

Improvement of antioxidant activities and yield of spring maize through seed priming and foliar application of plant growth regulators under heat stress conditions

Melhoria das atividades antioxidantes e do rendimento do milho primaveril através da aplicação de sementes e aplicação foliar de reguladores de crescimento em condições de estresse térmico

Ijaz Ahmad^{1*}; Shehzad Maqsood Ahmed Basra²; Muhammad Akram³; Allah Wasaya⁴; Muhammad Ansar⁵; Safdar Hussain⁶; Asif Iqbal⁷; Syed Azhar Hussain⁸

Abstract

Heat stress during reproductive and grain filling phases adversely affects the growth of cereals through reduction in grain's number and size. However, exogenous application of antioxidants, plant growth regulators and osmoprotectants may be helpful to minimize these heat induced yield losses in cereals. This two year study was conducted to evaluate the role of exogenous application of ascorbic acid (AsA), salicylic acid (SA) and hydrogen peroxide (H₂O₂) applied through seed priming or foliar spray on biochemical, physiological, morphological and yield related traits, grain yield and quality of late spring sown hybrid maize. The experiment was conducted in the spring season of 2007 and 2008. We observed that application of AsA, SA and H₂O₂ applied through seed priming or foliar spray improved the physiological, biochemical, morphological and yield related traits, grain yield and grain quality of late spring sown maize in both years. In both years, we observed higher superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activity in the plants where AsA, SA and H₂O₂ were applied through seed priming or foliar spray than control. Membrane stability index (MSI), relative water contents (RWC), chlorophyll contents, grain yield and grain oil contents were also improved by exogenous application of AsA, SA and H₂O₂ in both years. Seed priming of AsA, SA and H₂O₂ was equally effective as the foliar application. In conclusion, seed priming with AsA, SA and H₂O₂ may be opted to lessen the heat induced yield losses in late sown spring hybrid maize. Heat tolerance induced by ASA, SA and H₂O₂ may be attributed to increase in antioxidant activities and MSI which maintained RWC and chlorophyll contents in maize resulting in better grain yield in heat stress conditions.

Key words: Maize. Heat Tolerance. Antioxidants. Plant Growth Regulators. Osmoprotectants.

¹ Soil Conservation Officer, Soil Conservation Group of Agriculture Department, ADC Colony, Rawalpindi Road, Talagang, Pakistan. E-mail: ijazscogujrat@gmail.com

² Professor, Department of Crop Physiology, University of Agriculture Faisalabad, Pakistan. E-mail: shehzadbasra@gmail.com

³ Assistant Professor, Department of Environmental Sciences, COMSATS, Institute of Information Technology Vehari, Pakistan. E-mail: akramcp@gmail.com

⁴ Assistant Professor, Departments of Agronomy, Bahadar Campus of Bahauddin Zakryia University, Layyah, Pakistan. E-mail: wasayauaf@gmail.com

⁵ Professor, Department of Agronomy, Pir Mehr Ali Shah University of Arid Agriculture, Rawalpindi, Pakistan. E-mail: mohammad.ansar@uaar.edu.pk

⁶ Assistant Professor, Department of Agronomy, Ghazi University, Dera Ghazi Khan, Pakistan. E-mail: safdarb1184@yahoo.com

⁷ Executive District Officer Agriculture, City District Government, Lahore, Pakistan. E-mail: asif_wm@yahoo.com

⁸ Assistant Research Officer, Soil and Water Testing Laboratory for Research, Suleman Pura, Sargodha, Pakistan. E-mail: azee_uaf@yahoo.com

* Author for correspondence

Resumo

O estresse térmico durante as fases reprodutiva e de enchimento de grãos afeta negativamente o crescimento de cereais com redução do número e do tamanho do grão. A aplicação exógena de antioxidantes, reguladores de crescimento vegetal e osmoprotetores, pode ser útil para minimizar essas perdas. O objetivo foi avaliar o efeito da aplicação exógena de ácido ascórbico (ASA), ácido salicílico (SA) e peróxido de hidrogênio (H_2O_2) aplicada através de sementes ou pulverização foliar na indução de tolerância ao estresse térmico em milho híbrido semeado no final da primavera. A aplicação de AsA, SA e H_2O_2 através de sementes ou pulverização foliar melhora a fisiologia, bioquímica, morfologia e traços associados ao rendimento e qualidade de de grãos de milho no cultivo de Primavera tardia em ambos os anos. Foi observada maior superóxido dismutase (SOD), catalase (CAT) e atividade de peroxidase (POD) em plantas onde AsA, SA e H_2O_2 foram aplicados via sementes ou pulverização foliar em relação ao controle. O índice de estabilidade de membrana (MSI), conteúdo relativo de água (RWC), conteúdo de clorofila, rendimento de grãos e teor de óleo de grãos também foram melhorados pela aplicação de AsA, exógena SA e H_2O_2 em ambos os anos. A aplicação via sementes de AsA, SA e H_2O_2 foi igualmente eficaz à aplicação foliar. A embebição de sementes com AsA, SA e H_2O_2 pode ser utilizado para diminuir as perdas de rendimento induzida pelo calor em milho híbrido semeado no final da primavera. A indução de tolerância ao calor pela ASA, SA e H_2O_2 pode ser atribuído ao aumento de atividades antioxidantes e a MSI que manteve o RWC e o conteúdo de clorofila resultando em melhor rendimento de grãos em condições de estresse de calor.

