INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
14–029/2014/16–4–825–830
http://www.fspublishers.org

Full Length Article

Exogenous Application of Ascorbic acid, Salicylic acid and Hydrogen peroxide Improves the Productivity of Hybrid Maize at Low Temperature Stress

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Abstract

Maize being subtropical crop is sensitive to low temperature at early growth stages. Exogenous application of ascorbic acid (AsA), salicylic acid (SA) and hydrogen peroxide (H_2O_2) can improve the seedling growth of maize at early growth stages. In these studies, the effect of exogenous application of AsA, SA and H_2O_2 to improve the maize performance at sub-optimum temperatures was investigated in pots and field experiments. . In pot experiment, AsA, SA and H_2O_2 were foliage applied at 20 or 40 mg L^{-1} at 3rd leaf stage. In field experiment, these three substances were applied as seed priming or as foliar spray. In pot experiment, foliar application of AsA, SA and H_2O_2 at each concentration improved seedling growth, leaf relative water, chlorophyll *b* contents, membrane stability and enzymatic antioxidant activities in maize. In field experiment, application of these substances either through seed priming or foliar spray improved the morphological, yield related attributes and grain yield of spring maize; however, seed priming was more effective than foliar application. In conclusion, the productivity of hybrid maize can be improved by seed priming with AsA, SA and H_2O_2 under low temperature stress. © 2014 Friends Science Publishers

Keywords: Maize; Chilling injury; Antioxidants; Plant growth regulators; Osmoprotectants; Grain yield

Introduction

Maize (*Zea mays* L.) is a high yielding cereal crop and is ranked as third important cereal crop in Pakistan (Tariq *et al.,* 2002). It is 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). Maize is a warm season crop and is grown under extremely divergent climatic conditions ranging from tropical to temperate region. This crop can be successfully grown in areas where the night temperature does not go below 15ºC as crop stops growing below this level (AGRISNET, 2012). Maize crop is very sensitive to low temperature at early growth stages and studies have shown that low temperature below 15ºC suppresses photosynthetic capacity of maize's seedling through destroying cellular membranes, photosynthetic apparatus and cellular enzymes (Lukatkin, 2003; Apel and Hirt, 2004; Marccoo *et al*., 2005; Farooq *et al*., 2009), which may be due to increased production of reactive oxygen species (ROS) resulting in poor crop seedling stand establishment (Guan *et al*., 2009).

Plant species have evolved various mechanisms to cope with environmental stresses. For example, to mitigate chilling induced damage, plants may up-regulate various scavenging mechanism like enzymatic antioxidants (superoxide dismutase, peroxidase and catalase), non-enzymatic metabolites e.g., ascorbic acid (Gill and Tuteja, 2010), plant growth regulators (salicylic acid) and osmoprotectants (stress signaling substance) (Wahid *et al*., 2007; Farooq *et al*., 2008; Gautam and Singh, 2009; Ahmad *et al*., 2013). These chemicals protect membranes and photosynthetic apparatus from the injurious effects caused by environmental stresses (Foyer and Noctor, 2003).

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 and Wheeler, 2000; Farooq *et al*., 2013). AsA also modulates the tocopherol synthesis, which protects the plant from several environmental stresses (Conklin and Barth, 2004). Likewise, salicylic acid (SA) is an important signaling molecule in plants (Chen *et al*., 2009), which helps to regulate plant resistance against chilling stress (Farooq *et al*., 2008; Ahmad *et al*., 2013). Hydrogen peroxide (H_2O_2), acts as signaling molecule and assistant in triggering stress resistance mechanism (Kumar *et al*., 2010). Exogenous application of these chemicals helps in improving the

To cite this paper: Ahmad, I., S.M.A. Basra and A. Wahid, 2014. Exogenous application of ascorbic acid, salicylic acid and hydrogen peroxide improves the productivity of hybrid maize under at low temperature stress. *Int. J. Agric. Biol.,* 16: 825–830

resistance against chilling in field crops (Kumar *et al*., 2010; Ahmad *et al*., 2013). However, little is known about the proper concentration of these chemicals to mitigate chilling induced changes in plants. Moreover, field appraisal of exogenously applied chemicals is also lacking. This study was aimed to explore the role of exogenous application of these chemicals on morphology, biochemical attributes and yield related traits of spring hybrid maize planted at low temperature.

