Available online at www.sciencedirect.com
SCIENCE DIRECT®
Plant Science 166 (2004) 303–315
PLANT SCIENCE
www.elsevier.com/locate/plantsci

The important roles of reactive oxygen species in the relationship between ethylene and polyamines in leaves of spring wheat seedlings under root osmotic stress

Chao-Zhou Li [a,b], Jian Jiao [c], Gen-Xuan Wang [d,*]

[a] *Laboratory of Arid Agroecology, Lanzhou University, Lanzhou, Gansu Province 730000, PR China*
[b] *Faculty of Agriculture, Gansu Agricultural University, Lanzhou, Gansu Province 730070, PR China*
[c] *Faculty of Forestry, Gansu Agricultural University, Lanzhou, Gansu Province 730070, PR China*
[d] *College of Life Science, Zhejiang University, Hangzhou, Zhejiang Province 310027, PR China*

Received 3 June 2003; received in revised form 1 September 2003; accepted 17 September 2003

Abstract

The important roles of reactive oxygen species (ROS) in the relationship between ethylene (ETH) and polyamines (PAs) were investigated in leaves of spring wheat seedlings under root osmotic stress. The results showed that the increase in polyamine content reduced (while the decrease in polyamine content promoted) the production of ROS and ETH significantly in the deeply and not deeply stressed leaves. The promotion and inhibition in ETH production had no significant influence on the production of ROS, the activities of diamine oxidases (DAOs) and polyamine oxidases (PAOs), the content of putrescine (Put), spermidine (Spd) and spermine (Spm) in the not deeply stressed leaves. But in the deeply stressed leaves, the promotion in ETH production significantly promoted the production of ROS and the activities of DAOs and PAOs, thus reduced the content of these amines; the inhibition in ETH production significantly reduced the production of ROS and the activities of DAOs and PAOs, and thus alleviated the decline in the content of these amines that was caused by deep stress. It was also found that exogenous H_2O_2 promoted ETH production and the activities of DAOs and PAOs, and reduced the content of these amines in the deeply stressed leaves. The above results suggested that ROS played extremely important roles in the relationship between ETH and PAs: through reducing ROS levels, PAs inhibited ETH production in the deeply and not deeply stressed leaves; through promoting ROS levels, ETH promoted polyamine oxidation and hence reduced its content in the deeply stressed leaves; but in the not deeply stressed leaves, ETH had no significant influences on ROS levels and the activities of DAOs and PAOs, so the content of these amines was not significantly influenced.

Keywords: Ethylene; Osmotic stress; Polyamines; Reactive oxygen species; Spring wheat

1. Introduction

Ethylene (ETH) is a multipurpose signaling molecule in plants and is considered as a plant hormone; polyamines (PAs) are low molecular mass polycations found in all living organisms and implicate in a wide range of biological processes, such as growth, development and abiotic stress responses [1–4]. ETH and PAs (spermidine and spermine) share a common precursor *S*-adenosyl methionine (SAM), many evidence revealed that there are negative interactions between ETH and PAs, for instance, in Hiproly barley callus ACC production modulated the biosynthesis of PAs [5], in pea seedlings ETH inhibited the activities of arginine decarboxylase and SAM decarboxylase, and reduced the content of PAs [6], in tomato fruit PAs inhibited the accumulation of the wound inducible 1-aminocyclopropane 1-carboxylic acid (ACC) synthase transcript [7], hence the biosynthetic relationship between ETH and PAs are generalized as competitive demand for a limited pool of common precursor SAM, or the feedback inhibition of enzyme action system in one pathway by the products of the competing pathway

Abbreviations: ACC, 1-aminocyclopropane 1-carboxylic acid; AVG, aminoethoxyvinylglycine; DAOs, diamine oxidases; ETH, ethylene; MGBG, methylglyoxal bis-guanylhydrazone; MSI, membrane stability index; PAOs, polyamine oxidases; Put, putrescine; SAM, *S*-adenosyl methionine; Spd, spermidine; Spm, spermine; ROS, reactive oxygen species; TBARS, thiobarbituric acid reacting substances

* Corresponding author. Tel.: +86-931-8912893; fax: +86-931-8912893.
E-mail address: wanggx@lzu.edu.cn (G.-X. Wang).

0168-9452/$ – see front matter © 2003 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.plantsci.2003.09.019

[8]. Although the catabolism of PAs may have extremely important influences on their content under environmental stresses, few researches focused on it, let alone the influence of ETH on it, so in this aspect the linkage between ETH and PAs remains a moot point.

Environmental stresses, such as drought, wounding, heat and cold stress, may induce the plants undergo an oxidative stress caused by a rapid accumulation of ROS [9–11], in the other way, all aerobic organisms, especially plants, are endowed with an impressive array of antioxidant enzymes and antioxidants to cope with activated oxygen [12,13]. Reactive oxygen species (ROS) are produced in the reactions catalyzed by NAD(P)H oxydase and by some other specialized oxydase, and are also inevitable by-products of many redox reactions in eucaryotic cells [14]. At low concentrations ROS act as secondary messengers responsible for a signal transduction from extracellular signaling molecules and their membrane receptors to the intracellular regulatory systems, yet when the increase in ROS levels is above a certain threshold, the oxidative stress may be accompanied by the processes that are harmful for cell survival, such as lipid peroxidation and oxidative modification of proteins and nucleic acids [14]. Although ROS may have a close relation with the metabolism of PAs and ETH in stressed plant tissues, the information is scarce and the mechanism is unclear, so in this paper we investigated the influences of ROS, ETH and PAs on the activities of anti-oxidative enzymes, the production of ROS and ETH, the content of PAs, and even the influences of ROS and ETH on the activities of DAOs and PAOs, respectively, in this way we hope to elucidate the roles ROS played in the relationship between ETH and PAs in leaves of spring wheat seedlings under root osmotic stress.

2. Materials and methods

2.1. Plant culture and treatments

Seeds of spring wheat (*Triticum aestivum* L. cv. Ganchun 20) were surface sterilized with $HClO_4$ (8% active Cl_2) for 1 min, rinsed with distilled water, then immersed in distilled water for 24 h. The seeds were then cultured in a growth chamber at a temperature of $25 \pm 1\,^{\circ}C$, with a 13 h photoperiod and a photon flux density of 400 $\mu mol\,m^{-2}\,s^{-1}$, watered with 1/2 Hoagland solution [15] every day. In the 7th day the seedlings were taken out from the pots, the roots were rinsed with distilled water, then immersed in the following solutions, respectively: distilled water; −1.5 MPa polyethylene glucose (PEG 4000) solution; −1.5 MPa PEG solutions added with 0.5 $mmol\,l^{-1}$ Spm, 1.0 $mmol\,l^{-1}$ MGBG, 10 $\mu mol\,l^{-1}$ ACC or 2.0 $\mu mol\,l^{-1}$ AVG, respectively.

Thirty milliliters of each solution described above was put into a beaker with a volume of about 50 ml. Sponge slices, 25 cm long, 2 cm wide and 0.5 cm thick, were used to wrap up the base of the roots which being immersed in the solutions, then the seedlings were transferred into a growth chamber at a temperature of $25 \pm 1\,^{\circ}C$ and a continuous illumination of 400 $\mu mol\,m^{-2}\,s^{-1}$ photon flux density. After 4 h and 24 h of root osmotic stress, the expanded second leaves from the top of the seedlings were harvested for measurements.

The treatments with exogenous 10 $mmol\,l^{-1}$ H_2O_2 or distilled water were carried out by spraying each of them onto the leaves until the leaves were wet when the seedlings had suffered 24 h of root osmotic stress. After another 3 h of root osmotic stress, the expanded second leaves from the top of the seedlings were harvested for measurements.

2.2. Polyamine content

The extraction of polyamines and HPLC analysis were conducted according to the method of Flores and Galston [16], and authentic standards of Put, Spd and Spm (Sigma, St. Louis) were benzoylated following the procedure described by Flores and Galston [16]. The concentrations of these amines were measured using a programmable liquid chromatography (Model Waters 600E, Waters Inc., USA). The solvent system consisted of methanol: water (65% methanol) at a flow rate of 1mL min^{-1}. The benzoylated extracts were eluted at room temperature through a reverse-phase column (Waters Symmetry C_{18}, 3.9 mm × 150 mm, 5 μm in particle size) at 254 nm with a UV detector.

2.3. ETH production

ETH production was analyzed by the following procedure: After being picked from the seedlings, the leaves were placed into penicillin bottles then covered with rubber caps. After 1 h of retention in the bottles, the gas in the bottles was extracted for ETH concentration measurement. Gas-chromatography (Model GC-9A, Altex-Beckman Inc., Japan) with a column (Paropark) at a column temperature of 90 °C was used. N_2 was used as flow-gas.

2.4. Thiobarbituric acid reacting substances (TBARS) content and membrane stability index (MSI)

TBARS content was measured according to the method of Dhindsa and Matowe [17], the concentration of TBARS was calculated using its extinction coefficient of 155 $mmol\,l^{-1}\,cm^{-1}$. Membrane stability index (MSI) was determined according to the method of Sairam et al. [18].