Palavras-chave: Milho. Tolerância ao calor. Antioxidantes. Reguladores de crescimento. Osmoprotectores.

Introduction

Maize (*Zea mays* L.) is a high yielding cereal crop and is ranked as third important cereal crop in Pakistan and grown twice in a year in our country (spring and autumn) as primarily for grain production and secondarily for forage and fodder purposes (TARIQ et al., 2002). The yield potential of spring crop is much higher than autumn sown crop but high temperature at anthesis and grain filling stages is a severe constraint to achieve its yield potential (CHEIKH; JONES, 1994). The floral structure of maize, notably the separation of male and female floral organs and the near-synchronous development of florets on a single ear, borne on a single stem, is extremely sensitive to high temperature and moisture stress during anthesis (JOHNSON; HERRERO, 1981). In a study, Mitchell and Petolino (1988) reported that grain yield of maize was reduced on exposure to day time temperature of 38°C for 16 h. It has been also observed that high temperature decreases chlorophyll contents, net photosynthetic rate, pollen kernel setting, plant growth and seed filling duration, which results in smaller grain and lower seed yield (RAMADOSS et al., 2004). The

heat stress induces formation of toxic reactive oxygen species (ROS) (APEL; HIRT, 2004) and these ROS cause oxidative damage to chloroplast and mitochondria by damaging cellular structures (NOCTOR; FOYOR, 1998). The plants generate antioxidant defense system to overcome these ROS. Antioxidant defense system produces antioxidant compounds or antioxidant enzymes (APEL; HIRT, 2004), and it is well reported that that heat tolerance can be promoted by increasing antioxidant defense system in plants. To mitigate heat induced damage, plants may up-regulate various scavenging mechanism like enzymatic antioxidants (superoxide dismutase, peroxidase and catalase), non-enzymatic metabolites like ascorbic acid and plant growth regulators (salicylic acid) and osmoprotectants (AHMAD et al., 2015). These chemicals protect membranes and photosynthetic apparatus from the injurious effects caused by environmental stresses (FOYER; NOCTOR, 2003). For example, ascorbic acid (AsA) acts as a cofactor for several enzymes and regulates the phytohormone-mediating signaling processes (BARTH et al., 2006), and many physiological processes in plants (SMIRNOFF;

WHEELER, 2000). Ascorbic acid also modulates the tocopherol synthesis, which protects the plant from several environmental stresses (CONKLIN; BARTH, 2004). Likewise, salicylic acid (SA) is an important signaling molecule in plants, which helps to regulate plant resistance against chilling, drought and heat stresses (AHMAD et al., 2015). Hydrogen peroxide, acts as signaling molecule and assistant in triggering stress resistance mechanism (KUMAR et al., 2010). Exogenously application of these chemicals help in improving the resistance against environmental stresses in field crops; nonetheless exogenous application of these chemical in improving the growth and productivity of late spring sown hybrid maize in field conditions has never been evaluated. Thus, this study was aimed to explore the role of exogenous application of these chemicals on morphology, physiological, biochemical and yield related attributes, grain yield and grain quality of spring hybrid maize planted at low temperature.

Materials and Methods

This study was conducted at the research farm of department of Crop Physiology, University of Agriculture, Faisalabad (latitude 31°N, longitude 73°E and altitude 184.4 m), Pakistan during spring season of 2007 and 2008. The maize hybrid (Hi Sawn 9697) was used as experimental material during both years. The experiment soil was loamy having organic matter 0.61 and 0.66%, pH 7.39 and 7.41, ECe 0.387 and 0.393 dS m⁻¹, available potassium 335 and 350 ppm and available phosphorus 11.12 and 12.82 ppm during 2007 and 2008, respectively. The experiment was laid out in randomized complete block design with a net plot size of 4.2 m × 7.7 m, replicated thrice. The AsA, SA and H₂O₂ were applied either through seed priming (20 mg L⁻¹) or foliar spray (20 mg L⁻¹). For priming, maize seeds were soaked in solutions containing either of AsA, H₂O₂ and SA for 24 h maintaining seed to water ratio of 1:5. Primed and non-primed were

