BIOLOGIA PLANTARUM 60 (4): 686-694, 2016 DOI: 10.1007/s10535-016-0622-6

Salicylic acid and nitric oxide increase photosynthesis and antioxidant defense in wheat under UV-B stress

F. YAN[1], Y. LIU[1], H. SHENG[3], Y. WANG[3], H. KANG[3], and J. ZENG[1,2]*

College of Resources[1], *Institute of Natural Resources and Geographic Technology*[2], *and Institute of Triticeae Research*[3], *Sichuan Agricultural University, Chengdu, Sichuan, 611130, P.R. China*

Abstract

The effects of exogenous salicylic acid (SA), sodium nitropusside (SNP, a nitric oxide donor), or their combination on dwarf polish wheat (*Triticum polonicum* L.) seedlings under UV-B stress were studied. The UV-B stress significantly decreased plant height, shoot dry mass, pigment content, net photosynthetic rate, intercellular CO_2 concentration, stomatal conductance, transpiration rate, and variable to maximum chlorophyll fluorescence ratio (F_v/F_m) in all plants, but less in the presence of SA, SNP, and their combination. On the other hand, there were considerable increases in malondialdehyde (MDA), proline, $O_2^{\bullet-}$, and H_2O_2 content under the UV-B stress. When SA, SNP, and their combination were applied, content of MDA, proline, H_2O_2, and $O_2^{\bullet-}$ were less increased. Moreover, there were considerable increases in activities of superoxide dismutase, peroxidase, ascorbate peroxidase, and glutathione reductase under the UV-B stress and more in the presence of SA, SNP, and their combination. Therefore, it is considered that SA, SNP, and especially their combination could alleviate UV-B stress in dwarf polish wheat.

Additional key words: antioxidants, chlorophyll fluorescence, lipid peroxidation, net photosynthetic rate, stomatal conductance, transpiration rate, *Triticum polonicum.*

Introduction

The effects of increased UV-B radiation (280 - 320 nm) at the earth's surface on plant growth have been investigated extensively. Increases in solar UV-B have raised concerns about the damaging impact of UV-B radiation on crop plants (Caldwell *et al*. 2007). The UV-B radiation varies with time of day, time of year, latitude, and cloud cover (Rozema *et al*. 1997). A UV-B stress could induce over-production of free radicals resulting in oxidative stress (Yu *et al*. 2004). Harmful effects of enhanced UV-B are often related to the excessive production of reactive oxygen species (ROS) (Strid *et al*. 1994) and a resultant damage includes reduced photosynthesis, biomass accumulation, protein synthesis, and impaired chloroplast function (Ren *et al*. 2006). When plants are exposed to UV-B stress, they could induce some protective mechanisms. For example, increases in UV-B absorbing compounds, proline content, and activity of antioxidant enzymes have been reported (Prochazkova *et al*. 2001).

Salicylic acid (SA) is an important signal molecule, which plays an important role in modulating a number of physiological processes and plant response to stresses such as drought (Waseem *et al*. 2006, Saruhan *et al*. 2012), salinity (Mutlu *et al*. 2009), and heavy metals (Jamali *et al*. 2011). It has been suggested that SA is directly involved in signaling antioxidant responses (Larkindale and Knight 2002). In addition, application of SA was found to alleviate stress induced by water deficit and UV-B radiation (Bandurska and Ciéslak 2013).

Nitric oxide has been proved to be a signal molecule playing important roles in diverse physiological processes in plants, including growth and development (Paghussat *et al*. 2002), hormones modulation (Neil *et al*. 2002), and biotic and abiotic stresses (Modolo *et al*. 2002). Previous reports have suggested NO as stress-inducing agent (Antoniou *et al*. 2014), whereas others have assigned it as protective molecule (Tanou *et al*. 2012) functioning as antioxidant by scavenging ROS (Laspina *et al*. 2005). Moreover, NO can reduce superoxide anion formation and

Submitted 3 August 2015, *last revision* 20 December 2015, *accepted* 22 December 2015.

Abbreviations: APX - ascorbate peroxidase; Car - carotenoids; Chl - chlorophyll; c_i - intercellular CO_2 concentration; E - transpiration rate; GR - glutathione reductase; g_s - stomatal conductance; MDA - malondialdehyde; NBT - nitroblue tetrazolium chloride; PFD - photon flux density; P_N - net photosynthetic rate; POD - peroxidase; ROS - reactive oxygen species; SA - salicylic acid; SNP - sodium nitroprusside; SOD - superoxide dismutase.

Acknowledgement: The authors appreciate the support from the Natural Science Foundation of the Sichuan Agricultural University and the Bureau of Education of Sichuan Province, China.