2.5. Reactive oxygen species levels

5 g leaves were ground in an ice bath in 10 ml 250 $mmol\,l^{-1}$ phosphate buffer (pH 8.0), then centrifuged at 5000 × *g* for 10 min, the supernatant was used for the analysis of O_2^- and H_2O_2. The production rate of O_2^- and H_2O_2 was determined following the method of Ke et al. [19] and Manuel et al. [20], respectively. According to Ke et al., 0.1 ml hydroxylamine (10 $mmol\,l^{-1}$) was added into

1 ml of the above supernatant and incubated for 20 min at 20 °C, the color was developed by the addition of 1 ml 17 mmol l^{-1} ρ-amino-phenylsulfonic acid for 15 min. Three milliliters *n*-butanol was added into the reaction mixture, then centrifuged at 6000 × *g* for 10 min, the specific absorption at 530 nm was determined. Sodium nitrite was used as standard solution to calculate the content of O_2^-.

2.6. Anti-oxidative enzyme essays

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined spectrophotometrically as described by Spychalla and Desborough [21], One unit of SOD is defined as the amount of enzyme that inhibit the rate of ferrcytochrome c reduction by 50%. Activities of catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11) and guaiacol peroxidase (POD, EC 1.11.1.7) were measured following the methods described in our previous work [11]. CAT activity was determined by following the consumption of H_2O_2 (extinction coefficient 39.4 mmol l^{-1} cm^{-1}) at 240 nm for 2 min. APX activity was determined by following the decrease in A_{290} for 3 min (extinction coefficient 2.8 mmol l^{-1} cm^{-1}), corrections were made for oxidation of ascorbate in the absence of H_2O_2. POD activity was based on the determination of guaiacol oxidation (extinction coefficient 26.6 mmol l^{-1} cm^{-1}) at 470 nm by H_2O_2.

2.7. Activities of DAOs and PAOs

PAs can be oxidative degraded by the action of amine oxidases, which include diamine oxidases (DAOs; EC 1.4.3.6) and polyamine oxidases (PAOs; EC 1.5.3.3) [22]. The extraction and measurement of the activities of DAOs ad PAOs followed the method described by Béranger-Novat et al. [23]. Put, Spd and Spm are oxidatively deaminated by the action of DAOs and PAOs, respectively, and formed pyrroline and other substances, by measuring the [^{14}C] pyrroline formation from [^{14}C] putrescine or from [^{14}C] spermidine, the activities of DAOs and PAOs were assayed by a radiochemical method. LS. 1800 scintillation counter (Beckman) was used in the experiments.

2.8. Statistical analysis

All experiments were repeated at least three times and representative data were presented, all data were statistically analyzed using SPSS statistical software (SPSS for window 10), taking $P < 0.05$ as significant.

3. Results

3.1. Changes in ETH production and polyamine content in leaves under root osmotic stress

ETH production in leaves decreased after 4 h of root osmotic stress, but increased after 24 h of root osmotic stress (Fig. 1A). Put, Spd and Spm are the most abundant polyamines in leaves of spring wheat seedlings, after 4 h of root osmotic stress, the content of Put, Spd and Spm was significantly higher than the control (Fig. 1B–D); after 24 h of root osmotic stress, Put content reduced markedly but was still higher than the control (Fig. 1B), while the content of Spd and Spm reduced and became less than the control predominantly (Fig. 1C and D). Treatment with exogenous Spm significantly reduced ETH production in leaves after 4 and 24 h of root osmotic stress, while treatment with MGBG significantly promoted ETH production (Fig. 1A). Treatment with exogenous ACC promoted (while treatment with AVG reduced) ETH production in leaves after 4 and 24 h of root osmotic stress (Fig. 1A). Treatments with ACC and AVG had no significant influences on the content of Put, Spd and Spm in leaves when the stress lasted 4 h, but when the stress lasted 24 h exogenous ACC significantly accelerated the decreases in the content of these amines caused by deep osmotic stress, while AVG significantly alleviated the decreases of these amines (Fig. 1B–D). When exogenous H_2O_2 was sprayed onto the surface of the deeply stressed leaves (which had suffered 24 h of root osmotic stress) for 3 h, the increase in ETH production was promoted while the decrease in the content of Put, Spd and Spm was accelerated significantly (Table 1).

Table 1
Changes in production of ETH (pmol g^{-1} DW h^{-1}), content of Put, Spd and Spm (nmol g^{-1} DW) and activities of DAOs and PAOs (pK_at g^{-1} DW) in leaves of spring wheat seedlings

	After another 0 h of root osmotic stress	After another 3 h of root osmotic stress, the leaves were sprayed with distilled water	After another 3 h of root osmotic stress, the leaves were sprayed with 10 mmol l^{-1} H_2O_2
ETH production	104.4**	116.3*	139.7*
Put content	115.2*	111.3**	96.2*
Spd content	54.6**	47.7*	38.4*
Spm content	11.7*	11.2*	10.4*
Activity of DAOs	145.3*	167.9*	176.5*
Activity of PAOs	375.2*	388.2*	407.3*

The leaves were sprayed with 10 mmol l^{-1} H_2O_2 or distilled water after 24 h of −1.5 MPa root osmotic stressed ($n = 3$).
* $P < 0.05$.
** $P < 0.01$.

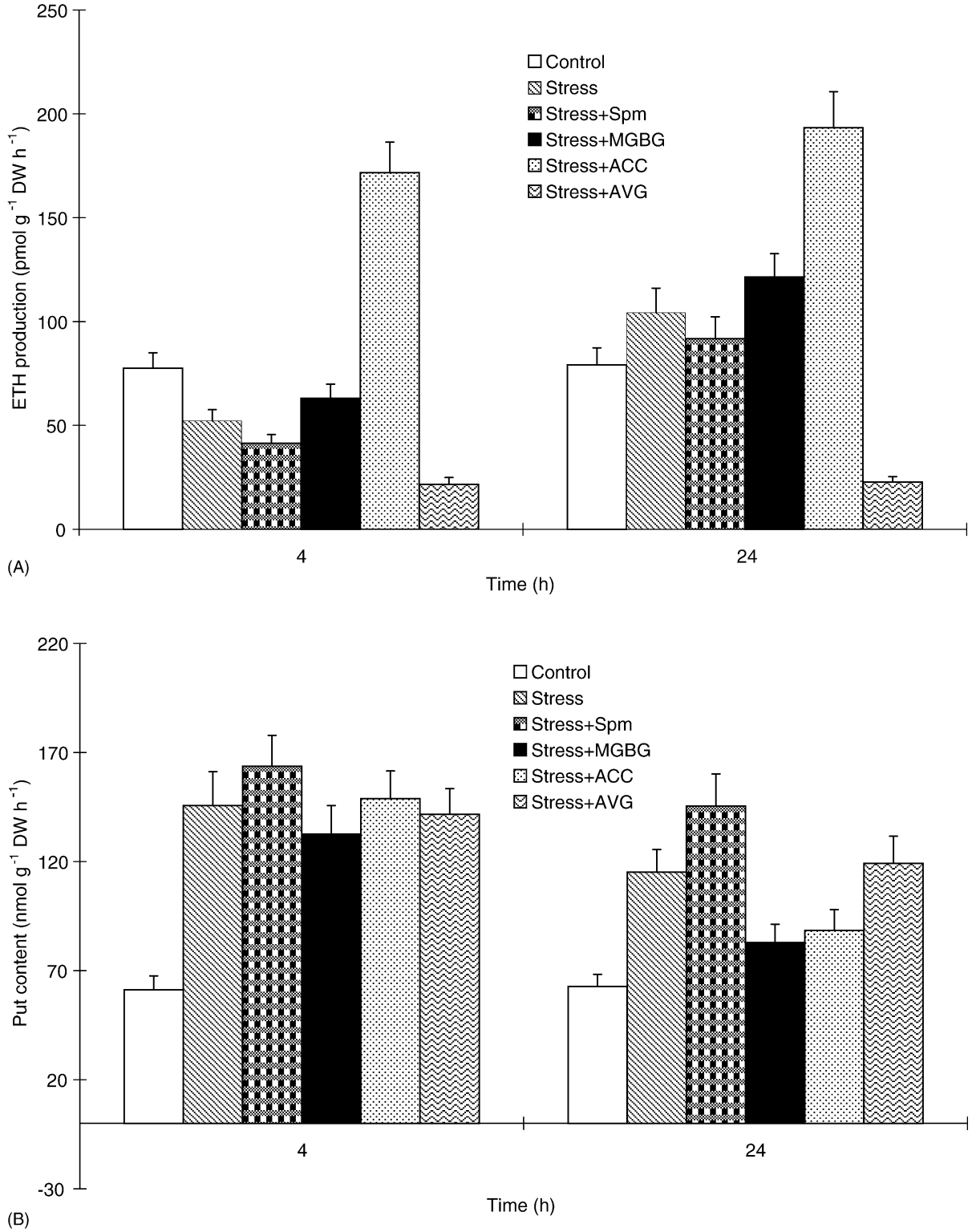
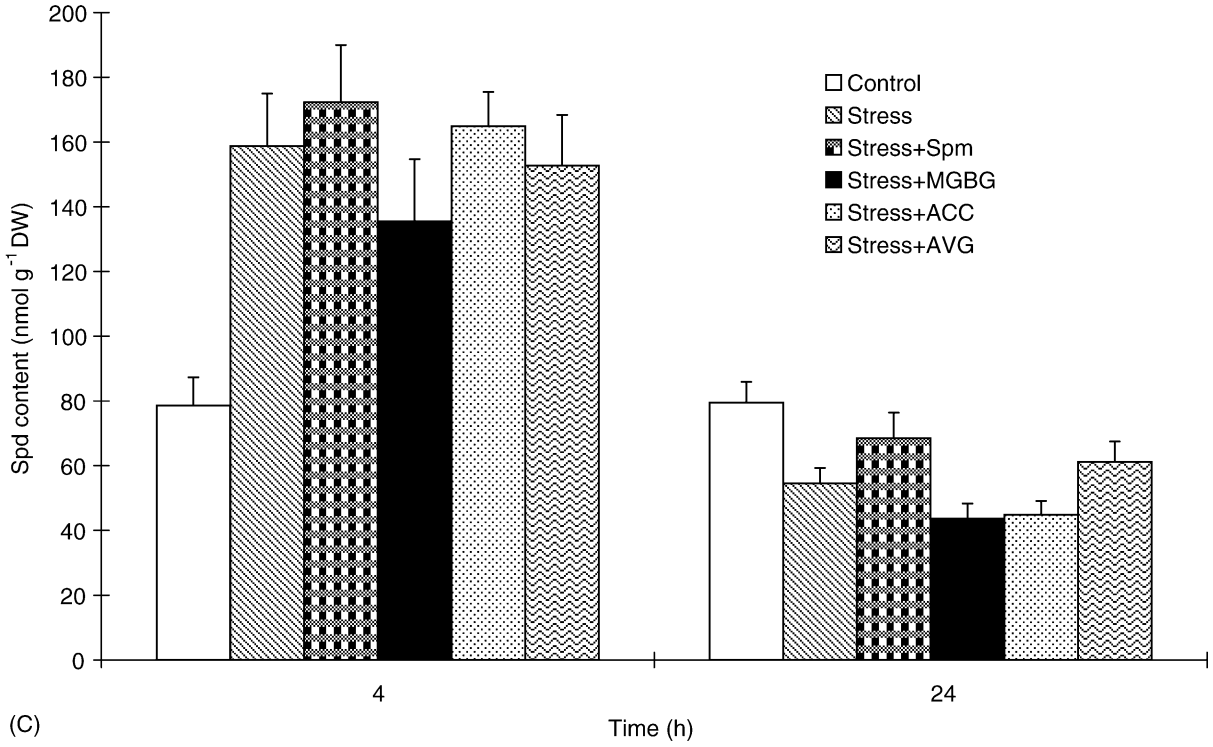