sown on 22nd March in 2007 and on 15th March in 2008. The crop was planted in 66 cm spaced rows with a dibbler with two seeds per hole maintaining 15 cm distance between two holes in both years. After uniform emergence, one healthy plant per hole was maintained. After eight weeks of sowing, each of AsA, H₂O₂ and SA were foliar applied at the rate of 20 mg L⁻¹. All other agronomic and plant protection measures were kept uniform. Samples of ear leaf were collected at tasseling to measure various biochemical attributes. Crop was harvested on 25th June in 2007 and 27th June in 2008.

Relative water contents

Leaf relative water contents were estimated by gravimetric method. Fresh leaves (0.5 g) were rinsed in a test tube until reached their turgidity and were weighed through electric balance. These turgid leaves were then dried in air and then in oven for 24 h at 80°C, were weighed and RWC were calculated by following formula (REDDY, 2004).

Membrane stability index

For determining the membrane stability index, 200 mg leaves sample was weighed through electric balance and taken in two sets of 10 cm³ test tube containing doubled distilled water (SAIRAM, 1994). One set of test tubes was heated at 40°C for half an hour while second set was boiled at 100°C in water bath for 10 minutes and their ECs were measured through Conductivity Bridge as C₁ and C₂, respectively.

Determination of chlorophyll contents

For determination of chlorophyll contents, segments of 0.5cm of fresh leaves were prepared and extracted overnight with 80% acetone at -10°C. The extracts of each sample were centrifuged at 14000 × g for 5 min and absorbance of supernatant were observed through spectrophotometer (T60)

at 645 and 663 nm and chlorophyll *a* and *b* contents were calculated using the formulae of Nagata and Yamashita (1992).

Extraction of antioxidant enzyme

To extract antioxidant enzymes, 0.5 g sample of fresh leaves from each pot was taken. It was grinded using a tissue grinder in 8 mL of cooled phosphate buffer having pH 7.0 (1% (w/v) polyvinyl pyrrolidone). Meanwhile, 0.2 g quartz sand was also added in test tubes. Then the homogenate was centrifuged at $15000 \times g$ for 20 min at 4°C. The supernatant was used for assays of enzyme activity.

Estimation of superoxide dismutase

The activity of SOD was detected by measuring its ability to inhibit photo reduction of nitro blue tetrazolium (NBT) using the method of Giannopolitis and Ries (1977). The reaction solution (3 mL) contained 50 μ M NBT, 1.3 μ M riboflavin, 13 mM methionine, 75 nM EDTA, 50 mM phosphate buffer (pH 7.8) and 50 μ L enzyme extract. Test tubes containing the reaction solution and leave were irradiated under light bank (15 fluorescent lamps) at $78 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 15 min. The absorbance of both irradiated and non-irradiated solutions were recorded at 560 nm by spectrophotometer (T60 spectrophotometer). One unit of SOD activity was defined as the amount of enzyme that would inhibit 50 of NBT photo reduction.

Catalase and Peroxidase

Activities of CAT and POD were measured using the method of Chance and Maehly (1955) with minor

modifications. Three mL of CAT reaction solution containing 50 mM phosphate buffer with pH 7.0, 15 mM H_2O_2 and 0.1 mL enzyme extract was used. Changes in absorbance of the reaction solution were recorded at 240 nm wavelength after every 20s. The POD reaction solution (3 mL) containing 50 mM Sodium Acetate buffer with pH 5.0, 20 mM Guaiacol, 40 mM H_2O_2 and 0.1 mL enzyme extract was used. Changes in absorbance of reaction solution at 470 nm were read after every 20 s. One unit CAT and POD activity was defined as an absorbance change of 0.01 units per minutes.

Yield and yield components

Before harvesting plant population per unit area was measured from two places in the plot and was averaged. Ten cobs were selected from each plot and were threshed to record number of grains per cob. The cobs from the whole plot were shelled with the help of maize sheller and grain yield per plot was recorded, which was later converted to Mega gram per hectare. Three sub samples of 100-grain were taken from each plot after shelling and their weight was measured on an electric balance to observe 100-grain weight.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) and least significance difference test at 0.05 probability was used to compare treatment means (STEEL et al., 1997).

Weather data

Weather data during the course of investigation of both studies is given in Figure 1 and 2.

Figure 1. Weekly meteorological data of the experimental site during 2007.