* Corresponding author; fax: (+86) 28 86290986, e-mail: zengjian@sicau.edu.cn

lipid peroxidation (Boveris *et al*. 2000). Therefore, we hypothesized that SA, NO, or their combination could ameliorate destructive effects of UV-B on plants.

Dwarf polish wheat (*Triticum polonicum* L., 2n = 4x = 28, AABB) may constitute a valuable genetic material for breeding new wheat cultivars characterized by a high nutritive value and satisfactory resistance to *Fusarium* head blight (Wiwart *et al*. 2013). However, several environmental factors, such as drought, salinity, heavy metals, and UV-B, throw a great threat to its survival. Therefore, the present study was designed to explore the role of SA, NO, and their combination in mediating damaging effects of UV-B radiation and elucidate possible physiological mechanisms in wheat.

Materials and methods

Plants and growth conditions: Dwarf polish wheat (*Triticum polonicum* L.) seeds were sterilized with 5 % (m/v) sodium hypochlorite for 15 min, washed extensively with distilled water, and then germinated on moist filter paper in the dark at 26 °C for 5 d. Each plastic pot (16 cm in diameter, 20 cm in height) was filled with *Vermiculite*, after which two uniform seedlings were transplanted into each pot. The seedlings were grown in chambers at a 16-h photoperiod, a photon flux density (PFD) of 500 μmol m^{-2} s^{-1}, day/night temperatures of 25/20 °C, and a 65 ± 5 % relative humidity. The seedlings were carefully irrigated in the morning of every third day with 20 cm^3 of Hoagland's nutrient solution. After four weeks, the seedlings were divided into eight treatments: *1*) C (control), *2*) U (UV-B), *3*) S (0.5 mM SA), *4*) N (NO; 0.5 mM sodium nitropruside, SNP), *5*) SN (SA + NO), *6*) US (UV-B + SA), *7*) UN (UV-B + NO), and *8*) USN (UV-B + SA + NO). The concentrations of SA and SNP were according to previous studies (Santa-Cruz *et al*. 2010, Li *et al*. 2014). One half of every group of the seedlings was irradiated with UV-B for 7 h a day (from 10:00 to 17:00) using UV fluorescent lamps (*TL 20 W/01 RS*). The irradiation was determined with a *USB2000 Fibre Optic* spectrometer (*Ocean Optics*, Dunedin, FL, USA) and weighed with the generalized plant action spectrum normalized to 300 nm (Caldwell 1971). Vertical polyester curtains were placed above the plants in order to prevent the UV-B radiation under the C treatment. The desired radiation dose rate was obtained by adjusting the distance between the lamps and the leaves. The total daily biological effective UV-B radiation was 21 kJ m^{-2} d^{-1} in this study (according to the method of Flint and Caldwell 2003a,b). The experiment was arranged in a randomized complete block design with three replicates.

After a week of the UV-B irradiation, photosynthetic parameters were measured and the plants were harvested. Leaves were washed thoroughly with running tap water followed by deionized water. Fresh samples of the top three fully expanded leaves were used for physiological indexes determination. For enzyme activities, the fresh material was frozen in liquid nitrogen and stored at -70 °C. For determination of plant dry matter, the plants were dried to a constant mass at 80 °C for 48 h.

Photosynthetic parameters: Content of chlorophyll (Chl) *a*, Chl *b*, and total carotenoids (Car) were determined according to Lichtenthaler (1987). The pigments were extracted for 48 h from 0.2 g of fresh leaf tissue using 25 cm^3 of 80 % (v/v) chilled acetone in the dark and then measured with a spectrophotometer (*UV-1700*, *Shimadzu*, Kyoto, Japan) by reading absorbances at 663, 646, and 470 nm.

Net photosynthetic rate (P_N), intercellular CO_2 concentration (c_i), stomatal conductance (g_s), and transpiration rate (E) were measured in the three fully expanded top leaves from 09:00 to 11:00 using an *LI-6400* portable photosynthesis system (*Li-Cor*, Lincoln, NE, USA). All the measurements were taken at a constant flow rate of 500 μmol s^{-1}, a PFD of 500 μmol m^{-2} s^{-1}, an ambient CO_2 concentration of approximately 350 μmol mol^{-1}, and a temperature of about 25 °C.

Chlorophyll fluorescence images were taken with a fluorometer *Imaging-PAM* (*MAXI-version*, *Heinz Walz GmbH*, Effeltrich, Germany) after keeping leaf samples in darkness for 15 min. Fluorescence intensities F_0 and F_m were determined from *PAM* kinetics measured at the central part of the fully expanded leaves before and after a saturation pulse, respectively, and the maximal photochemical quantum yield of photosystem (PS) II was calculated from these values as $F_v/F_m = (F_m-F_o)/F_m$.