Fig. 1. Changes in ETH production (pmol g^{-1} DW h^{-1}) and the content of Put, Spd and Spm (nmol g^{-1} DW) in leaves of spring wheat seedlings under root osmotic stress. Treatments were carried out by immersing the roots in the following solutions. Control: distilled water; stress: −1.5 MPa PEG solution; stress + Spm: −1.5 MPa PEG solution with 0.5 mmol l^{-1} Spm; stress + MGBG: −1.5 MPa PEG solution with 1 mmol l^{-1} MGBG; stress + ACC: −1.5 MPa PEG solution with 10 μmol l^{-1} ACC; stress + AVG: −1.5 MPa PEG solution with 2.0 μmol l^{-1} AVG. Vertical lines in each point show ± S.E. ($n = 3$) ($P < 0.05$).



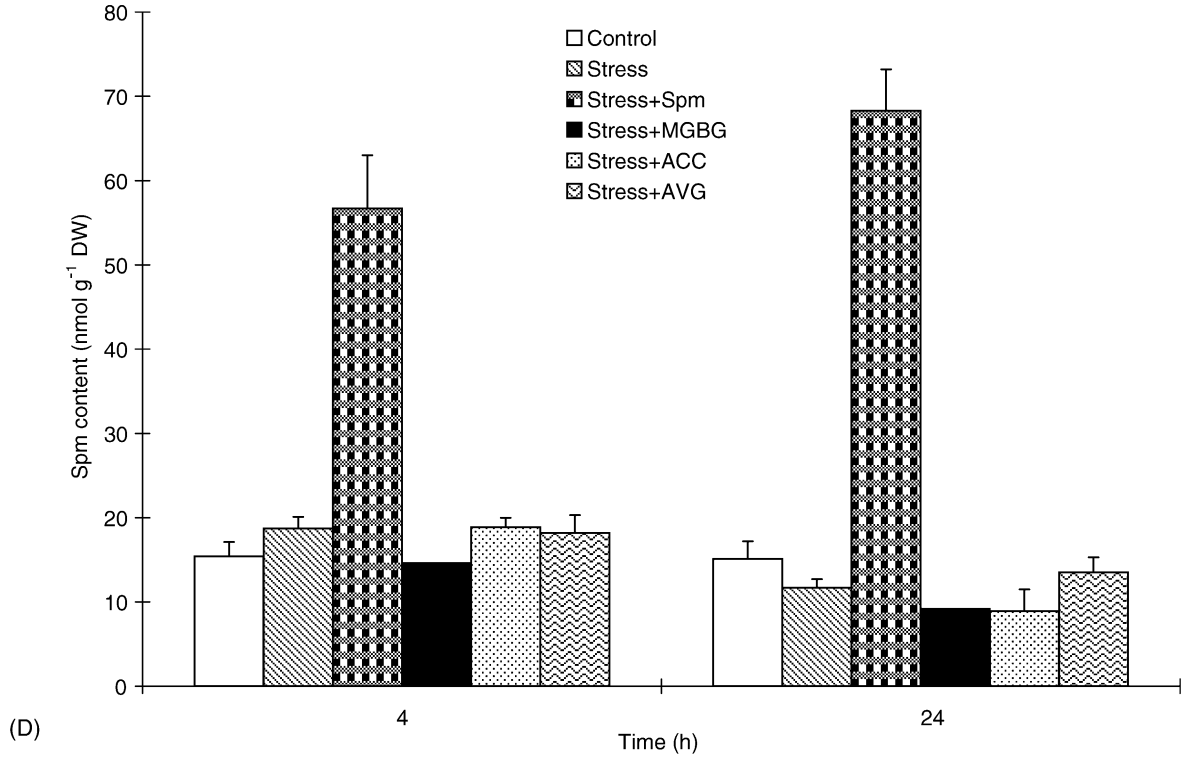
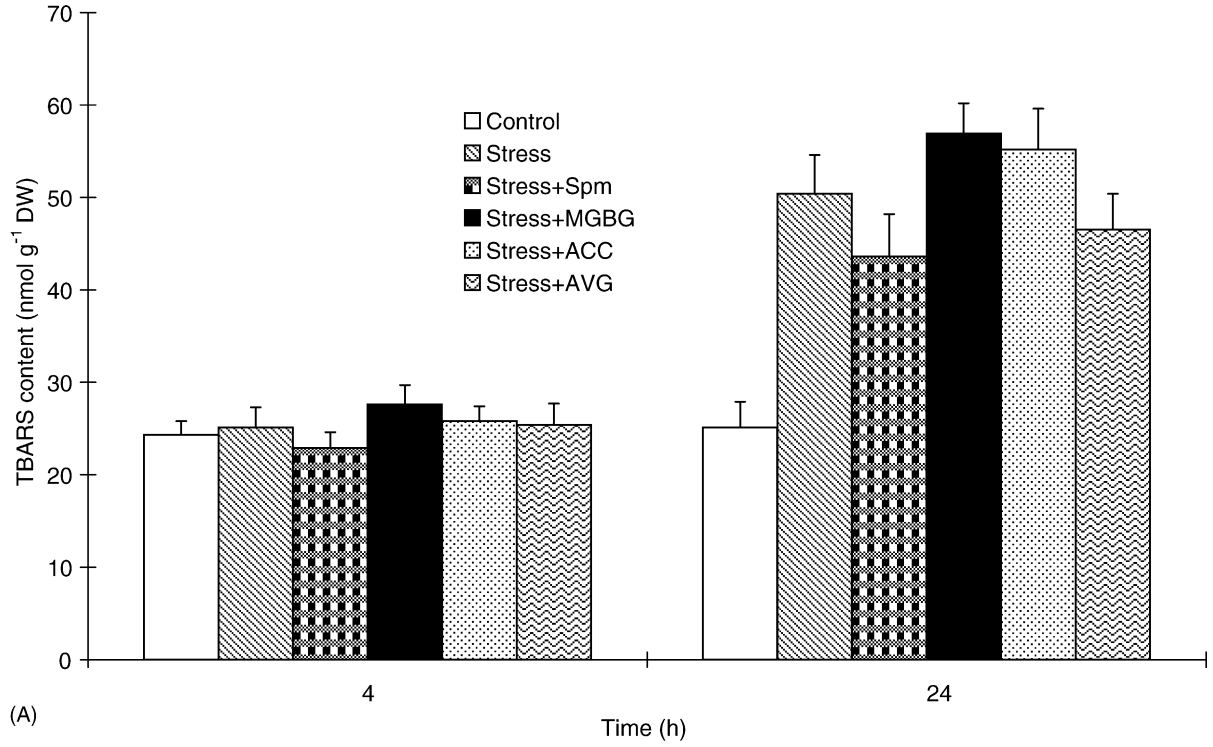


Fig. 1. (*Continued*).