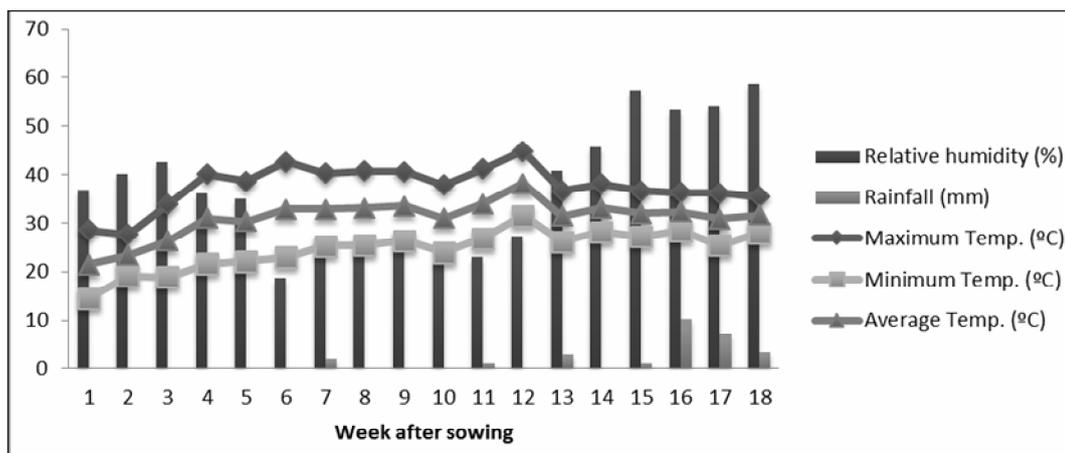
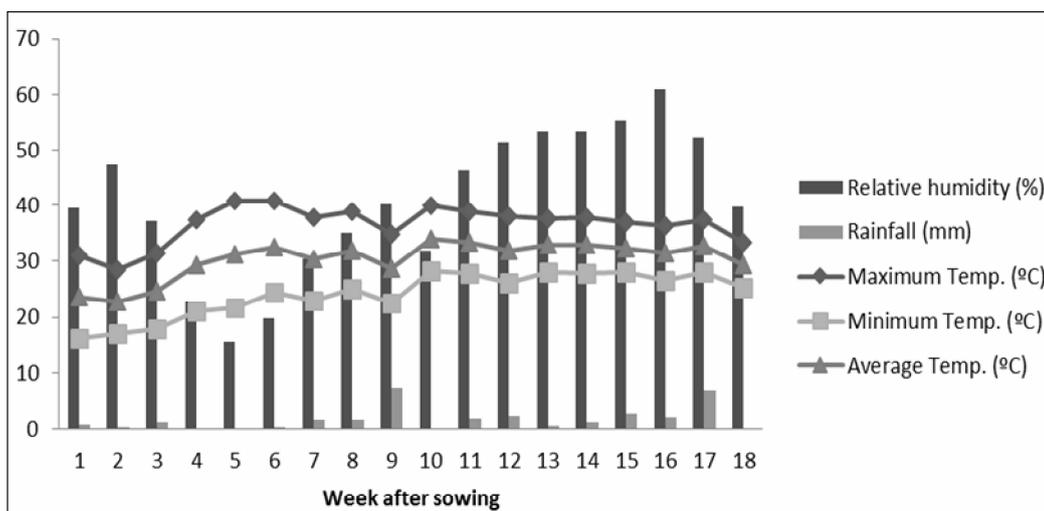


Figure 2. Weekly meteorological data of the experimental site during 2008.



Results

The results indicated that exogenous application of ascorbic acid (AsA), salicylic acid (SA) and hydrogen peroxide (H₂O₂) either through seed priming or foliar spray improved the physiological, biochemical, morphological and yield related traits and grain yield of spring sown hybrid maize in both years (Table 1-3).

In the first year, the maximum SOD activity was recorded when seeds were primed with H₂O₂ and

it was statistically at par with all other treatments (Table 1), while SOD activity was lowest in the seeds which were neither primed nor foliar sprayed with any chemical in the first year (Table 1). Maximum SOD activity in the second year was recorded when seeds were primed with SA while it was lowest when seeds were either primed or foliar sprayed with AsA (Table 1). During both years, maximum CAT activity was recorded when plants were foliar sprayed with SA followed by weed priming with H₂O₂, while it was lowest in control (Table 1).

Table 1. Impact of exogenous application of ascorbic acid, salicylic acid and hydrogen peroxide on chlorophyll contents and activities of superoxide dismutase, catalase and peroxidase, in heat stressed spring sown hybrid maize in both years.

