INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
11–194/2013/15–1–95–100
http://www.fspublishers.org

Full Length Article

Growth Improvement in Spring Maize through Exogenous Application of Ascorbic Acid, Salicylic Acid and Hydrogen Peroxide

Ijaz Ahmad[1*], Shahzad M.A. Basra[1], Irfan Afzal[1], M. Farooq[2] and Abdul Wahid[3]
[1]*Departments of Crop Physiology, University of Agriculture, Faisalabad-38040, Pakistan*
[2]*Departments of Agronomy, University of Agriculture, Faisalabad-38040, Pakistan*
[3]*Departments of Botany, University of Agriculture, Faisalabad-38040, Pakistan*
*For Correspondence: ejazahmad2007@hotmail.com

Abstract

Early plantation of spring maize crop is practiced to avoid heat stress during seed set but low temperature causes poor and erratic stand establishment. Foliar application of antioxidants, plant growth regulators and osmoprotectant can improve early seedling growth. Foliar spray of ascorbic acid (AsA), salicylic acid (SA) and hydrogen peroxide (H_2O_2) @ 20 and 40 mg L^{-1} was applied on spring maize seedlings at 3rd leaf stage. Foliar application improved shoot and root lengths, which was associated with higher superoxide dismutase (SOD), chlorophyll and nutrients contents. A positive correlation was observed between shoot length and shoot N, P and K contents, leaf SOD and chlorophyll contents, while root length was positively correlated with root N, P and K contents. It is concluded that early seedling establishment can be improved by exogenous application of AsA, SA and H_2O_2 owing to enhanced antioxidants defense system and nutrient homeostasis. © 2013 Friends Science Publishers

Keywords: Maize; Foliar spray; Superoxide dismutase; Nutrients

Introduction

Temperature below 10°C induces damage through production of reactive oxygen species (ROS), which cause oxidative damage to various macromolecules and cellular structures (Apel and Hirt, 2004; Marocco *et al*., 2005) and resultantly leads to poor seedling establishment (Guan *et al*., 2009). Previous reports indicated that most conspicuous changes occurred in cellular membrane, photosynthetic apparatus and enzymes under low temperature stress conditions (Lukatkin, 2003; Marocco *et al*., 2005). Several endogenous defense mechanisms against ROS include both enzymes i.e., superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Noctor and Foyer, 1998) and non-enzymatic metabolites like ascorbic acid (AsA) (Farooq *et al*., 2008), salicylic acid (SA) (Gautam and Singh, 2009) and low concentration of H_2O_2 (Wahid *et al*., 2007). They quench oxygen radicals and protect membranes and other cellular structures from their injurious effects (Foyer and Noctor, 2003).

AsA is an antioxidant, which acts as a cofactor for some of the enzymes affecting phytohormone-mediating signaling processes (Noctor and Foyer, 1998; Barth *et al*., 2006). AsA and its associated enzyme, ascorbate peroxidase, play diverse roles in several physiological processes in plants (Smirnoff and Wheeler, 2000). Furthermore, ascorbate enhanced α-tocopherol synthesis, which protects the plant from programmed cell death induced by ROS (Conklin and Barth, 2004). Similarly, salicylic acid is an important commonly occurring signaling molecule in plants (Chen *et al*., 2009) response to adverse environmental conditions like low temperature (Ahmad *et al*., 2012; Farooq *et al*., 2008) and salinity stress (Khan *et al*., 2010). Exogenously applied salicylic acid helps plants to regulate several functions including systemic acquired resistance (SAR) and plant resistance to chilling stress in maize (Farooq *et al*., 2008).