3.2. Changes in lipid peroxidation and ROS levels in leaves under root osmotic stress

The stress degrees were measured in terms of TBARS content using thiobarbituric acid reaction and membrane stability index (MSI). TBARS content and MSI showed little changes in leaves after 4 h of root osmotic stress, yet after 24 h of root osmotic stress, TBARS content increased while MSI reduced significantly (Fig. 2A and B), the results showed that the leaves were not deeply stressed after 4 h of



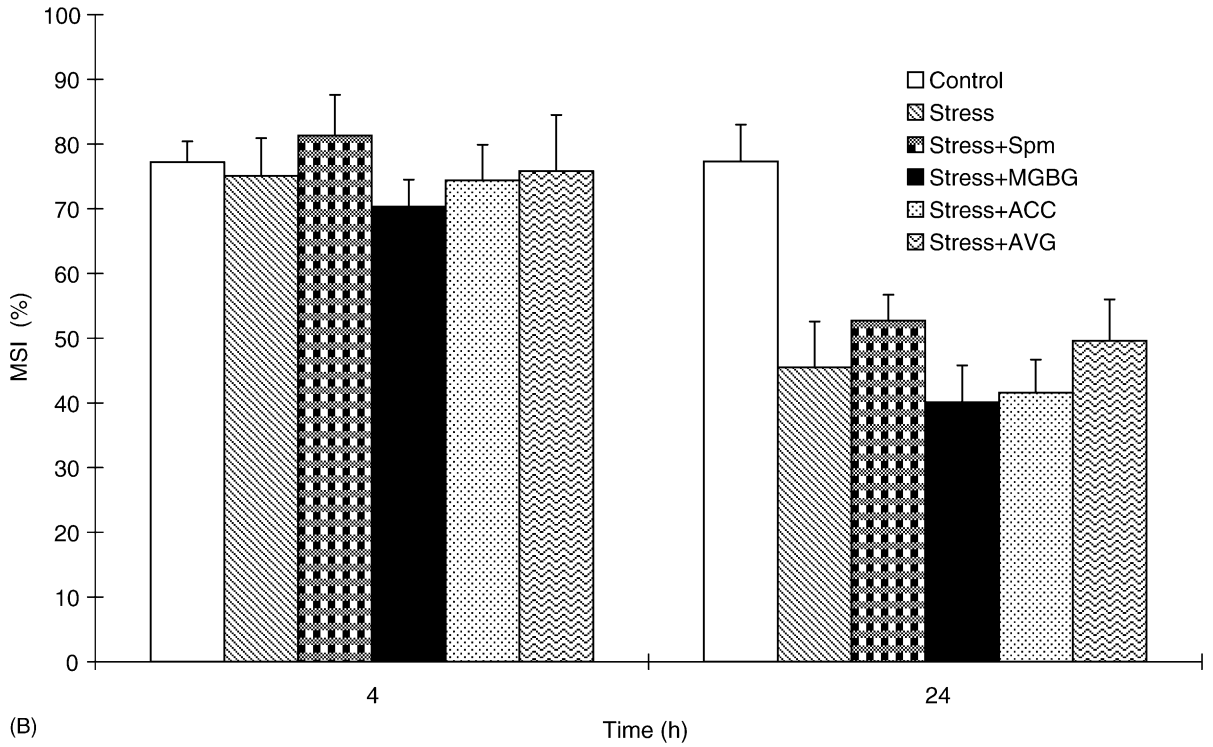
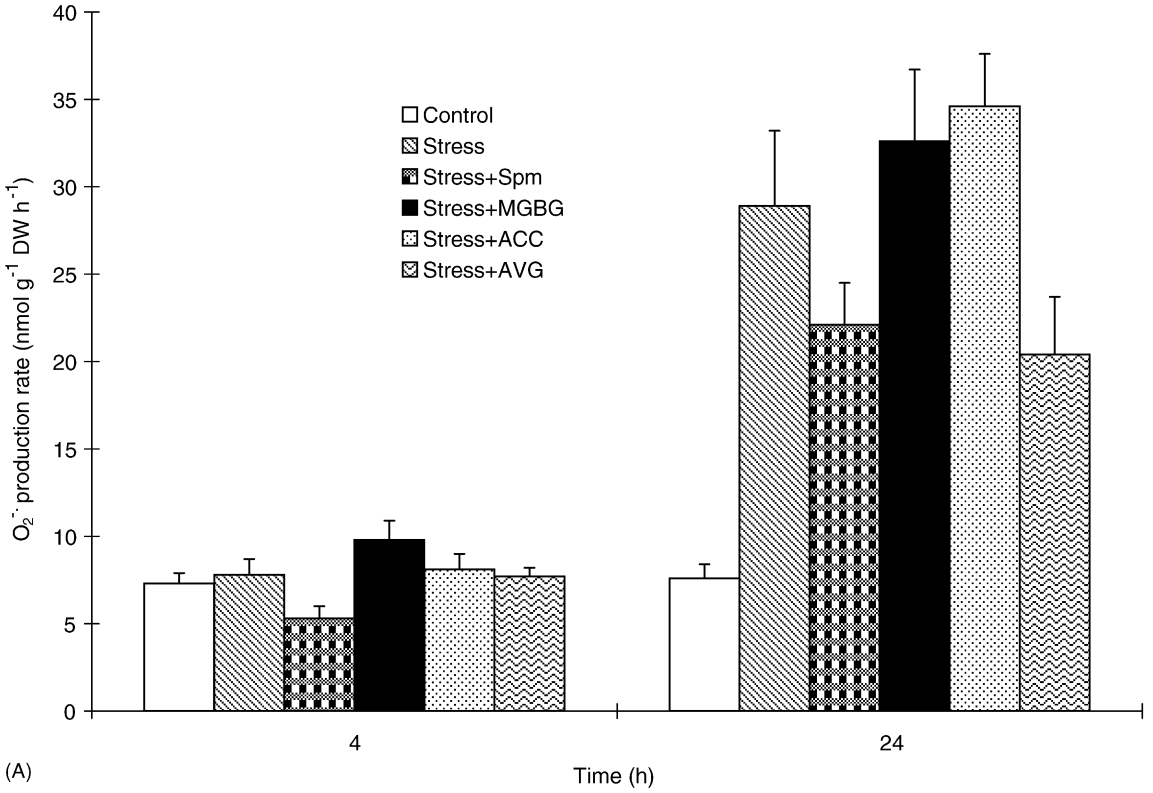


Fig. 2. Changes in TBARS content (nmol g^{-1} DW) and membrane stability index (%) in leaves of spring wheat seedlings under root osmotic stress. Treatments were carried out by immersing the roots in the following solutions. Control: distilled water; stress: −1.5 MPa PEG solution; stress + Spm: −1.5 MPa PEG solution with 0.5 mmol l^{-1} Spm; stress + MGBG: −1.5 MPa PEG solution with 1 mmol l^{-1} MGBG; stress + ACC: −1.5 MPa PEG solution with 10 μmol l^{-1} ACC; stress + AVG: −1.5 MPa PEG solution with 2.0 μmol l^{-1} AVG. Vertical lines in each point show ± S.E. ($n = 3$) ($P < 0.05$).