Treatments	Superoxide dismutase (mg ⁻¹ pr.)		Catalase (mg ⁻¹ pr.)		Peroxidase (mg ⁻¹ pr.)		Chlorophyll a (mg 100mL ⁻¹)		Chlorophyll b (mg 100mL ⁻¹)	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Control	16.45b	14.79cd	2.13e	1.13d	1.79c	0.80d	2.30d	1.65e	0.96b	0.85e
SP-AsA	20.94a	13.94d	2.60d	1.50c	2.88ab	0.88b	2.51c	1.47f	1.25a	0.79f
FS-AsA	21.28a	13.94d	2.61d	1.61c	2.90a	0.90a	2.70b	1.67d	1.22a	0.80f
SP-SA	21.57a	17.90a	2.97c	1.97b	2.87b	0.87c	2.77ab	1.63e	1.19a	1.71a
FS-SA	21.36a	16.36b	3.18a	2.18a	2.88ab	0.88b	2.85a	1.82c	1.23a	1.12c
SP-H ₂ O ₂	22.08a	16.74ab	3.11ab	2.11a	2.90a	0.90a	2.82ab	1.95a	1.19a	1.68b
FS-H ₂ O ₂	21.56a	15.90bc	2.99bc	1.95b	2.89a	0.89a	2.86a	1.86b	1.23a	1.00d
LSD	1.215	1.293	0.128	0.119	0.017	0.0098	0.136	0.023	0.08	0.019

Means sharing a letter in a column did not differ significantly $P < 0.05$; LSD: Least significant difference. SP-ASA: Seed primed with ascorbic acid, FS-ASA: Foliar application of ascorbic acid SP-SA: seed primed with salicylic acid, FS-SA: Foliar application of salicylic acid, SP-H₂O₂: seed primed with hydrogen peroxide, FS-H₂O₂: Foliar application of hydrogen peroxide; Pr.= Protein.

We noted that POD activities were significantly higher and similar when AsA was foliar applied or seeds were primed with H₂O₂ and it was followed by foliar application of H₂O₂ in both years (Table 1). In the first year, maximum chlorophyll contents were observed when H₂O₂ was foliar applied followed by foliar application of SA, while it was highest when seeds were primed with H₂O₂ in the second year (Table 1). In the first year, maximum chlorophyll *b* contents were recorded when seeds were primed with AsA and it was statistically similar with the all other treatments except control, while during second year chlorophyll *b* contents were highest when seeds were primed with SA (Table 1). In both years, minimum POD activity and chlorophyll *a* and *b* contents were recorded in the seeds which were neither primed nor foliar sprayed (Table 1). Similarly, the highest number of plants per unit area was observed when seeds were primed with AsA, SA or H₂O₂ in the first year (Table 2). However, in the second year plants per unit area were maximum when seeds were primed with AsA (Table 2). Cobs per plant were highest when seeds were primed with H₂O₂ and it was statistically similar when seeds were primed with SA and seed priming and foliar spray of AsA in the first year (Table 2). During the second year, cobs per plant

were maximum and similar when H₂O₂ was foliar sprayed or seeds were primed with SA (Table 2). In both years, grains per cob were highest when seeds were primed with H₂O₂, however it was statistically similar with the all rest of treatments in the first year (Table 2). Seed priming with H₂O₂ produced maximum con length in the first year, while during the second year, cob length was highest when seeds were primed with AsA. However, all the seed priming and foliar spray treatments were statistically similar with each other except control (Table 1). 100-grain weight was maximum when seeds were primed with AsA or were foliar sprayed with H₂O₂ in the first and second year respectively but both all treatments were statistically par with each other except control which gave lowest grain weight (Table 2). Grain yield was maximum when seeds were foliar sprayed with H₂O₂ or AsA in the first and second year respectively, but all treatments were statistically at par with other in both years except control where seeds were neither primed nor foliar sprayed (Table 2). Highest protein contents were recorded when seeds were neither primed nor foliar sprayed in both years while they were lowest when seeds were primed with AsA followed by foliar application of SA in both years (Table 3). In the first year, maximum grain oil contents were

recorded when seeds were primed with AsA or were foliar sprayed with H₂O₂ but they were statistically at par with the other treatments except control (Table 3). In the second year, maximum grain oil contents were recorded when seeds were primed with AsA (Table 3). Membrane stability index and relative water contents were highest when seeds

were foliar sprayed with SA in both years, however, it was followed by seed priming with H₂O₂ in the first year and was statistically at par with all the treatment except control in the second year (Table 3). Minimum MSI and RWC were recorded when seeds were neither primed nor foliar sprayed with any chemical (Table 3).

Table 2. Impact of exogenous application of ascorbic acid, salicylic acid and hydrogen peroxide on morphological and yield related traits and grain yield of heat stressed spring sown hybrid maize in both years.