Materials and Methods

Two independent experiments (pot and field) were carried out to investigate the role of exogenous application of AsA, SA and H_2O_2 on morphology, biochemical attributes and grain yield of spring maize. The first experiment was conducted in sand-filled pots during February-March, 2008 in the net house of Department of Crop Physiology, University of Agriculture, Faisalabad, Pakistan (latitude = 31°30'N, longitude =73°10'E and altitude = 184.4 m). The maize hybrid Hi Sawn 9697 was used as experimental material. This experiment was laid down in completely randomized design with three replications. Crop was sown on 01 February, 2008. Initially 15 seeds were sown in each pot, containing 10 kg thoroughly washed sand, which were thinned to maintain 10 plants after uniform emergence. Hoagland solution was applied to nourish the plants. Foliar spray of AsA, SA and H_2O_2 (each 0, 20 and 40 mgL^{-1}) was applied at 3 leaf seedling stage (18 days after sowing). After 35 days of sowing, five uniform seedlings were harvested from each pot sowing and were analyzed for seedling vigor and antioxidants activity. Fresh weight of shoots and roots of 5 plants was recorded and was averaged. These shoots and roots samples were over dried till constant weight to record dry weight of shoot and root. Leaf area was measured with a simple ruler. The field experiment was conducted at Experimental Station, University of Agriculture, Faisalabad, during 2008. The experimental soil was loamy having organic matter 0.66, pH 7.41, ECe 0.393 dS m^{-1}, available K 350 ppm and available P 12.82 ppm. This experiment was laid out in randomized complete block design with three replications and net plot size of 7.7 m × 4.2 m. The experiment consisted of following treatments viz. AsA, SA and H_2O_2 were applied as seed priming or foliar spray. For priming, maize seeds were soaked in solutions containing 20 mg L^{-1} either of AsA, H_2O_2 and SA for 24 h maintaining seed to water ratio of 1:5. Primed and non-primed were sown on 1st February 2008. The crop was planted in 60 cm spaced rows with a dibbler with two seeds per hole maintaining 15 cm distance between two holes. After uniform emergence, one plant per hole was maintained. After 58 days of sowing, 20 mgL^{-1} each of AsA, H_2O_2 and SA were foliar applied. 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 7th June 2008. Before harvesting plant population per unit area was measures 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 t ha^{-1}. 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. Weather data during the course of investigation of both studies is made (Table 1).

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 80ºC, 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 cm^3 test tube containing doubled distilled water (Sairam, 1994). One set of test tubes was heated at 40ºC for half an h while second set was boiled at 100ºC in water bath for 10 min and their ECs were measured through Conductivity Bridge as C_1 and C_2, respectively.

Determination of Chlorophyll Contents

For determination of chlorophyll contents, segments of 0.5 cm of fresh leaves were prepared and extracted overnight with 80% acetone at -10ºC. The extracts of each sample were centrifuged at14000 × *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 × *g* for 20 min at 4ºC. The supernatant was used for assays of enzyme activity.

Table 1: Meteorological data during the experimental period

Weeks after sowing	Maximum Temp. (°C)	Minimum Temp. (°C)	Average Temp. (°C)	Relative Humidity (%)	Rainfall (mm)	Sunshine (h)
1	16.2	4.5	10.4	43.4	0.9	4.0
2	17.0	2.9	10.0	32.1	0.1	7.5
3	24.3	7.3	15.8	39.0	0.0	8.8
4	23.4	7.7	15.5	34.6	0.0	7.0
5	28.9	12.2	20.6	43.1	0.0	8.4
6	29.0	13.1	21.1	40.1	0.0	8.3
7	31.9	15.4	23.7	34.6	0.0	9.5
8	31.9	15.3	23.6	35.1	0.0	9.2
9	32.7	16.9	24.8	36.4	0.8	7.5
10	27.4	16.4	21.9	50.3	0.3	6.5
11	31.4	18.4	24.9	38.7	1.2	8.3
12	36.1	20.6	28.4	24.1	0.0	10.1
13	40.5	21.1	30.8	16.3	0.0	10.4
14	41.6	24.9	33.3	15.9	0.0	8.7
15	37.2	22.6	28.2	32.9	1.8	9.8
16	39.7	25.2	32.5	31.7	1.6	6.5
17	34.1	22.4	28.3	43.7	7.3	8.8
18	39.6	27.3	33.5	30.9	0.0	9.4