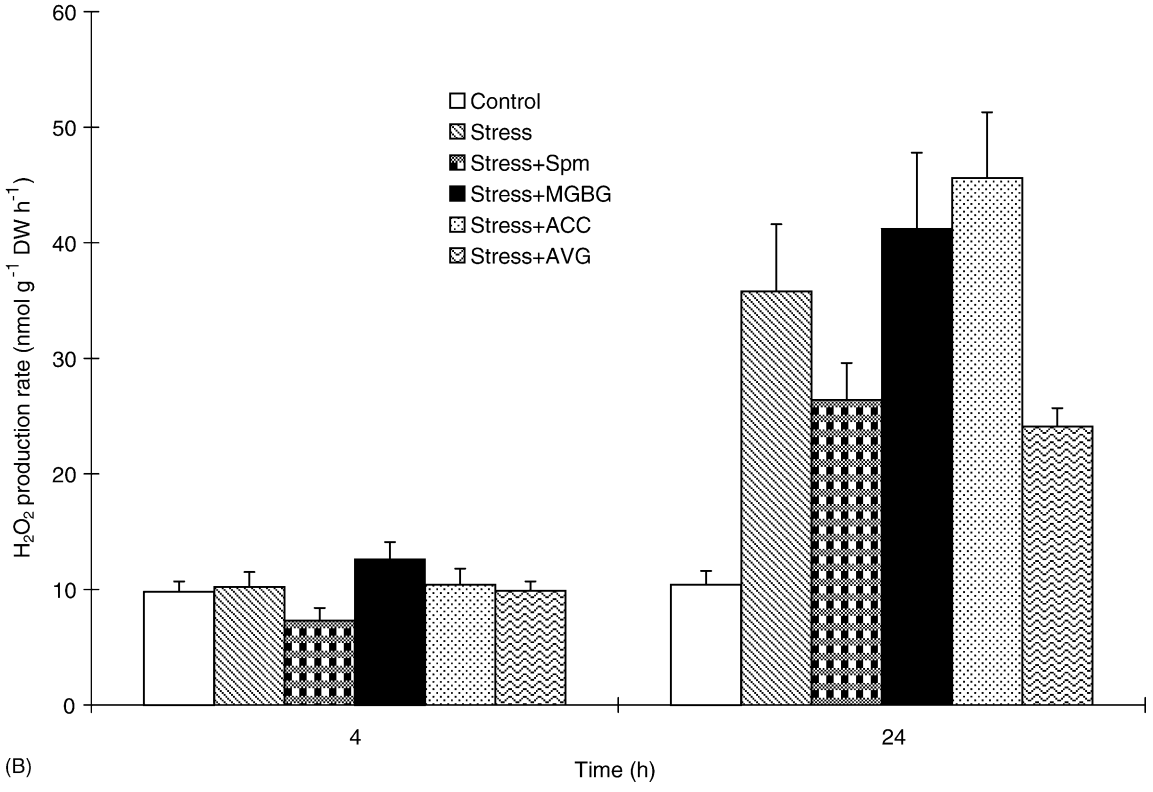


Fig. 3. Changes in the production rate of O_2^- and H_2O_2 (nmol g^{-1} DW h^{-1}) in leaves of spring wheat seedlings under root osmotic stress. Treatments were carried out by immersing the roots in the following solutions. Control: distilled water; stress: −1.5 MPa PEG solution; stress + Spm: −1.5 MPa PEG solution with 0.5 mmol l^{-1} Spm; stress + MGBG: −1.5 MPa PEG solution with 1 mmol l^{-1} MGBG; stress + ACC: −1.5 MPa PEG solution with 10 μmol l^{-1} ACC; stress + AVG: −1.5 MPa PEG solution with 2.0 μmol l^{-1} AVG. Vertical lines in each point show ± S.E. ($n = 3$) ($P < 0.05$).

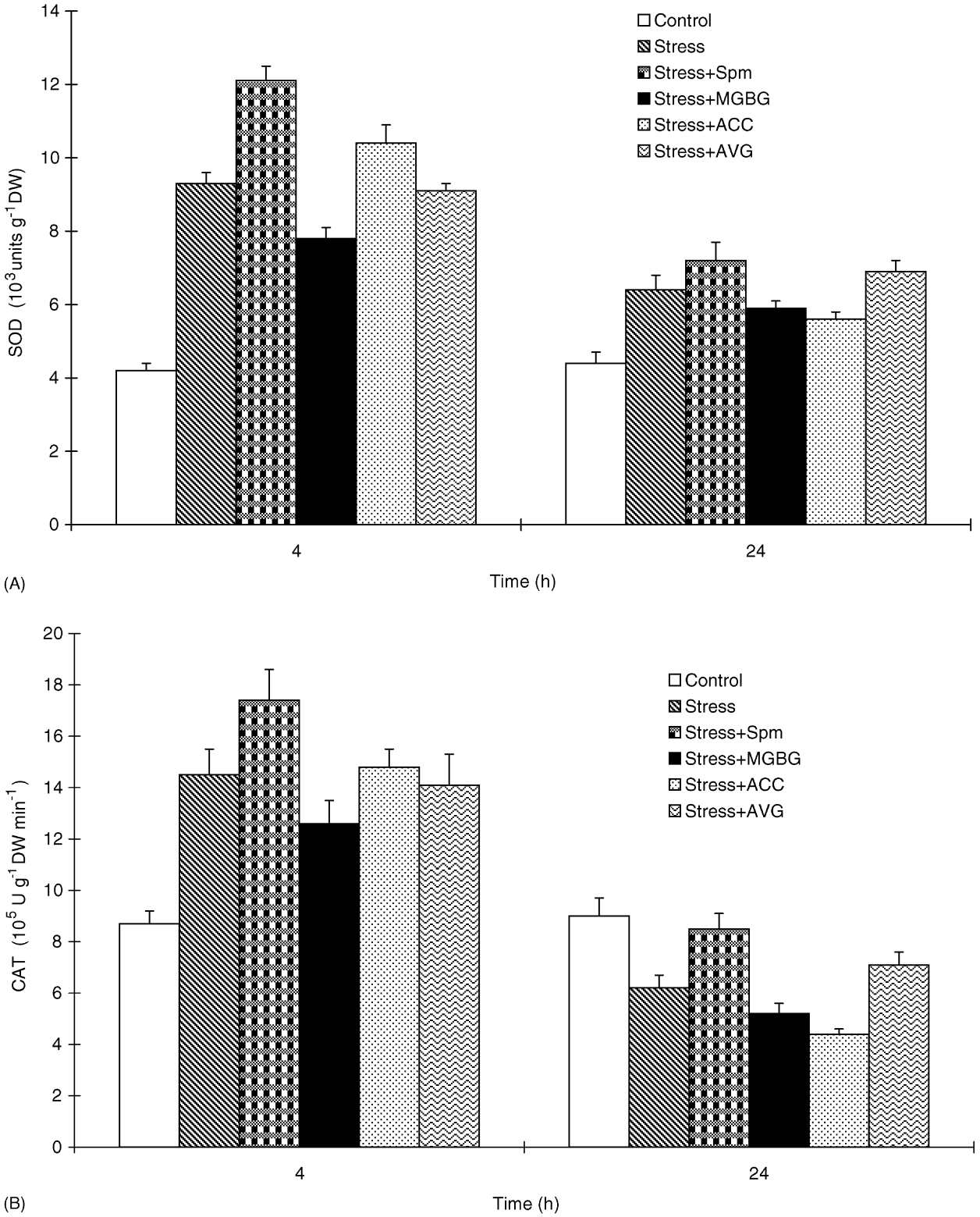
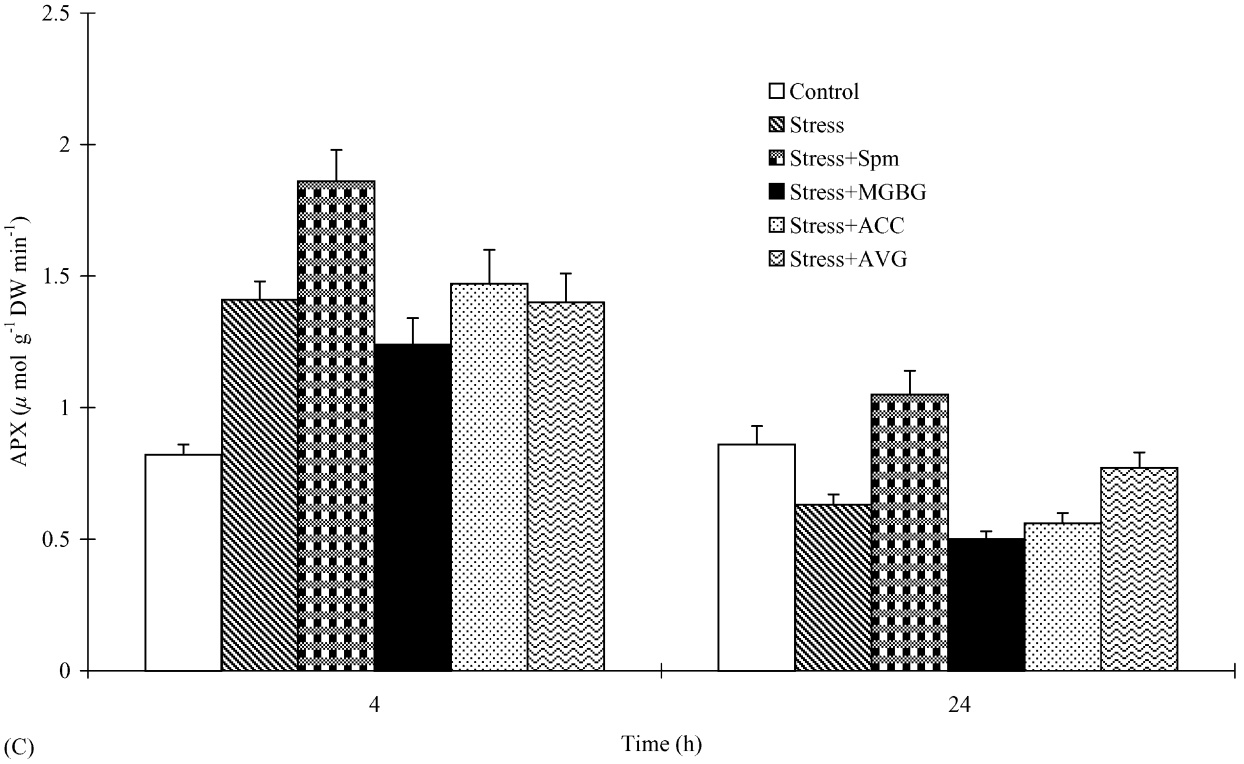