Treatments	Plants per unit area (m ⁻²)		Cobs per plant		Grains per cob		Cob length (cm)		100-grain weight (g)		Grain yield (Mgha ⁻¹)	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Control	10.0b	10.0b	1.07b	1.20b	357.7b	349.7c	16.3b	16.2b	22.7b	22.4a	3.73b	3.63b
SP-AsA	11.0a	10.7a	1.27a	1.33ab	389.0a	369.7bc	18.6a	18.4a	23.9a	22.2a	4.66a	4.30a
FS-AsA	10.0b	10.3ab	1.27a	1.33ab	394.0a	380.3b	18.7a	18.2a	23.7a	22.2a	4.65a	4.50a
SP-SA	10.7a	10.3ab	1.27a	1.40a	398.3a	393.3ab	18.6a	18.1a	23.5a	22.1a	4.52a	4.33a
FS-SA	10.0b	10.0b	1.20ab	1.20b	402.7a	376.7bc	18.8a	18.2a	23.6a	21.9a	4.63a	4.20a
SP-H ₂ O ₂	10.7a	10.3ab	1.33a	1.27ab	400.0a	410.3a	18.8a	18.2a	23.5a	21.8a	4.56a	4.40a
FS-H ₂ O ₂	10.0b	10.0b	1.20ab	1.40a	399.7a	386.3ab	18.7a	18.3a	23.5a	20.9b	4.81a	4.13a
LSD	0.501	0.614	0.179	0.161	13.9	27.95	0.27	0.47	0.5	0.8	0.33	0.46

Means sharing a letter in a column did not differ significantly $P < 0.05$; LSD: Least significant difference. SP-ASA: Seed primed with ascorbic acid, FS-ASA: Foliar application of ascorbic acid SP-SA: seed primed with salicylic acid, FS-SA: Foliar application of salicylic acid, SP-H₂O₂: seed primed with hydrogen peroxide, FS-H₂O₂: Foliar application of hydrogen peroxide.

Table 3. Impact of exogenous application of ascorbic acid, salicylic acid and hydrogen peroxide on grain quality, ear leaf membrane stability index and relative water contents of heat stressed spring sown hybrid maize in both years.

Treatments	Grain protein contents (%)		Grain oil contents (%)		Ear leaf membrane stability index (%)		Relative water contents (%)	
	2007	2008	2007	2008	2007	2008	2007	2008
Control	7.90a	8.06a	4.28b	4.32b	57.5c	50.1b	69.5c	70.1b
SP-AsA	7.71c	7.64c	4.45a	4.41a	61.4b	52.4a	73.4b	72.4a
FS-AsA	7.80bc	7.84b	4.44a	4.35ab	61.5b	52.4a	73.5b	72.4a
SP-SA	7.86ab	7.89b	4.44a	4.37ab	61.7ab	52.4a	73.7ab	72.4a
FS-SA	7.78bc	7.64c	4.44a	4.37ab	62.0a	52.6a	74.0a	72.6a
SP-H ₂ O ₂	7.84ab	7.84b	4.44a	4.34ab	61.97a	52.3a	74.0a	72.3a
FS-H ₂ O ₂	7.85ab	7.86b	4.45a	4.32b	61.53b	52.3a	73.5b	72.2a
LSD	0.09	0.09	0.06	0.07	0.40	0.53	0.4	0.5

Means sharing a letter in a column did not differ significantly $P < 0.05$; LSD: Least significant difference. SP-ASA: Seed primed with ascorbic acid, FS-ASA: Foliar application of ascorbic acid SP-SA: seed primed with salicylic acid, FS-SA: Foliar application of salicylic acid, SP-H₂O₂: seed primed with hydrogen peroxide, FS-H₂O₂: Foliar application of hydrogen peroxide.

Discussion

This study clearly depicted that exogenous application of antioxidants (AsA), plant growth regulators (SA) and osmoprotectants (H_2O_2) improved the physiological, biochemical, morphological and yield related traits and grain yield of spring sown hybrid maize under late sown conditions when crop was subjected to high temperature stress at its reproduction stage which was visible through enhanced MSI, RWC and chlorophyll contents, higher catalase and peroxidase activities, enhanced grain weight and number thus resulting in increased yield (Table 1-3). We observed that heat stress at anthesis in late sown spring hybrid maize negatively influenced the growth, physiology and grain yield of maize which might be due to over production of reactive oxygen species (ROS) which may have impaired balance between light absorption and its utilization ultimately inhibiting Calvin cycle activity of carbon fixation (LOGAN et al., 2006), over reduction of respiratory electron transport chain (HU et al., 2008) and reduction in the Rubisco activity (ZHOU et al., 2006). However, in this study we observed that exogenous application of AsA, SA or H_2O_2 either through seed priming or foliar spray improved seedling growth, physiological and biochemical, yield attributes and grain yield which might be due to mitigation of injurious effects of ROS on light harvesting apparatus and fixation of CO_2 in maize due to exogenous application of AsA, SA and H_2O_2 (LOGAN et al., 2006). In the present study, chlorophyll *a* and *b* contents were increased with application of AsA, SA and H_2O_2 in heat stressed conditions (Table 1), which might be due to enhancement in antioxidants which may have protected chlorophyll from degradation. In some other studies, exogenous application of AsA, SA and H_2O_2 as seed priming and foliar application increased chlorophyll contents in crop plants (RIVAS-SAN; PLASENCIA, 2011). In a study, Sakr and Arafa (2009) documented that chlorophyll contents were increased with exogenous application

