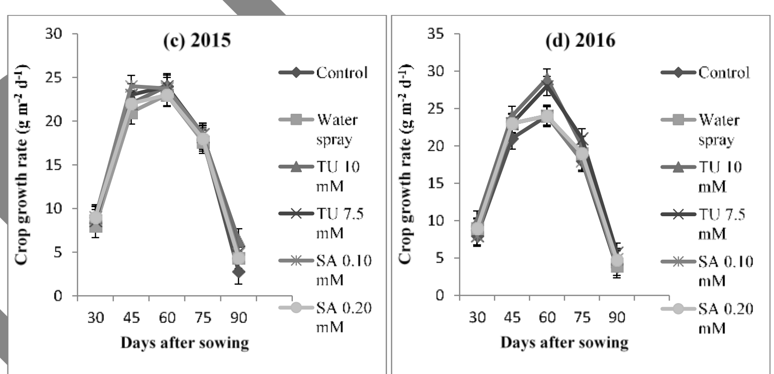

Fig. 4: Effect of bio-regulators on crop growth rate of maize during 2015 (c) and 2016 (d)
TU= Thiourea; SA= Salicylic acid

during both years (Table 2). Heat tolerant hybrid had more number of grain rows and grains per row per cob. Application of TU@ 10 m*M* had maximum of these traits followed by SA @ 0.10 m*M* in both years of study. However, interaction was non-significant between hybrids and bio-regulators for these traits (*P<0.01*) during both years (Table 2). Hybrids and bio-regulators significantly (*P<0.01*) affected the TGW during both years (Table 3). Heat tolerant hybrid expressed highest TWG and among bio-regulators TU @ 10 m*M* had significantly (*P<0.01*) maximum TGW during 2015 and for TU @ 7.5 m*M* for 2nd year. Highest stover yield was recorded for heat tolerant hybrid and maize plants sprayed with different bio-regulators had significantly different stover yield during both years. In first year, maximum stover yield was recorded in plants treated with TU @ 7.5 m*M* and for TU application @ 10 m*M* during 2nd year.

Table 3: Effect of thiourea and salicylic acid on thousand grain weight, stover yield, grain yield, grain starch content and grain protein content of maize

Treatments	Thousand grain weight (g)		Stover yield (t ha^{-1})		Grain yield (t ha^{-1})		Grain starch content (%)		Grain protein content (%)	
Year	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Hybrids (H)										
YH1898	320.46 a	319.36 a	11.31 a	11.81a	7.64 a	7.87 a	72.27 a	70.28 a	8.29 a	8.73 a
33M-15	288.96 b	288.77 b	8.79 b	10.06b	3.27 b	4.03 b	62.37 b	61.29 b	6.93 b	7.56 b
LSD ($p \leq 0.05$)	3.95	2.97	0.21	0.25	0.13	0.35	1.02	1.24	0.08	0.13
Treatments (T)										
Control	298.90 c	297.95 d	9.72 c	10.38b	4.91 d	5.45 d	62.28 d	61.64 b	6.92 c	7.54 b
Water spray	302.26 bc	301.82 d	9.85 bc	10.47b	5.07 d	5.78 c	64.62 c	62.98 b	7.18 b	7.73 b
TU 10 mM	310.25 a	307.18ab	10.1abc	11.40a	5.92 a	6.40 a	70.19 a	68.13 a	7.92 a	8.52 a
TU 7.5 mM	306.51 ab	309.67 a	10.43 a	11.26a	5.76 ab	6.13 b	67.84 b	67.47 a	7.80 a	8.38 a
SA 0.10 mM	306.06 ab	304.10abc	10.22ab	11.08a	5.67 b	5.99bc	70.12 a	67.31 a	7.91 a	8.36 a
SA 0.20 mM	304.28ab	303.07bcd	9.98 bc	11.05a	5.43 c	5.95bc	68.87ab	67.29 a	7.94 a	8.33 a
LSD ($p \leq 0.05$)	6.84	5.15	0.38	0.44	0.23	0.21	1.77	2.16	0.14	0.23
Interaction (H×T)										
LSD ($p \leq 0.05$)	NS	NS	NS	NS	0.002	NS	NS	NS	NS	NS

Means sharing the same case letter for a parameter do not differ significantly at $p \leq 0.05$; TU= Thiourea; SA= Salicylic acid; NS= non-significant