Likewise, it is widely accepted that H_2O_2 acts as plant signaling molecule, mediating the acquisition of tolerance to abiotic stresses and acquired resistance (Slesak *et al*., 2007; Wahid *et al*., 2007). One of consequences of many stresses is an increase in the cellular concentration of ROS, which are subsequently converted to H_2O_2. It was previously reported that exogenously applied H_2O_2 at high concentration caused oxidative stress while low level of H_2O_2 improves chilling tolerance by enhancing antioxidants activities in *Brassica juncea* (Kumar *et al*., 2010) and maize (Chen *et al*., 2009; Wang *et al*., 2010; Ahmad *et al*., 2012) and induced salt tolerance by enhanced activities of antioxidants and reduced peroxidation of membrane lipids in leaves and roots of maize (Azevedo-Neto *et al*., 2005).

It is evident from the above that AsA, SA and H_2O_2 are promising chemicals for foliar application, while their role in low temperature tolerance is scarcely studied. We hypothesize that foliar application of these agents may profoundly modulate the physiological activities and

To cite this paper: Ahmad, I., S.M.A. Basra, I. Afzal, M. Farooq and A. Wahid, 2013. Growth improvement in spring maize through exogenous application of ascorbic acid, salicylic acid and hydrogen peroxide. *Int. J. Agric. Biol*., 15: 95–100

improve low temperature tolerance in maize. The present study was therefore designed to explore the role and optimized the level of AsA, SA and H_2O_2 as foliar agents in alleviation of oxidative damage and improved chilling tolerance of maize.

Materials and Methods

The experiment was conducted on hybrid maize cv. Hi Sawn 9697 in the net-house by using completely randomized design (CRD) with three replications. Ten seeds were sown on 1st February, 2008 in each pot containing sand. Hoagland solution with full strength was used to nourish the plants. Foliar spray with varying concentrations (20 and 40 mg L^{-1}) of ascorbic acid (AsA), salicylic acid (SA) and hydrogen peroxide (H_2O_2) were applied at 3rd leaf seedling stage. Seedlings were harvested at 4th leaf stage on 8th March, 2008 and were used for seedling vigor and antioxidants analysis. Maximum and minimum temperatures started from sowing to harvesting were recorded and weekly mean temperature calculated and presented (Table 1).

Determination of Chlorophyll Contents

The fresh leaves were cut into 0.5 cm segments and extracted overnight with 80% acetone at -10°C. The extract was centrifuged at 14000 × *g* for 5 min and the absorbance of supernatant was read at 645 and 663 nm using a spectrophotometer (T60 U spectrophotometer PG Instruments, Limited). The chlorophyll *a* and *b* contents were calculated by using the following formulae (Nagata and Yamashita, 1992):

Chlorophyll *a* = 0.999 A_{663}- 0.0989A_{645}
Chlorophyll *b* = -0.328 A_{663} + 1.77 A_{645}
Total Chlorophyll = Chl *a* + Chl *b*

Detection of Superoxide Dismutase

To extract antioxidant enzymes, 0.5 g fresh leaves randomly sampled from plants in each pot were ground using a tissue grinder in 8 mL of cooled phosphate buffer (pH 7.0, containing 1% (w/v) polyvinylpyrrolidone) and 0.2 g quartz sand in test tubes that were placed in an ice bath. The homogenate was centrifuged at 15000 × *g* for 20 min at 4°C. The supernatant was used for assays of enzyme activity. The activity of SOD was determined by measuring its ability to inhibit the photo-reduction of nitrobluetetrazolium (NBT) following 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 n*M* EDTA, 50 m*M* 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 irradiated and non-irradiated solution at 560 nm was determined with spectrophotometer (T_{60} spectrophotometer). One unit of SOD activity was defined as the amount of enzyme that would inhibit 50 of NBT photo reduction.

Nutrient Analysis

Total nitrogen was determined through micro-Kjeldhal distillation apparatus by taking Ten mL of digested sample along with 10 mL of NaOH (40%) was taken in Kjeldhal flask and immediately connected flask with distillation apparatus. Afterward ten mL of 4% boric acid along with mixed indicator were taken in 100 mL conical flask when distillate was approximately 40–50 mL then flask was removed. The distillate was cooled for a few minutes and titrated against 0.01N H_2SO_4 up to the appearance of light pink color (end point) of the indicator solution. Nitrogen was calculated by the formula given by Singh *et al*. (2005), while phosphorus and potassium were determined by using spectrophotometer (Spectrophotometer AnA-720 W Japan) and flame photometer-410 (Corning Model), respectively by method given by Singh *et al*. (2005).