Detection of Superoxide Dismutase

The activity of SOD was detected by measuring its ability to inhibit photoreduction of nitro blue tetrazolium (NBT) using the method of Giannopolitis and Ries (1977). The reaction solution (3 mL) contained 50 µM NBT, 1.3 µM riboflavin, 13 mM methionine, 75 nM EDTA, 50 mM phosphate buffer (pH 7.8) and 50 µL enzyme extract. Test tubes containing the reaction solution and leave were irradiated under light bank (15 flouresant lamps) at 78 µmol m^{-2} 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 20 sec. 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 sec. One unit CAT and POD activity is defined as an absorbance change of 0.01 units/min.

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*., 1996).

Results

Pot Experiment

The results indicated that foliar application of AsA, H_2O_2 and SA improved the growth of maize than control, which was apparent through increased fresh and dry weights of shoots and roots (Table 2). Maximum shoot fresh weight was recorded when H_2O_2 was applied at 40 mL L^{-1} followed by SA (20 mg L^{-1}), H_2O_2 (20 mL L^{-1}), AsA (20 mg L^{-1}), AsA (40 mg L^{-1}) and SA (40 mg L^{-1}); however all these treatments were at par with each other (Table 2). Similarly, foliar spray of H_2O_2, SA and AsA improved shoot dry weight than control but it was maximum from the application of AsA at 40 mg L^{-1} followed by AsA (20 mg L^{-1}), H_2O_2 (40 L L^{-1}), SA (20 mg L^{-1}), H_2O_2 (20 mL L^{-1}) and SA (40 mg L^{-1}) (Table 2). Similarly, root dry weight was maximum when SA was applied at 40 mg L^{-1} followed by H_2O_2 (40 mL L^{-1}), AsA (40 mg L^{-1}), H_2O_2 (20 mL L^{-1}), SA (20 mg L^{-1}) and AsA (20 mg L^{-1}). Maximum leaf area per plant was recorded when H_2O_2 was applied @ 20 mL L^{-1} followed by H_2O_2 (40 mL L^{-1}) and SA (20 mg L^{-1}), while lowest leaf area per plant was recorded in control treatment (Table 2).

Foliar application of AsA, H_2O_2 and SA improved biochemical traits of maize as indicated by increased membrane stability index, leaf relative water contents, chlorophyll b contents and higher catalase and peroxidase activities (Table 2). Maximum relative leaf water contents were recorded when H_2O_2 was applied @ 40 mL L^{-1} followed by AsA (20 mg L^{-1}), H_2O_2 (20 mL L^{-1}), SA (40 mg L^{-1}), SA (20 mg L^{-1}) and AsA (40 mg L^{-1}), while lowest leaf relative water contents were recorded in control (Table 2). Similarly, highest membrane stability index was achieved when SA was applied @ 20 mg L^{-1} followed by H_2O_2 (20 mL L^{-1}), AsA (40 mg L^{-1}) and AsA (20 mg L^{-1}). However lowest membrane stability index was recorded in control followed by SA application @ 40 mg L^{-1} (Table 2). Foliar application of H_2O_2 was applied @ 20 mg L^{-1} resulted in maximum choloropyll b contents, which was followed by SA or AsA (40 mg L^{-1}), SA or H_2O_2 (20, 40 mL L^{-1} respectively), and AsA (20 mg L^{-1}). Lowest choloropyll b contents were observed in control (Table 2). Maize plants without foliar application of these chemicals showed highest chlorophyll *a:b* ratio followed by foliar application of H_2O_2@ 40 mL L^{-1}, H_2O_2 (20 mL L-1) and AsA (40 mg L^{-1}). Chlorophyll *a:b* ratio was lowest when SA was applied @ 20 mg L^{-1} (Table 2). Catalase activity was recorded maximum when H_2O_2 was applied @ 40 mL L^{-1} followed by AsA (20 mg L^{-1}), SA or H_2O_2 (40, 20 mg/mL L^{-1} respectively), SA (20 mg L^{-1}) and AsA (40 mg L^{-1}), while lowest catalase activity was observed in control (Table 2).