Fig. 4. Changes in the activities of SOD (10^3 U g^{-1} DW), CAT (10^5 U g^{-1} DW min^{-1}), APX (umol g^{-1} DW min^{-1}) and POD (mmol g^{-1} DW min^{-1}) in leaves of spring wheat seedlings under root osmotic stress. Treatments were carried out by immersing the roots in the following solutions. Control: distilled water; stress: −1.5 MPa PEG solution; stress + Spm: −1.5 MPa PEG solution with 0.5 mmol l^{-1} Spm; stress + MGBG: −1.5 MPa PEG solution with 1 mmol l^{-1} MGBG; stress + ACC: −1.5 MPa PEG solution with 10 μmol l^{-1} ACC; stress + AVG: −1.5 MPa PEG solution with 2.0 μmol l^{-1} AVG. Vertical lines in each point show ± S.E. ($n = 3$) ($P < 0.05$).



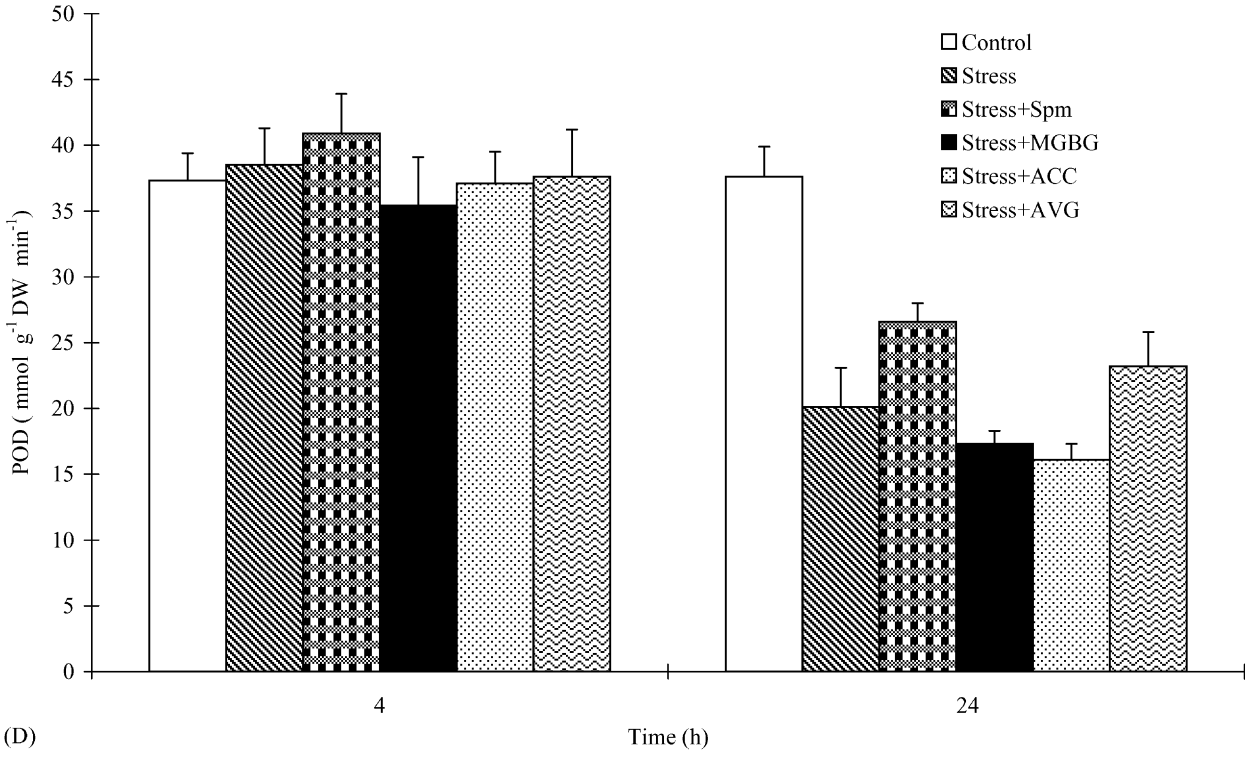
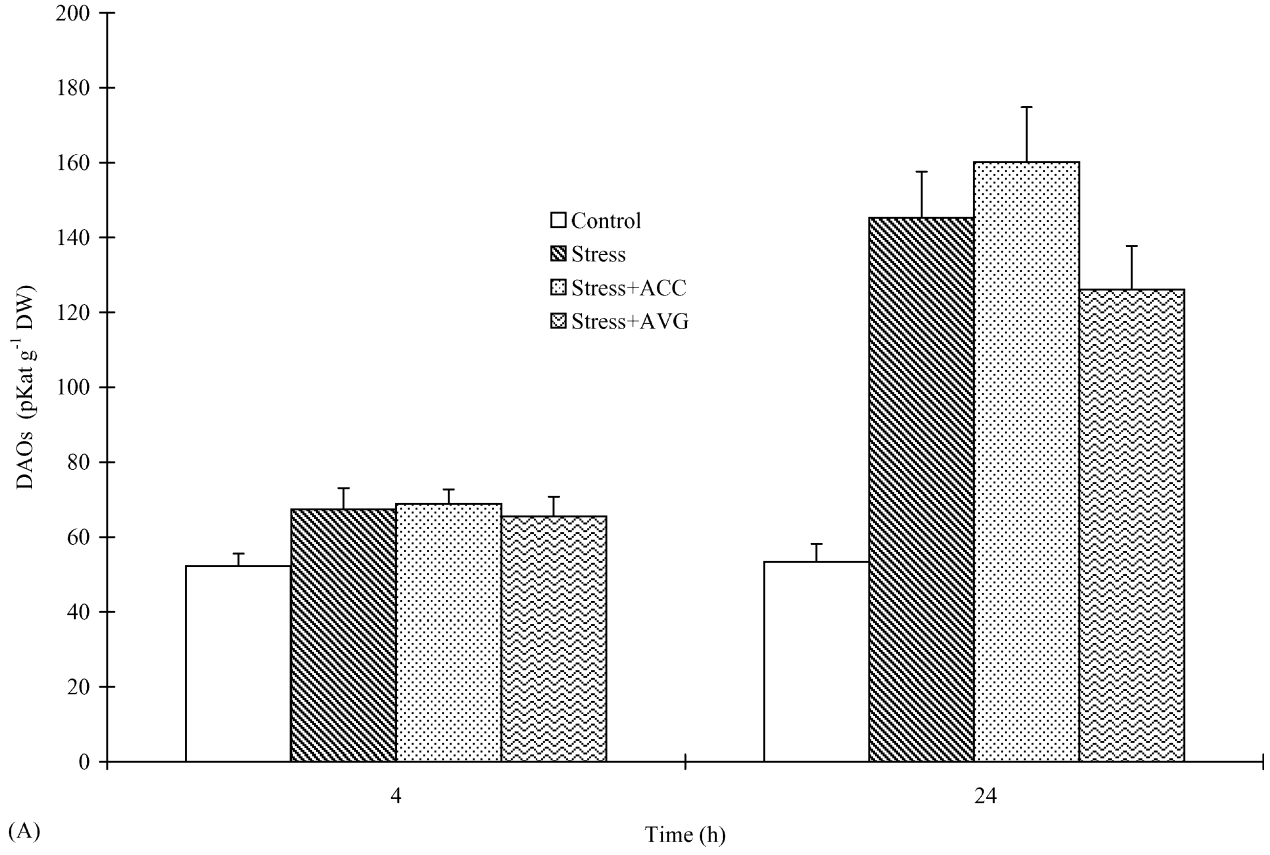


Fig. 4. (*Continued*).

root osmotic stress, but after 24 h of root osmotic stress the leaves were deeply stressed. Treatment with Spm reduced TBARS content and promoted MSI significantly after 4 and 24 h of root osmotic stress, while treatment with MGBG showed the reversed influences on both TBARS content and MSI (Fig. 2A and B). The results also showed that ACC and AVG had no significant influence on TBARS content and MSI after 4 h of root osmotic stress. After 24 h of root