of antioxidants in stress conditions as was observed in this study. Moreover, exogenous application of AsA, SA and H_2O_2 improved ear leaf RWC and membrane stability index (MSI) in late sown spring maize, which might be due to protective effects of AsA, SA and H_2O_2 on membrane degradation of late sown hybrid maize under heat stressed conditions. In earlier studies, it is well documented that exogenously application of AsA, SA and H_2O_2 stabilizes the internal membrane system under heat stress (GONG et al., 2001). We observed an increase in antioxidants activities. Recent study reported that SOD, POD and CAT activities were increased with SA, AsA and H_2O_2 at low concentration in finger millet (APPU; MUTHUKRISHNAN, 2014).

In this study, the salient components of grain yield showed that seed priming and foliar spray of AsA, SA and H_2O_2 were beneficial in improving growth, yield related traits and grain yield of maize under heat stressed conditions during both years. Improvement in yield related traits and grain yield in this study might be due to amelioration of the injurious effects of chilling injury on light harvesting apparatus and CO_2 fixation in maize plants due to exogenous application of these chemicals (LOGAN et al., 2006), which maintained maize growth in stress conditions. Moreover, improved grain yield due to exogenous application of these chemicals may be due to membrane stabilization and improved antioxidant activity which may have helped the maize crop to maintain normal photosynthesis under heat stress thus resulting in more grain number per cob and increased grain weight, which ultimately resulted in higher grain yield. Moreover, grain quality traits like grain oil contents were improved through exogenous application of these effectors compounds. More grain oil contents might be due to increased antioxidants activities while lower grain protein contents might be due involvement of proteins molecule in signaling transaction during stressful environmental conditions.

Conclusions

In conclusion, the exogenous application of AsA, H₂O₂ and SA through seed priming or foliar spray ameliorated the adverse effects of high temperature stress in late sown spring maize by improving growth, stabilization of membranes and by induction of antioxidants activities of SOD, POD and CAT enzymes. These positives effects resultantly improved grain yield through increase in grain number and size under heat stressed conditions during both years.