Table 2: Influence of foliar applied ascorbic acid (AsA), salicylic acid (SA) and hydrogen peroxide (H_2O_2) on seedling growth and biochemical parameters of spring hybrid maize

Treatments	Shoot Fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root shoot ratio	Leaf area per plant (cm^2)	Relative water contents (%)	Membrane stability index (%)	Chlorophyll *a:b* ratio	Chlorophyll *b* (mg 100 mL^{-1})	Catalase activity (unit 100 mg^{-1} Pr.)	Peroxidase activity (unit 100 mg^{-1} Pr.)	SOD/[CAT+POD] activity ratio
Control	52.23 b	7.98 b	6.37 b	0.91 b	0.122 a	140.67 d	78.22 b	48.85 e	2.62 a	0.93 b	15.87 b	4.42 b	0.635 a
AsA (20 mg L^{-1})	67.53 a	9.57 a	7.69 a	1.05 a	0.110 b	176.00 c	79.92 a	54.77 ab	2.52 b	1.06 a	19.82 a	5.78 a	0.483 e
AsA (40 mg L^{-1})	67.35 a	9.75 a	7.82 a	1.07 a	0.110 b	178.67 c	79.56 a	54.90 ab	2.55 ab	1.09 a	19.75 a	5.75 a	0.496 de
SA (20 mg L^{-1})	67.61 a	9.46 a	7.60 a	1.06 a	0.112 b	186.00 ab	79.67 a	55.32 a	2.41 c	1.08 a	19.77 a	5.91 a	0.505 cd
SA (40 mg L^{-1})	67.15 a	9.42 a	8.05 a	1.10 a	0.116 ab	182.67 bc	79.77 a	52.92 c	2.51 b	1.09 a	19.81 a	5.97 a	0.509 cd
H_2O_2 (20 mL L^{-1})	67.54 a	9.44 a	7.82 a	1.07 a	0.114 b	190.67 a	79.83 a	55.26 a	2.55 ab	1.10 a	19.81 a	5.91 a	0.535 b
H_2O_2 (40 mL L^{-1})	67.65 a	9.48 a	7.94 a	1.09 a	0.115 ab	189.00 ab	80.11 a	53.57 b	2.58 ab	1.08 a	19.83 a	5.99 a	0.517 c
LSD	*0.33*	*0.38*	*0.16*	*0.07*	*0.007*	*6.93*	*0.65*	*1.34*	*0.08*	*0.08*	*0.45*	*0.30*	*0.02*

Figures sharing same letter did not differ significantly at 0.05 level of probability (Pr. = Protein)

Table 3: Influence of seed priming and foliar application of ascorbic acid (AsA), salicylic acid (SA) and hydrogen peroxide (H_2O_2) on morphological, biochemical, yield related traits and grain yield of spring maize in field conditions at low temperature

Treatments	Leaf area index	Chlorophyll b contents (mg 100 mL^{-1})	Chlorophyll a:b ratio	Superoxide dismutase (units mg^{-1} Pr.)	Peroxidase (units mg^{-1} Pr.)	Catalase (units mg^{-1} Pr.)	SOD/[CAT+POD] activity ratio	Plants per unit area	Grains per cob	100-Grain weight (g)	Grain yield (Mg ha^{-1})
Control	3.68c	0.61 f	1.21 a	1.33 d	0.80 b	1.33 d	7.13 A	8.3 b	412 c	28.50 b	3.94 c
FS-AsA	4.28ab	0.67 e	0.71 c	1.70 c	0.80 b	1.70 c	5.57 B	9.3 a	441 bc	29.06 ab	4.85 ab
FS-H_2O_2	4.32 a	0.68de	0.72 bc	2.13ab	0.90 a	2.13 ab	5.41 B	9.3 a	484 a	28.91 ab	4.70 b
FS-SA	4.27ab	1.39 a	0.72 bc	2.20 a	0.90 a	2.20 a	5.38 B	9.3 a	426 c	28.90 ab	4.79 ab
SP-AsA	4.33 a	0.78 c	0.73 bc	1.80 bc	0.83 b	1.80 bc	5.29 B	10.0 a	442 bc	28.52 b	4.87 ab
SP-H_2O_2	4.26ab	0.69 d	0.73 bc	2.10 ab	0.80 b	2.10 ab	5.73 B	10.0 a	472 ab	28.76 ab	4.90 ab
SP-SA	4.22 b	1.24 b	0.74 b	1.80 bc	0.80 b	1.80 bc	6.86 A	10.0 a	443 bc	29.28 a	4.94 a
LSD	*0.09*	*0.01*	*0.03*	*0.35*	*0.04*	*0.36*	*0.76*	*0.67*	*40.11*	*0.58*	*0.21*