Statistical Analysis

Data obtained were subjected to Fisher's analysis of variance (ANOVA), and treatment means showing F-values significant compared using least significance difference at 0.05 probability level (Steel *et al*., 1997).

Results

Seedling Vigor and Biochemical Analysis

Foliar application of ascorbic acid, salicylic acid and hydrogen peroxide significantly improved seedling growth of spring maize (Table 2). All foliar sprays increased shoot and root lengths, however maximum shoot length was recorded in plants sprayed with 40 mg L^{-1} AsA (Table 2). Moreover, root shoot ratio was not significantly increased by all foliar treatments. Minimum root shoot ratio was recorded in plants sprayed with higher concentration of AsA (Table 2). Exogenous application of AsA, SA and H_2O_2 significantly improved SOD activity of maize seedlings as compared to control (Table 2). Different levels of foliar applications of AsA, SA and H_2O_2 significantly increased leaf *Chl a* (Table 2). Moreover, exogenous application of H_2O_2, AsA and SA did not increased total chlorophyll content significantly (Table 2).

Shoot Nutrient Contents

Foliar applications of AsA, SA and H_2O_2 significantly increased nitrogen, phosphorus and potassium contents in the shoot of spring maize (Fig. 1a, b and c). Maximum shoot N (1.063) and K (1.29) contents were observed in maize sprayed with AsA-40 (Fig. 1a and c). Similarly, P contents in the shoots of spring maize were also improved by all foliar agents (Fig. 1b).

Table 1: Air temperatures recorded during course of study

Development stage	Days after sowing	Atmospheric temperature (°C)		
		Maximum	Minimum	Mean
Sowing	0	18.50	0.00	9.25
Germination stage	7	15.20	5.60	10.40
	14	18.60	2.50	10.55
	21	22.10	6.80	14.45
Seedling stage	28	22.60	6.90	14.25
	35	26.00	12.90	18.45
Final harvest	36	27.00	13.20	20.10

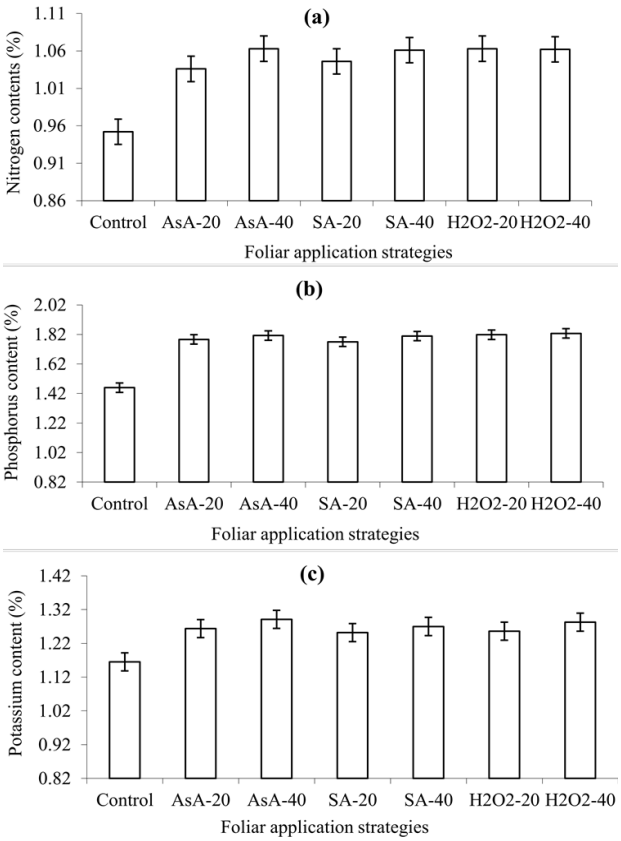

Fig. 1: Shoot nitrogen (a), phosphorus (b) and potassium (c) content of maize as influenced by different levels of foliar application of ascorbic acid, salicylic acid and hydrogen peroxide