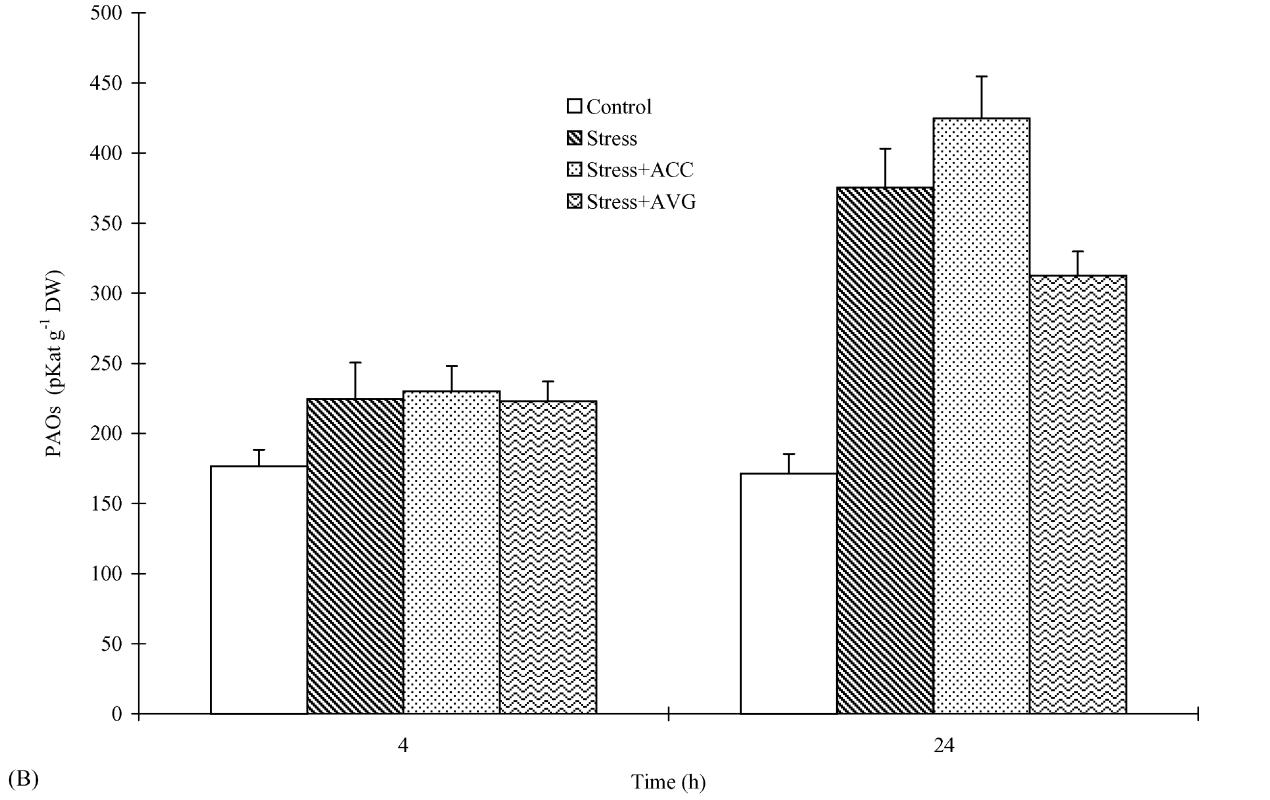


Fig. 5. Changes in the activities ($pK_a t\,g^{-1}$ DW) of DAOs and PAOs in leaves of spring wheat seedlings under root osmotic stress. Treatments were carried out by immersing the roots in the following solutions. Control: distilled water; stress: −1.5 MPa PEG solution; stress + ACC: −1.5 MPa PEG solution with 10 μmol l^{-1} ACC; stress + AVG: −1.5 MPa PEG solution with 2.0 μmol l^{-1} AVG. Vertical lines in each point show ± S.E. ($n = 3$) ($P < 0.05$).

osmotic stress, exogenous ACC promoted TBARS content and reduced MSI, while AVG reduced TBARS content and increased MSI significantly (Fig. 2A and B).

O_2^- and H_2O_2 are two kinds of important ROS. The production rat of O_2^- and H_2O_2 in leaves had negligible changes after 4 h of root osmotic stress; but it increased markedly after 24 h of root osmotic stress (Fig. 3A and B). Treatment with exogenous Spm significantly reduced the production rate of O_2^- and H_2O_2, while treatment with MGBG significantly promoted it after 4 h of root osmotic stress. After 24 h of root osmotic stress, treatment with exogenous Spm significantly alleviated the increases in the production rate of O_2^- and H_2O_2 caused by stress, while treatment with MGBG significantly promoted the increases (Fig. 3A and B). Treatments with ACC and AVG had no significant influences on the production rate of O_2^- and H_2O_2 in leaves when the stress lasted 4 h, but when the stress lasted 24 h, treatment with ACC significantly promoted the increases in the production rate of O_2^- and H_2O_2, while AVG significantly alleviated the increases (Fig. 3A and B).

3.3. Changes in the activities of antioxidant enzymes, DAOs and PAOs in leaves under root osmotic stress

The activities of SOD, CAT and APX in leaves increased significantly after 4 h of root osmotic stress, while the activity of POD increased not significantly ($P > 0.05$); after 24 h of root osmotic stress, the activities of all the four anti-oxidative enzymes declined significantly (Fig. 4A–D). Treatment with Spm promoted the activities of SOD, CAT, APX and POD significantly after 4 and 24 h of root osmotic stress, while treatment with MGBG significantly reduced the activities of these four enzymes (Fig. 4A–D). After 4 h of root osmotic stress, treatments with ACC and AVG had no significant influences on the activities of CAT, APX and POD, while SOD activity increased significantly with the treatment of ACC and decreased not significantly with the treatment of AVG; after 24 h of root osmotic stress, treatment with ACC significantly accelerated the decreases in the activities of SOD, CAT, APX and POD caused by stress, while treatment with AVG significantly alleviated the decreases (Fig. 4A–D).

The activities of DAOs and PAOs increased significantly after 4 and 24 h of root osmotic stress (Fig. 5A and B). Treatments with ACC and AVG had no significant influences on the activities of DAOs and PAOs after 4 h of root osmotic stress; but after 24 h of root osmotic stress, treatment with ACC significantly promoted the increases in the activities of DAOs and PAOs caused by stress, while treatment with AVG significantly alleviated the increases in the activities of the two enzymes (Fig. 5A and B). When exogenous H_2O_2 was sprayed onto the surface of the deeply stressed leaves for 3 h, the activities of DAOs and PAOs in leaves were both promoted significantly (Table 1).

4. Discussion

Since the discovery in 1952 that Put content increased in plants subjected to K^+ deficiency [24], several other environmental stresses such as osmotic stress have been found to exert the same effects [25,26]. It was reported that the biosynthesis of Spd from Put and SAM was not blocked in high osmotic stressed rape leaf discs, but the content of Spd declined, the reason may be the promotion of polyamine oxidation [25]. Our study attested that the activities of DAOs and PAOs increased significantly in the deeply stressed leaves (Fig. 5A and B), it should be an important reason for the decline in the content of Put, Spd and Spm (Fig. 1B–D).

As a plant hormone, the functions of ethylene have a close relation with plant developmental stages, in young plant tissues, ethylene is supposed to stimulate growth, alternatively in stressed tissues, ethylene may behave as a growth inhibitor and promote senescence, for example, ETH promoted the superoxide production in carrot cells during carbon starvation [27], and enhanced ROS production in the deeply stressed leaves (Fig. 3A and B). Our study also found that ETH promoted the activities of DAOs and PAOs in the deeply stressed leaves (Fig. 5A and B), we would point out that to our knowledge this is the first report that ETH promotes the activities of DAOs and PAOs, and this may be an important reason that ETH reduces the content of Put, Spd and Spm in the deeply stressed leaves.

AVG and exogenous ACC had no significant influences on ROS levels in the not deeply stressed leaves (Fig. 3A and B), the reason of it may be of the high activities of anti-oxidative enzymes (Fig. 4A–D). Furthermore, in the not deeply stressed leaves, the activities of DAOs and PAOs (Fig. 5A and B) and the content of Put, Spd and Spm were not significantly affected by the treatment of ACC and AVG (Fig. 1B–D), the results showed that ETH had no significant influences on ROS levels and polyamine catabolism in the not deeply stressed leaves.

It was also reported that ROS participated in the synthesis of ETH [28], and our results showed that exogenous H_2O_2 promoted ETH production in the deeply stressed leaves (Table 1); on the other hand, ETH promoted the production of ROS in the deeply stressed leaves (Fig. 5A and B), so it could be concluded that ETH and ROS reinforced the production of the other in the deeply stressed leaves.