Figures sharing same letter did not differ significantly at 0.05 level of probability (Pr. = Protein)

Similarly, peroxidase activity was highest when H_2O_2 was applied @ 40 mL L^{-1} followed by SA (40 mg L^{-1}), SA or H_2O_2 (20 mg/mL L^{-1}), SA (20 mg L^{-1}), AsA (20 mg L^{-1}) and AsA (40 mg L^{-1}) while control treatment showed lowest peroxidase activity (Table 2). Results indicated that maximum SOD/CAT+POD ratio was recorded in control while among the foliar appications; it was lowest when AsA was applied @ 20 mg L^{-1}followed by AsA application at rate of 40 mg L^{-1} (Table 2).

Field Experiment

Morphological, Biochemical and Yield Related Traits

The results indicated that foliar application of seed priming with AsA, SA and H_2O_2 or foliar application of these chemicals improved the morphology, biochemical and yield related attributes and grain yield of maize (Table 3). Maximum leaf area index was recorded when seeds were primed with AsA which was followed by foliar application of H_2O_2. However, minimum leaf area index was recorded in control where the seeds were neither primes, nor the plants were foliar sprayed (Table 3). Similarly, chlorophyll b contents were in the plants in which SA was foliage sprayed, while, minimum chlorophyll b contents were recorded in control (Table 3). Likewise, maximum chlorophyll a:b ratio was noted in control, while it was minimum when AsA was foliar applied on maize plants (Table 3). Regarding biochemical attributes, maximum SOD activity was observed with the application of SA as foliar spray which was followed by foliar spray of H_2O_2 and seed priming of seeds with the same chemical. However, minimum SOD activity was observed in control where the seeds were neither primes, nor the plants were foliar sprayed (Table 3). Moreover, maximum POD activity was recorded when SA and H_2O_2 were applied as foliar spray respectively, while it was minimum in control and this was statistically at par with all the seed priming and foliar application treatments of all chemicals (Table 3). Similarly, enhancement in CAT activity was observed with the foliar application of SA which was followed by foliar spray and seed priming with H_2O_2, while minimum CAT activity was observed in control (Table 3). SOD/CAT+POD ratio was highest in control, which was statistically similar with the priming of seeds with SA, and it was minimum when seeds were primed with AsA and this was at par with all

treatments (Table 3). Seed priming enhanced the plant population than control and foliar treatments. Maximum plant population per unit area was recorded in seeds when they were either primed with ASA, SA and H_2O_2 respectively, while it was minimum in control and it was at par with foliar application of all chemicals (Table 3). Regarding yield related traits, grains per cob were maximum when H_2O_2 was foliar applied which was followed by seed priming with H_2O_2. However, minimum grains per cob were recorded in control followed by foliar application of SA (Table 3). Maximum 100-grain weight was observed when seeds were primed with SA which was followed by foliar application of AsA. However, minimum 100-grain weight was recorded in control (Table 3). Moreover, maximum grain yield was recorded when seeds were primed with SA which was followed by seed priming with of H_2O_2, seed priming with AsA, foliar application of AsA, foliar application of SA and foliar application of H_2O_2 (Table 3).