Root Nutrient Contents

Exogenous application of AsA, SA and H_2O_2 remarkably increased nitrogen, phosphorus and potassium contents in the roots of spring maize (Fig. 2a, b and c). However, different effectors with various levels responded similarly with each. However, minimum N, P and K contents were observed in control without any foliar application strategies (Fig. 2a, b and c).

Correlation and Regression Studies

Shoot length was significantly positively correlated with shoot N, P, K, superoxide dismutase activity and

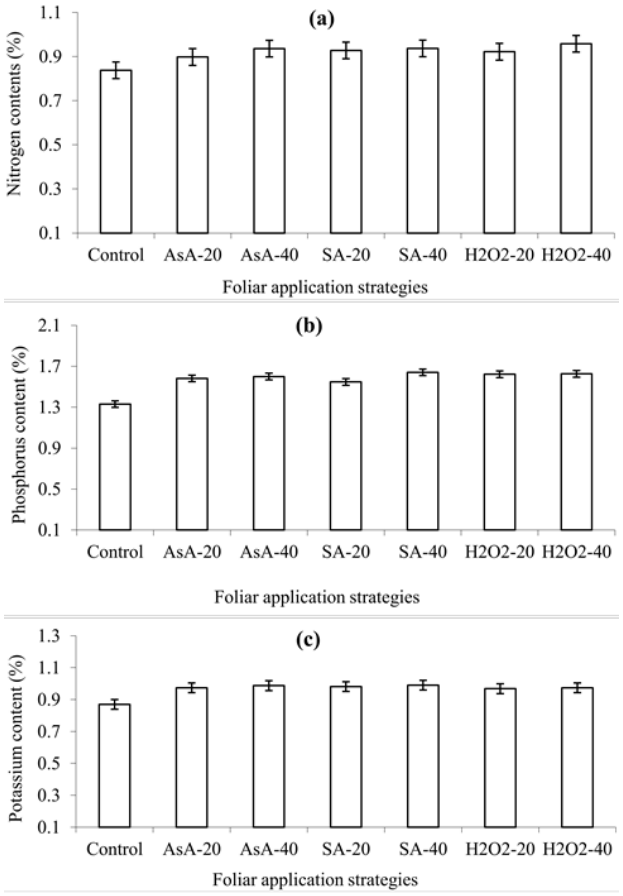

Fig. 2: Root nitrogen (a), phosphorus (b) and potassium (c) content of maize as influenced by different levels of foliar application of ascorbic acid, salicylic acid and hydrogen peroxide

chlorophyll *a* contents, which were illustrated through simple linear regression models (Fig. 3a, b, c, d and e), respectively. While root length was strongly positively related with N, P and K contents in roots, which were also depicted through linear regression models (Fig. 4a, b and c).

Discussion

Acclimation of chilling/low temperature stress involved responses, which are operating physiological and biochemical make of crop plant resistance toward adverse environment. It has been observed that chilling stress increase the production of ROS in maize (Apel and Hirt, 2004). In the present study, foliar application of AsA, SA and H_2O_2 improved plant growth by mitigating the adverse effects of low temperature (Table 2). Furthermore, shoot length was positively related with SOD activity (Fig. 4e). In the present study, chilling stress caused reduction in SOD activity but exogenous but these effectors enhanced it. Higher SOD activity in leaves of plant subjected to foliar application of AsA, SA and H_2O_2 at suboptimal temperature

Table 2: Seedling vigor and biochemical attributes of spring maize as influenced by foliar application of different levels of ascorbic acid (AsA), salicylic acid (SA) and hydrogen peroxide (H_2O_2)