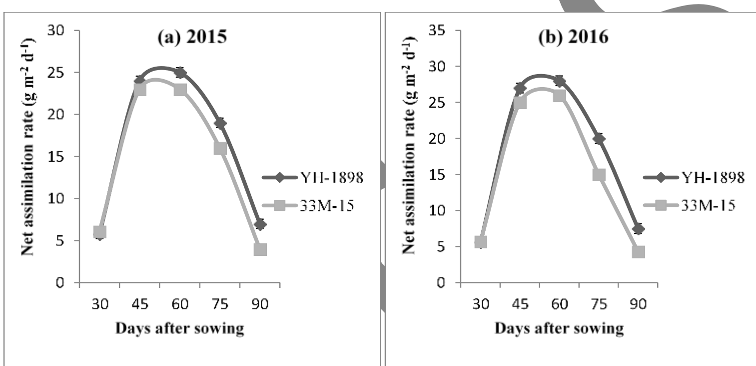

Fig. 5: Effect of hybrids on net assimilation rate of maize during 2015 (a) and 2016 (b)

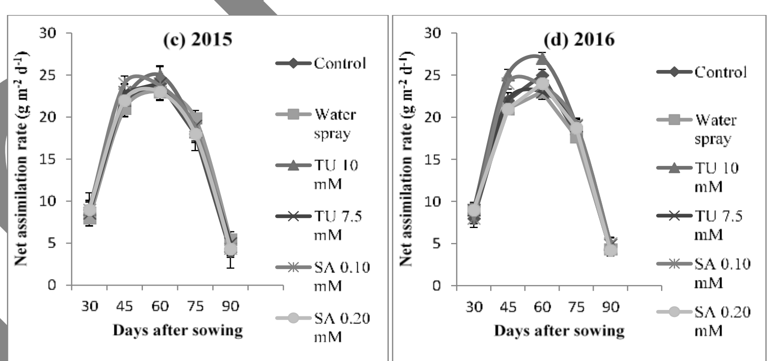

Fig. 5: Effect of bio-regulators on net assimilation rate of maize during 2015 (c) and 2016 (d)
TU= Thiourea; SA= Salicylic acid

Effect of hybrids and bio-regulators was significant on grain yield of maize (Table 3). However, interaction of hybrids and bio-regulators for grain yield was significant (*P<0.01*) for 2015 and non-significant for 2016 (Fig. 5). Heat tolerant hybrid had more grain yield during both years and application of TU @ 10 m*M* expressed (*P<0.01*) maximum grain yield (5.92 t ha^{-1}) that was statistically at par with TU @ 7.5 m*M*. During 2015, heat tolerant hybrid gave highest yield by application of TU @ 7.5 m*M*.

Grain Starch and Protein Contents

Significant (*P<0.01*) difference of starch contents for hybrids and bio-regulators was observed during both years (Table 3) while their interaction was non-significant (*P>0.05*). Heat tolerant hybrid had higher starch content than heat sensitive hybrid during both years. During first year, TU@ 10 m*M* applied plants had significantly (*P<0.01*) maximum grain starch content (70.19%) which followed

Table 4: Net income and benefit-cost ratio (BCR) of different treatments during 2015 and 2016

Treatments	Gross income ($Rs\ ha^{-1}$)		Total Cost ($Rs\ ha^{-1}$)		Net Income ($Rs\ ha^{-1}$)		B:C ratio	
Year	2015	2016	2015	2016	2015	2016	2015	2016
Control	116612	129437	86371	86371	30241	43066	1.35	1.50
Water spray	120412	137275	86871	86871	33541	50404	1.39	1.58
TU 10 mM	140600	152000	88474	88474	52126	63526	1.59	1.72
TU 7.5 mM	136800	145587	88198	88198	50402	57389	1.57	1.65
SA 0.10 mM	134662	142262	87393	87393	47269	54869	1.54	1.63
SA 0.20 mM	128962	141312	87416	87416	41546	53896	1.48	1.62

B:C ratio= Benefit cost ratio; TU= Thiourea; SA= Salicylic acid

by SA @ 0.10 m*M* and statistically at par with plants grain starch contents treated with SA @ 0.20 m*M*. Significant ($P<0.01$) difference for protein contents in grains of different hybrids and bio-regulators was found during both years (Table 3), while their interaction was non-significant ($P>0.05$). Heat tolerant hybrid had showed higher protein content than 33 M-15 during both years. During first year, SA treatment @ 0.20 m*M* had significantly ($P<0.01$) maximum protein contents (7.94%) and during second year, TU @ 10 m*M* had significantly ($P<0.01$) maximum grain protein content (8.52%).