Discussion

The study clearly depicted that exogenous application of antioxidant (AsA), plant growth regulator (SA) and osmoprotectants (H_2O_2) improved the morphological, biochemical and yield related attributes and grain yield of maize at low temperature which was visible through increased fresh and dry weights of shoots and roots, enhanced membrane stability index, leaf relative water contents, chlorophyll b contents and higher catalase and peroxidase activities, enhanced grain weight, more grain number and increased yield (Tables 2 and 3). However, performance of hybrid maize in terms of growth and bio-chemical attributes was poor at low temperature in pot as well as in field experiment. In previous studies it has documented that chilling stress limits growth of crop plants through over production of ROS which impairs balance between light absorption and its utilization, inhibits Calvin cycle activity (Logan *et al*., 2006), enhances over reduction of respiratory electron transport chain (Hu *et al*., 2008) and reduces the activity of Rubisco (Zhou *et al*., 2006). However, in our recent studies we observed that application of AsA, SA, H_2O_2 either through seed priming or as foliar spray improved seedling growth, morphology, biochemical and yield related traits and grain yield of maize at low temperature (Tables 2, 3), which 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). In this study, AsA, H_2O_2 and SA at any rate improved the fresh and dry weight of shoots and roots at low temperature than control in sand filled pots media (Table 2). Earlier, it has been documented that H_2O_2 at low concentration as a signal molecule in cells in plants (Neill *et al*., 2002), while SA regulates cell growth encouraging cell expansion and save cell structure in stress conditions (Kang *et al*., 2007) which may be the possible reason for seedling growth improvement in maize. In present study, root-shoot ratio was lower in plants where H_2O_2, SA and AsA were exogenously applied than non-treated plants (Table 2). In another study, Roy and Srivastava (1999) also observed that, root and shoot length was improved when AsA was applied in wheat, but root-shoot ratio was decreased. Moreover, this decrease in root-shoot ratio in our study may be due to greater osmotic adjustment in shoots than roots. In present study, chlorophyll *b* contents were increased with exogenous application of AsA, SA and H_2O_2 in maize in pot media as well as in field conditions (Tables 2, 3). This increase in chlorophyll *b* contents might be due to enhancement in antioxidant production at low temperature which may have protected chlorophyll from degradation. In some other studies, foliar application of AsA, salicylic acid and H_2O_2 increased chlorophyll *b* contents in maize (Khodary, 2004) and wheat (Khan, 2007) in stressful conditions. In another study, Sakr and Arafa (2009) documented that chlorophyll contents were increased with the application of antioxidants in canola on a saline soil.

This study also predicted that exogenous application of AsA, SA and H_2O_2 improved the leaf relative water contents and membrane stability index in spring sown hybrid maize (Table 2). This increase in leaf relative water contents might be due to protective effects of exogenous application of these chemicals on membrane degradation in maize subjected to low temperature. It is well documented that exogenously applied AsA, SA and H_2O_2 stabilizes the internal membrane system under stressful conditions. Moreover, chilling caused reduction in CAT and POD activities of maize but the foliar application of AsA, SA and H_2O_2 enhanced the activities of these enzymes in pot as well as in field conditions (Table 2, 3). Higher CAT, SOD and POD activities along with lower SOD/[CAT+POD] due to exogenous application of AsA, SA and H_2O_2 at suboptimal temperature might be due to development of an efficient scavenging system which may have protected membranes from injurious effect of chilling injury through scavenging superoxide radical into H_2O_2 and converting H_2O_2 to O_2. Previously, it is reported that activities of antioxidants enzymes increases with exogenous application of SA (Waseem, 2006), AsA (Khan *et al*., 2006), or H_2O_2 (Gong *et al*., 2001) in several cereal crops. Several recent studies also reported that antioxidant activity was increased with exogenous application of SA, AsA and H_2O_2 at low concentration under stressful environment (Appu and Muthukrishnan, 2014).

Moreover, yield related traits and yield was improved through exogenous application of these chemicals. More 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 low temperature stress thus resulting in more grain number per cob and increased grain weight, which ultimately resulted in higher grain yield.

In conclusion, exogenous application of AsA, H_2O_2 and SA mitigated the adversities of low temperature at early stages of maize by improving growth, stabilizing membranes and by increasing the activity of catalase and peroxidase enzymes. These positives changes eventually resulted in improved maize yield under low temperature stress.

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(Received 11 January 2014; Accepted 3 March 2014)