Foliar application	Shoot length (cm)	Root length (cm)	Root shoot ratio	Chlorophyll *a* (mg 100 mL^{-1})	Total chlorophyll (mg 100 mL^{-1})	SOD (units mg^{-1} protein)
Control	46.00 c	18.03 b	0.392 a	2.36 b	3.29	12.86 b
AsA (20 mg L^{-1})	55.32 ab	20.23 a	0.366 ab	2.69 a	3.75	15.74 a
AsA (40 mg L^{-1})	59.70 a	19.87 a	0.333 c	2.71 a	3.80	15.67 a
SA (20 mg L^{-1})	56.33 ab	19.87 a	0.353 bc	2.68 a	3.76	15.88 a
SA (40 mg L^{-1})	57.22 ab	19.68 a	0.344 bc	2.71 a	3.80	15.77 a
H_2O_2 (20 mL L^{-1})	55.99 b	19.92 a	0.354 bc	2.72 a	3.82	15.78 a
H_2O_2 (40 mL L^{-1})	55.44 b	20.31 a	0.366 ab	2.72 a	3.80	15.76 a
LSD	4.0440	1.6406	0.0337	0.1128	NS	0.9549

Figures sharing same letter did not differ significantly at 0.05 level of probability

stress suggests a more efficient scavenging system, which may protect membrane from injurious effects of ROS (Foyer and Noctor, 2003). Previously, antioxidant activity was reported to increase with exogenous application of SA, AsA and H_2O_2 at low concentration were observed under stressful environment (Khan, 2007; Wahid *et al*., 2007; Noreen *et al*., 2009; Ahmad *et al*., 2012).

Low temperature below 15°C reduces photo-inhibition characterized by poor photosynthetic capacity (Marocco *et al*., 2005) but higher shoot growth than root growth may be due to improved photosynthetic by protecting photosynthetic apparatus through detoxifying injurious effects of ROS. Increased shoot growth with foliar application of AsA, SA and H_2O_2 might be due to up-regulating of photosynthetic capacity through protecting cell by enhancing SOD activity (Farooq *et al*., 2008; Kumar *et al*., 2010; Ahmad *et al*., 2012). Anti-stress properties of AsA, SA and H_2O_2 have been studies by various workers on different crop like maize (Ahmad *et al*., 2012), cucumber (Guatam and Singh, 2009) and *Brassica* spp (Kumar *et al*., 2010). Results of present study, root shoot ratio was lower in H_2O_2, SA and AsA treated pots than non-treated ones, which indicated the facilitation of shoot growth over root growth (Table 2).

Low temperature below 15°C reduces plant growth but foliar application of AsA, SA and H_2O_2 had ameliorative effect (Table 2). Increased chlorophyll contents might be due to protective effects on chlorophyll degradation in maize subjected suboptimal temperature (Fig. 3e). These results are in accordance with those of exogenously applied ascorbic acid, salicylic acid and hydrogen peroxide increased chlorophyll *a* in wheat (Khan, 2007; Wahid *et al*., 2007) and canola (Sakr and Arafa, 2009) under stressful conditions.

Low temperature reduce root growth (Stamp *et al*., 1997) but foliar application of ascorbic acid (AsA), salicylic acid (SA) and hydrogen peroxide (H_2O_2) on maize seedling under suboptimal temperature increased root length (Table 2). Furthermore, correlation analysis showed that a strong and positive association between root length and root N, P and K contents (Fig. 4a, b and c). Improvement in root length of maize with application of effectors might be due to stimulating effects on signal transduction of H_2O_2 (Neill *et al*., 2002), up-regulating number and size by SA (Kang *et al*., 2007) and up-regulating of antioxidants molecule in biological system by ascorbic acid (Shalata and Neumann, 2001). Low temperature limit root and shoot nutrient uptake (Enns *et al*., 2006), while foliar application of AsA, SA and H_2O_2 on maize increased nutrient absorption in root (Fig. 1a, b and c) and uptake in shoot (Fig. 2a, b and c) in spring at suboptimal temperature. The well-developed roots encourage absorption of N, P and K efficiently (Fig. 4a, b and c). These results are consistent with Khan *et al*. (2010) for maize who found out that exogenous SA applications stimulated N, P and K uptake.