At cellular pH value PAs behave as cations [3,29], when PAs were bound to membrane targets they might induce conformational changes [30], so it was suggested that PAs could impair the functionality of ETH synthesizing system so as to inhibit ETH production in aged orange peel discs [30]. In the other way, many studies showed that PAs reduced ROS levels, for instance, in wheat seedlings exogenous PAs inhibited the accumulation of O_2^- and H_2O_2 under osmotic stress [31]; the pretreatment of Spd prevented chill-induced increase of H_2O_2 content in leaves and NADPH-dependent superoxide generation in microsome

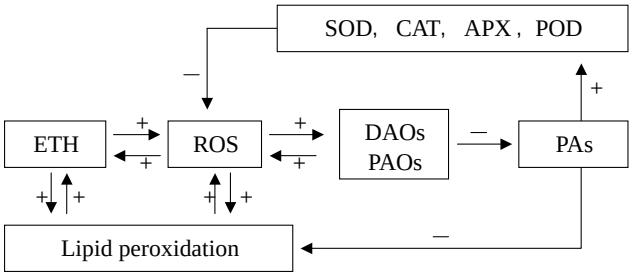


Fig. 6. The roles of ROS in the relationship between ETH and PAs in the deeply stresses leaves of spring wheat seedlings. "+" standing for promoting the content (or activity, or production), "−" standing for reducing the content (or activity, or production).

[4]; our results also attested that PAs reduced the production of O_2^- and H_2O_2 (Fig. 3A and B), these results above were in consistence with the discovery that PAs acted as direct free radical scavengers [32] or acted as radical scavengers by interacting with other molecule [33]. So through reducing ROS levels PAs restrained lipid peroxidation and protected cell membrane in the deeply stressed leaves (Fig. 2A and B). It should also be pointed out that H_2O_2 can also be produced during the oxidation of PAs [25], in this way the oxidation of PAs may promote the increase in ROS levels.

From the above results it could be concluded that ROS played extremely important roles in the relationship between ETH and PAs in the stressed leaves: through reducing ROS levels, PAs inhibited the production of ETH in both the deeply and not deeply stressed leaves; through promoting ROS levels, ETH promoted the activities of DAOs and PAOs, hence reduced the content of Put, Spd and Spm in the deeply stressed leaves (Fig. 6); in the not deeply stressed leaves, ETH had no significant influences on ROS levels and the activities of DAOs and PAOs, therefore, the content of these amines was not significantly influenced.

Acknowledgements

The work was supported by National Key Basic Research Special Funds (G1999011705) and National Natural Science Foundation of China (30170161).

References

[1] A.W. Galston, R.K. Sawhney, Polyamines in plant physiology, Plant Physiol. 94 (1990) 406–410.
[2] A. Bouchereau, A. Aziz, F. Larher, J. Martin-Tanguy, Polyamines and environmental challenges: recent development, Plant Sci. 140 (1999) 103–125.
[3] A. Tassoni, F. Antoghoni, M.L. Battistini, O. Sanvido, N. Bagni, Characterization of spermidine binding to solubilized plasma membrane proteins from zucchini hypocotyls, Plant Physiol. 117 (1998) 971–977.
[4] W.Y. Shen, K. Nada, S. Tachibana, Involvement of polyamines in the chilling tolerance of cucumber cultivars, Plant Physiol. 124 (2002) 431–440.
[5] Y. Katoh, T. Hasegawa, T. Suzuki, T. Fujji, Effect of 1-aminocyclopropane 1-carboxylic acid production on the changes in the polyamine levels in Hiproly barley callus after auxin withdrawal, Agric. Biol. Chem. 51 (1987) 2457–2463.
[6] A. Apelbaum, A. Goldlust, I. Icekson, Control by ethylene of arginine decarboxylase activity in pea seedlings and implication for hormonal regulation of plant growth, Plant Physiol. 79 (1985) 635–640.
[7] N. Li, B.R. Parsons, D. Liu, A.K. Mattoo, Accumulation of wound inducible ACC synthase transcripts in tomato fruit is inhibited by salicylic acid and polyamines, Plant Mol. Biol. 18 (1992) 477–487.
[8] S. Pandey, S.A. Ranade, P.K. Nagar, N. Kumar, Role of polyamines and ethylene as modulators of plant senescence, J. Biosci. 25 (2000) 291–299.
[9] T. Gaspar, T. Franck, B. Bisbis, C. Kevers, L. Jouve, J.F. Hausman, J. Dommes, Concepts in plant stress physiology. Application to plant tissue cultures, Plant Growth Regul. 37 (2002) 263–285.
[10] M. Becana, J.F. Moran, I. Iturbe-Ormaetxe, Iron-dependent oxygen free radical generation in plants subjected to environmental stress: toxicity and antioxidant protection, Plant and Soil 201 (1998) 137–147.
[11] J.S. Lin, G.X. Wang, Double CO_2 could improve the drought tolerance better in sensitive cultivars than in tolerant cultivars in spring wheat, Plant Sci. 163 (2002) 627–637.
[12] Asada K. Radical production and scavenging in the chloroplasts. In: Baker NR, editor. Photosynthesis and environment. Dordrecht: Kluwer Academic Publishers; 1996. p. 123–50.
[13] V.V. Zykova, A.V. Kolesnichenko, V.K. Voinikov, Involvement of reactive oxygen species in plant mitochondrial response to low-temperature stress, Russ. J. Plant Physiol. 49 (2002) 269–276.
[14] K.T. Turpaev, Reactive oxygen species and regulation of gene expression, Biochem. (Moscow) 67 (2002) 339–352.
[15] Hoagland DR, Arnon DI. California agriculture experiment station, Circular 1957;347.
[16] H.E. Flores, A.W. Galston, Analysis of polyamines in higher plants by high performance liquid chromatography, Plant Physiol. 69 (1982) 701–706.
[17] R.S. Dhindsa, W. Matowe, Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation, J Exp. Bot. 32 (1981) 79–91.
[18] R.K. Sairam, D.S. Siiukla, D.C. Saxena, Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes, Biol. Plant. 40 (1997/1998) 357–364.
[19] D.S. Ke, A.G. Wang, G.C. Sun, L.F. Dong, The effect of active oxygen on the activity of ACC synthase induced by exogenous IAA, Acta Bot. Sinica 44 (2002) 551–556.
[20] B. Manuel, A. Pedro, J.I. Juan, S. Manuel, Some enzymes of hydrogen peroxide metabolism in leaves and root nodules of *Medicago sativa*, Plant Physiol. 82 (1986) 1169–1171.
[21] J.P. Spychalla, S.L. Desborough, Superoxide dismutase, Plant Physiol. 94 (1990) 1214–1218.
[22] J. Martin-Tanguy, Metabolism and function of polyamines in plants: recent development (new approaches), Plant Growth Regul. 34 (2001) 135–148.
[23] N. Béranger-Novat, J. Monin, J. Jassey, J. Martin-Tanguy, Polyamine catabolism in dormant embryos of spindle tree (*Euonymus europaeus* L.) and in dormancy break obtained after treatment with gibberellic acid, Plant Growth Regul. 21 (1997) 65–70.
[24] F.J. Richards, E.G. Coleman, Occurrence of putrescine in potassium deficient barley, Nature 170 (1952) 460–461.
[25] A. Aziz, J. Martin-Tanguy, F. Larher, Plasticity of polyamines metabolism associated with high osmotic stress in rape leaf discs and with ethylene treatment, Plant Growth Regul. 21 (1997) 153–163.
[26] H.E. Flores, A.W. Galston, Polyamines and plant stress: activation of putrescine biosynthesis by osmotic shock, Science 217 (1982) 1259–1261.

[27] H.S. Chae, W.S. Lee, Ethylene- and enzyme-mediated superoxide production and cell death in carrot cells grown under carbon starvation, Plant Cell Rep. 20 (2001) 256–261.
[28] A. Apelbaum, S.F. Yang, Biosynthesis of stress ethylene by water deficit, Plant Physiol. 68 (1981) 594–596.
[29] O. Heby, L. Persson, Molecular genetics of polyamine synthesis in eukaryotic cells, Trends Biochem. Sci. 15 (1990) 153–158.
[30] A. Apelbaum, A.C. Burgoon, J.D. Anderson, M. Liberman, R. Ben-Arie, A.K. Mattoo, Polyamines inhibit biosynthesis of ethylene in higher plant tissue and fruit protoplasts, Plant Physiol. 68 (1981) 453–456.
[31] Y.C. Xu, J. Wang, X. Dong, M.M. Li, L. Shan, Effect of exogenous polyamines on glycolate oxidase activity and active oxygen species accumulation in wheat seedlings under osmotic stress, Isr. J. Plant Sci. 49 (2001) 173–178.
[32] G. Drolet, E.B. Dumbroff, R.L. Legge, J.E. Thompson, Radical scavenging properties of polyamines, Phytochemistry 25 (1986) 367–371.
[33] W. Bors, C. Langebartels, C. Michel, H. Sandermann, Polyamines as radical scavengers and protectants against ozone damage, Phytochemistry 28 (1989) 1589–1595.