References

- AHMAD, I.; BASRA, S. M. A.; HUSSAIN, S.; HUSSAIN, S. A.; HAFEEZ-UR-REHMAN; REHMAN, A.; ALI, A. Priming with ascorbic acid, salicylic acid and hydrogen peroxide improves seedling growth of spring maize at suboptimal temperature. *Journal of Environmental and Agricultural Sciences*, Sarghoda, v. 3, p. 14-22, 2015.
- APEL, K.; HIRT, H. Reactive oxygen species: metabolism, oxidative stress and signal transductions. *Annual Review of Plant Biology*, Palo Alto, v. 55, n. 1, p. 373-399, 2004.
- APPU, M.; MUTHUKRISHNAN, S. Foliar application of salicylic acid stimulates flowering and induce defense related proteins in finger millet plants. *Universal Journal of Plant Science*, Tamil Nadu, v. 2, n. 1, p. 14-18, 2014.
- BARTH, C.; DE-TULLIO, M.; CONKLIN, P. L. The role of ascorbic acid in the control of flowering time and the onset of senescence. *Journal of Experimental Botany*, Oxford, v. 57, n. 8, p. 1657-1665, 2006.
- CHANCE, M.; MAEHLI, A. C. Assay of catalases and peroxidases. *Methods Enzymology*, New York, v. 2, p. 764-775, 1955.
- CHEIKH, N.; JONES, R. J. Disruption of maize kernel growth and development by heat stress. *Plant Physiology*, Florence, v. 106, n. 1, p. 45-51, 1994.
- CONKLIN, P. L.; BARTH, C. Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens and the onset of senescence. *Plant Cell and Environment*, Logan, v. 27, n. 8, p. 959-971, 2004.
- FOYER, C. H.; NOCTOR, G. Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiology Plantarum*, Denmark, v. 119, p. 355-364, 2003.
- GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiology*, Florence, v. 59, n. 2, p. 309-314, 1977.
- GONG, M.; CHEN, B.; LI, Z. G.; GOU, L. H. Heat-shock-induced cross adaptation to heat, chilling, drought and salt in maize seedlings and involvement of H₂O₂. *Journal of Plant Physiology*, Stuttgart, v. 158, n. 9, p. 1125-1130, 2001.
- HU, W. H.; SONG, X. S.; SHI, K.; XIA, X. J.; ZHOU, Y. H.; YU, J. Q. Changes in electron transport, superoxide dismutase and ascorbate peroxidase isoenzymes in chloroplasts and mitochondria of cucumber leaves as influenced by chilling. *Photosynthetica*, Rozvojová, v. 46, n. 4, p. 581-588, 2008.
- JOHNSON, R.; HERRERO, M. P. Corn pollination under moisture and high temperature stress. In: CORN AND SORGHUM INDUSTRY RESEARCH CONFERENCE, 1981, Chicago. *Proceedings* Chicago: American Seed Trade Association, Washington, 1981. p. 66-77.
- KUMAR, M.; SIRHINDI, G.; BHARDWAJ, R.; KUMAR, S.; JAIN, G. Effect of exogenous H₂O₂ on antioxidant enzymes of *Brassica juncea* L. seedlings in relation to 24-epibrassinolide under chilling stress. *Indian Journal of Biochemistry and Biophysics*, New Delhi, v. 47, n. 6, p. 378-382, 2010.
- LOGAN, B.A.; KORNIEYEV, D.; HARDISON, J.; HOLADAY, A. S. The role of antioxidant enzymes in photoprotection. *Photosynthesis Research*, Baton Rouge, v. 88, n. 2, p. 119-132, 2006.
- MITCHELL, J. C.; PETOLINO, J. F. Heat stress effects on isolated reproductive organs of maize. *Journal of Plant Physiology*, Stuttgart, v. 133, n. 5, p. 625-628, 1988.
- NAGATA, M.; YAMASHITA, I. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Journal of Japan Society of Food Science and Technology*, Tokyo, v. 39, n. 10, p. 925-928, 1992.
- NOCTOR, G.; FOYOR, C. H. Ascorbate and glutathione: keeping active oxygen under control. *Annual of Review of Plant Physiological and Molecular Biology*, Palo Alto, v. 49, n. 1, p. 249-279, 1998.
- RAMADOSS, M.; BIRCH, C. J.; CARBERRY, P. S.; ROBERTSON, M. Water and high temperature stress effects on maize production. In: INTERNATIONAL CROP SCIENCE CONGRESS, 4., 2004, Brisbane. *Proceedings...* Brisbane: [s.n.], 2004.
- REDDY, S. R. *Principles of crop production*. 2th ed. New Delhi: Kalyani Publishers, 2004. 354 p.

RIVAS-SAN, M.M. V.; PLASENCIA, J. Salicylic acid beyond defense: its role in plant growth and development. *Journal of Experimental Botany*, Oxford, v. 62, n. 10, p. 3321-3338, 2011.

SAIRAM, R. K. Effect of moisture stress on physiological activities of two contrasting wheat genotypes. *Indian Journal of Experimental Biology*, New Dehli, v. 32, p. 594-597, 1994.

SAKR, M. T.; ARAFA, A. A. Effect of some antioxidants on canola plants grown under soil salt stress condition. *Pakistan Journal of Biological Sciences*, Faisalabad, v. 12, n. 7, p. 582-588, 2009.

SMIRNOFF, N.; WHEELER, G. L. Ascorbic acid in plants: biosynthesis and function. *Critical Review of Biochemistry and Molecular Biology*, Bethesda, v. 35, n. 4, p. 291-314, 2000.

STEEL, R. G. D.; TORRIE, T. H.; DICKEY, D. A. Principles and procedures of statistics. A biometrical approach. 3thed. Singapore: McGraw Hill Book International Co. Inc. New York, 1997. p. 400-428.

TARIQ, M.; KHAN, M. A.; PARVEEN, S. Response of maize to applied soil Zinc. *Asian Journal of Plant Science*, Peshawar, v. 1, n. 4, p. 476-477, 2002.

ZHOU, Y. H.; YU, J. Q.; MAO, W. H.; HUANG, L. F.; SONG, X. S.; NOGUES, S. Genotypic variation of Rubisco expression, photosynthetic electron flow and antioxidant metabolism in the chloroplasts of chill-exposed cucumber plants. *Plant Cell Physiology*, Zhejiang, v. 47, n. 2, p. 192-199, 2006.