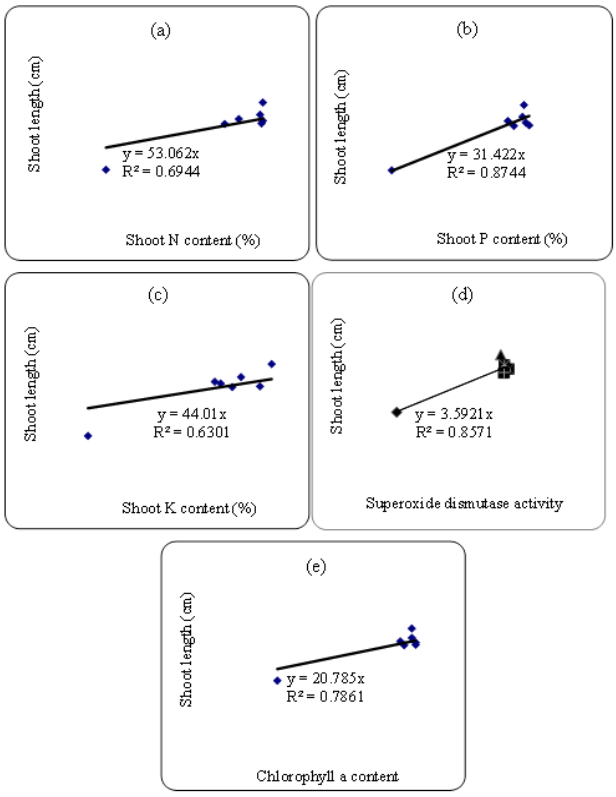

Fig. 3: Association of shoot length with shoot nitrogen (a), phosphorus (b) and potassium (c) contents, superoxide dismutase activity (d) and chlorophyll a (e)

In present study, chilling stress caused reduction in SOD activity of maize but the exogenous application of AsA, SA and H_2O_2 enhanced SOD activity (Table 2).

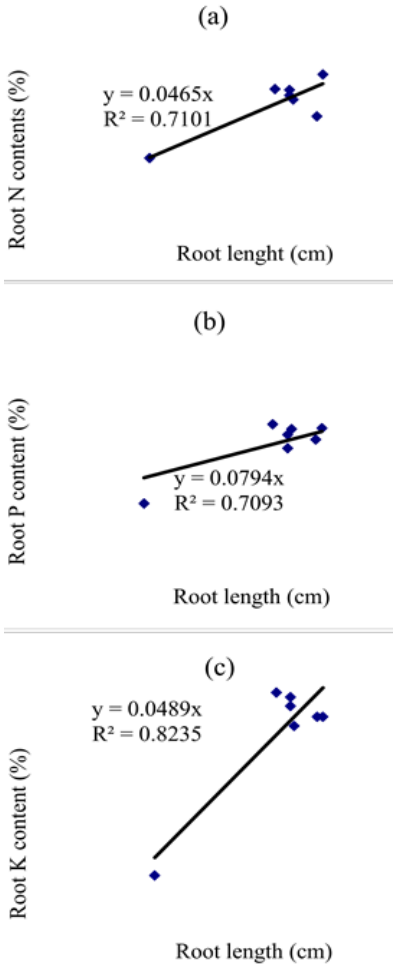

Fig. 4: Association of root length with root nitrogen (a), phosphorus (b) and potassium (c) contents

Increased activity of SOD with application of with AsA, SA and H_2O_2 has been found to be associated with improved chilling tolerance in maize (Janda *et al*., 1994). Higher SOD activity in leaves of plants subjected to foliar application of AsA, SA and H_2O_2 at suboptimal temperature stress suggests a more efficient scavenging system, which may protect membranes from injurious effect of ROS (Foyer and Noctor, 2003). Previously, SOD activity was reported to increase with exogenous application of AsA (Khan, 2007) and H_2O_2 at low concentration under stressful environment (Wahid *et al*., 2007).