Economic Analysis

Net income and benefit-cost ratio (BCR) of different treatments for both years showed highest net income (52126 $Rs\ ha^{-1}$) for (TU 10 m*M*) which followed TU 7.5 m*M* (50402 $Rs\ ha^{-1}$). Minimum net income was calculated for control (30241 $Rs\ ha^{-1}$). Benefit cost ratio was also highest for TU 10 m*M* (1.58) followed by TU 7.5 m*M* (1.57) and minimum (1.35) in control treatment. Similar trend was estimated for net income and benefit cost ratio during 2016 (Table 4).

Discussion

Average maize yield of Pakistan is low compared to many agricultural countries of world. Major factors responsible for low yield include poor agronomic practices, low quality seed, pest infestation, lack of balanced nutrition, water and temperature stresses (Lawlor, 2002; Ammani *et al*., 2012; Naveed *et al*., 2014; Noor *et al*., 2016). Bio-regulators like thiourea and salicylic acid have been found effective to improve the performance of many crops including maize under normal growing and stressful conditions (Akladious, 2013; Amin *et al*., 2013; Asthir *et al*., 2013). In present study, thiourea and salicylic acid application increased the LAI (Fig. 2c & d). This was might be due to their stimulatory effects on cell division, chlorophyll contents and net photosynthesis rate which resulted in improved vegetative growth (Garg *et al*., 2006; Anjum *et al*., 2008; Amin *et al*., 2013; Perveen *et al*., 2015). Increased leaf area provided more surface area for photo-assimilate production and partitioning towards sink (Garg *et al*., 2006) and resulted in higher CGR and NAR (Fig. 4 & 5). Improved crop growth by foliar applied thiourea and salicylic acid could be attributed to higher photosynthetic efficiency (Anjum *et al*., 2011). Increased leaf chlorophyll contents were found on thiourea and salicylic acid application in present study, also previously reported for different field crops including maize (Akladious, 2013; Amin *et al*., 2013; Asthir *et al*., 2013). Cell membrane thermostability increased in plants treated with bio-regulators due to their role in scavenging reactive oxygen species and reducing lipid peroxidation (Mahatma *et al*., 2009; Akladious, 2013). More cell membrane thermostability of tolerant hybrid during both years indicated the possible character of hybrid involved in stress tolerance (Asthir *et al*., 2013). Bio-regulators exogenous application improved growth and reduced the adverse effects of high temperature on maize which resulted in better yield attributes like cob length, cob diameter, number grain rows per cob, number of grains per cob, thousand grain weight and grain yield (Amin *et al*., 2013). Both hybrids showed varied response to yield components and tolerant hybrid maintained better response in all tested yield attributes (Table 2). Thiourea and salicylic acid have potential role in increasing growth of reproductive organs of maize (Amin *et al*., 2013) and wheat (Asthir *et al*., 2013). Cob length and cob diameter were substantially increased by application of bio-regulators (Table 2). The thicker and longer cobs resulted in increased number of grains per cob in both years. The increase in thousand grain weight by application of bio-regulators could be attributed to improved growth attributes like higher LAI, LAD, CGR and NAR of maize particularly at flowering and grain filling stages which leaded to delayed senescence and ultimately more assimilates translocation towards grains (Jagetiya and Kaur, 2006). Higher number of grains per cob and higher thousand grain weight by application of bio-regulators ultimately increase yield over control. The increase in number of grains per cob and thousand grain weight chould be attributed to the role of bio-regulators to maintain higher photosynthetic rate, delayed senescence and increased partioning of assimilates to the grains (Khodary, 2004; El-Tayeb, 2005; Anjum *et al*., 2008; Asthir *et al*., 2013). Improved higher protein and starch contents with application of bio-regulators in maize grains might be due to their involvement in synthesis of sulphur containing amino acids such as methionine, cysteine and cystine which increases protein contents (Haneklaus *et al*.,1999).

Exogenous application of bio-regulators especially thiourea not only improved crop growth, yield and quality attributes of maize but also found more economical over control due to higher net income and benefit cost ratio (Table 4). Such improvement in thiourea economic returns had been also reported in canola (Rehman *et al.*, 2013). Therefore, use of these bio-regulators is an economical strategy to boost production and income of spring maize.

Conclusion

This study demonstrated that maize hybrid YH-1898 performed better than 33M-15 under spring conditions. Foliar application of bio-regulators particularly TU @ 10 m*M* maintained the higher chlorophyll contents and cell membrane themostability at reproductive stages of maize and likely growth and yield traits of both hybrids were significantly improved by application of TU. Economically, highest net returns and benefit cost ratio were calculated for TU @ 10 m*M*. Thus, exogenous application of thiourea @ 10 m*M* was found cost effective to improve the productivity of spring planted maize heat tolerant hybrids.

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