Conclusion

Low temperature induced inhibition in growth of maize can be ameliorated by foliar application of ascorbic acid, salicylic acid and hydrogen peroxide. On the basis of results obtained from this investigation it may be concluded maize responded similarly to foliar strategies. Exogenous application with either 20 mg L^{-1} or 40 mg L^{-1} solution of H_2O_2, AsA and SA improved seedling establishment in maize through protecting photosynthetic pigments by inducing antioxidant activity and nutrient homeostasis.

References

Ahmad, I., T. Khaliq, A. Ahmad, S.M.A. Basra, Z. Hussain and A. Ali, 2012. Effect of seed priming with ascorbic acid, salicylic acid and hydrogen peroxide on emergence, vigor and antioxidant activities of maize. *Afr. J. Biotechnol*., 11: 1127–1132

Apel, K. and H. Hirt, 2004. Reactive oxygen species: metabolism, oxidative stress and signal transductions. *Annu. Rev. Plant Biol*., 55: 373–399

Azevedo-Neto, A.D., J.T. Prisco and J. Eneas-Filho, 2005. Hydrogen peroxide pre-treatment induces stress acclimation in maize plants. *J. Plant Physiol.*, 162: 1114–1122

Barth, C., M. De-Tullio and P.L. Conklin, 2006. The role of ascorbic acid in the control of flowering time and the onset of senescence. *J. Exp. Bot.*, 57: 1657–1665

Chen, X.Y., X. Ding, S. Xu, R. Wang and W.B. Shen, 2009. Endogenous hydrogen peroxide plays a positive role in the upregulation of heme oxygenase and acclimation to oxidative stress in wheat seedling leaves. *J. Integr. Plant Biol*., 51: 951–960

Chen, Z., Z. Zheng, J. Huang, Z. Lai and B. Fan, 2009. Biosynthesis of salicylic acid in plants. *Plant Signaling Behaviour*, 4: 493–496

Conklin, P.L. and C. Barth, 2004. Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens and the onset of senescence. *Plant Cell Environ.*, 27: 959–971

Enns, L.C., M.E. McCully and M.J. Canny, 2006. Branch roots of young maize seedlings, their production, growth and phloem supply from the primary root. *Funct. Plant Biol.*, 33: 391–399

Farooq, M., T. Aziz, S.M.A. Basra, M.A. Cheema and H. Rehman, 2008. Chilling tolerance in hybrid maize induced by seed priming with salicylic acid. *J. Agron. Crop Sci.*, 194: 161–168

Foyer, C.H. and G. Noctor, 2003. Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol. Plant..*, 119: 355–364

Gautam, S. and P.K. Singh, 2009. Salicylic acid induced salinity tolerance in corn under NaCl stress. *Acta Physiol. Plant.*, 31: 1185–1190

Giannopolitis, C.N. and S.K. Ries, 1977. Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiol*., 59: 309–314

Guan, Y.J., J. Hu, X.J. Wang and C.X. Shao, 2009. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *J. Zhejang Univ. Sci*., 10: 427–433

Janda, T., G. Szalai, I.J. Kissimon, E. Paldi, C. Marton and Z. Szigeti, 1994. Role of irradiance in the chilling injury of young maize plants studied by chlorophyll florescence induction measurements. *Photosynthetica*, 30: 293–299

Kang, G.Z., Z.X. Wang, K.F. Xia and G.C. Sun, 2007. Protection of ultrastructure in chilling-stressed banana leaves by salicylic acid. *J. Zhejang Univ. Sci*., 8: 277–282

Kaspar, T.C. and W.L. Bland, 1992. Soil temperature and root growth. *Soil Sci.*, 154: 290–299

Kaydan, D., M. Yağmur, N. Okut and G. Tarihi, 2007. Effects of salicylic acid on the growth and some physiological characters in salt stressed wheat (*Triticum aestivum* L.). *Tarim Bilimleri Dergisi* 2007, 13: 114–119

Khan, A., 2007. Influence of exogenously applied ascorbic acid on growth and physiological attributes of hexaploid wheat (*Triticum aestivum* L.) under salt stress. *Ph. D. Dissertation*. Department of Botany, University of Agriculture, Faisalabad, Pakistan

Khan, N.A., S. Syeed, A. Masood, R. Nazar and N. Iqbal, 2010. Application of salicylic acid increases the contents of nutrient metabolism in mungbean and alleviate adverse effects of salinity. *Int. J. Plant Biol.*, 1: ei

Lukatkin, A.S., 2003. Contribution of oxidative stress to the development of cold-induced damage to leaves of chilling-sensitive plants: 3. Injury of cell membrane by chilling temperatures. *Russ. J. Plant Physiol.*, 50: 243–246

Marocco, A., C. Lorenzoni and Y. Fracheboud, 2005. Chilling stress in maize. *Daydica*, 50: 571–580

Nagata, M. and I. Yamashita, 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *J. Jpn. Soci. Food Sci. Technol.*, 39: 928–928

Neill, S.J., R. Desikan, A. Clarke, R.D. Hurst and J.T. Hancock, 2002. Hydrogen peroxide and nitric oxide as signaling molecules in plants. *J. Exp. Bot.*, 53: 1237–1247

Noctor, G. and C.H. Foyer, 1998. Ascorbate and glutathione: Keeping active Oxygen under control. *Annu. Rev. Plant Physiol. Mol. Biol.*, 49: 249–279

Sakr, M.T. and A.A Arafa, 2009. Effect of some antioxidants on canola plants grown under soil salt stress condition. *Pak. J. Biol. Sci.*, 12: 582–588

Sedghi, M., A. Nemati and B. Esmailepour, 2010. Effect of seed priming on germination and seedling growth of two medicinal plants under salinity. *Emmirat J. Food Agric*., 22: 130–139

Shalata, A. and P.M. Neumann, 2001. Exogenous ascorbic acid (Vitamin C) increases resistance to salt tolerance and reduced lipid peroxidation. *J. Exp. Bot.*, 364: 2207–2211

Singh, D., P.K. Chhakar and B.S. Dwivedi, 2005. *Manual on Soil Plant and Water Analysis*, pp: 77–92. Westville Publishing House, New Dehli, India

Slesak, I., M. Libik, B. Karpnska, S. Karpinski and Z. Miszaski, 2007. The role of hydrogen peroxide in regulation of plant metabolism and cellular signaling in response to environmental stresses. *Acta Biochim. Polonica*, 54: 39–50

Smirnoff, N. and G.L. Wheeler, 2000. Ascorbic acid in plants: biosynthesis and function. *Crit. Rev. Biochem. Mol. Biol.*, 35: 291–314

Stamp, P., B. Feil, M. Schortemeyer and W. Richner, 1997. Responses of roots to low temperatures and nitrogen forms. *In*: *Plant Roots from Cells to Systems*, pp: 143–154. Anderson, H.M. (ed.). Kluwer Academic Publishers: Dordrecht, The Netherlands

Steel, R.G.D., T.H. Torrie and D.A. Dickey, 1997. *Principles and Procedures of Statistics: A Biometrical Approach*, 3rd edition, pp: 204–232. McGraw Hill Book International Co. Inc., Singapore

Wahid, A., M. Perveen, S. Gelani and S.M.A. Basra, 2007. Pretreatment of seed with H_2O_2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *J. Plant Physiol.*, 164: 283–294

Wang, Y., J.L. Li, J.Z. Wang and Z.K. Li, 2010. Exogenous H_2O_2 improves the chilling tolerance of manilagrass and mascarenegrass by activating the antioxidative system. *Plant Growth Regul.*, 61: 195–204

(Received 22 march 2012; Accepted 18 December 2012)