Histochemical detection of $O_2^{\bullet-}$ and H_2O_2: Superoxide ($O_2^{\bullet-}$) and H_2O_2 in leaves were visualized based on the method of Wang *et al*. (2011) with little modification. Briefly, the fresh three fully expanded leaves were stained in 0.25 mM nitroblue tetrazolium chloride (NBT) or 1 % (m/v) 3,3-dimethoxybenzidine (DAB) for 10 h under a PFD of about 100 μmol m^{-2} s^{-1} and a temperature of 25 °C for visualizing $O_2^{\bullet-}$ and H_2O_2, respectively. After incubation in NBT or DAB, the leaves were washed with distilled water and then decolorized in boiling 95 % (v/v) ethanol which allowed detection of blue insoluble formazan (for $O_2^{\bullet-}$) or deep brown polymerization product (for H_2O_2).

Examination of proline and malondialdehyde content: Proline content was determined according to the method of Bates *et al*. (1973). After extraction at room temperature with a 3 % (m/v) 5-sulfosalicylic acid solution, proline content was determined from a standard curve and calculated on fresh mass basis.

The sample leaves (0.2 g) were soaked into 1 cm^3 of distilled water, boiled for 30 min, and centrifuged at 5 000 *g* for 4 min. Lipid peroxidation was measured as 2-thiobarbituric acid reactive metabolites, mainly malondialdehyde (MDA), following the modified method of

Heath and Packer (1968). The frozen samples were homogenized in a pre-chilled mortar and pestle with two volumes of ice cold 0.1 % (m/v) trichloroacetic acid (TCA) and centrifuged at 15 000 *g* for 15 min. An assay mixture containing 1 cm^3 aliquot of the supernatant and 2 cm^3 of 0.5 % (m/v) 2-thiobarbituric acid in 20 % (m/v) TCA was heated to 95 °C for 30 min and then rapidly cooled in an ice-bath. After centrifugation (10 000 *g* and 4 °C for 10 min), supernatant absorbance was read at 532 nm and a value corresponding to nonspecific absorption (600 nm) was subtracted. Content of MDA was calculated using its coefficient of absorbance of 155 mM^{-1} cm^{-1}.

Determination of enzyme activities: Samples (0.3 g of the frozen tissue) were homogenized on ice with 5 cm^3 of an ice-cold 50 mM Na-phosphate buffer (pH 7.8), containing 0.1 mM EDTA and 1 % (m/v) polyvinyl pyrrolidone. The mixture was then centrifuged at 15 000 *g* and 4 °C for 20 min to obtain the supernatant for enzyme activity determinations. Superoxide dismutase (SOD) activity was assayed by monitoring the inhibition of photochemical reduction of NBT according to the method of Beauchamp and Fridovich (1971). One unit of SOD activity was defined as the amount of the enzyme that inhibited 50 % of NBT photoreduction. Peroxidase (POD) activity was measured by an increase in absorbance at 470 nm due to guaiacol oxidation (Nickel and Cunningham 1969). One unit of POD activity was defined as the amount of the enzyme which produced an absorbance change of 0.1 per minute at 470 nm. Ascorbate peroxidase activity determination followed the procedures of Nakano and Asada (1981). Glutathione reductase activity was assayed based on the description of Foyer and Halliwell (1976) with minor modifications.

Statistical analysis: Each measurement was replicated at least three times. Data are expressed as means ± standard deviations (SDs). Statistical comparisons were carried out by the *SPSS v. 19.0* software. Before the statistical analyses, homogeneity of variances and normality of distributions were tested for each variable. Tukey's test was used to detect possible differences among the treatments at $\alpha = 0.05$.

Results

Compared with the control, the plants treated with the UV-B exhibited 14.0, 27.9, and 37.5 % reductions in plant height, total biomass, and root/shoot ratio, respectively (Table 1). However, there was no significant difference in root length between the U and C treatments. The S and N treatments reduced root dry mass and root/shoot ratio, whereas the SN treatment increased root/shoot ratio as compared to the C treatment. On the other hand, the US, UN, and USN treatments induced increases in plant height, shoot dry biomass, root dry biomass, and root/shoot ratio compared with the U treatment. The greatest increases were in plant height and root/shoot ratio (15.0 and 50.0 %, respectively, under the USN treatment).

The UV-B stress significantly reduced Chl content (Table 2). Also under the S, N, and SN treatments, the wheat plants showed significant decreases in Chl *a*, Chl *b*, and Car content compared with the C. Parallel changes in Chl *a* and Chl *b* led to non-significant changes in Chl *a*/*b* ratio under all the treatments. However, there was a higher content of Chl *a*, Chl *b* and Car in the plants grown under the US, UN and USN than in the plants grown under the U alone.

Compared with the C treatment, the S and N treatments significantly decreased P_N, g_s, and E (Table 3) but the values of g_s, and E in the SN treatment were higher than those in the C treatment. The wheat plants subjected to the UV-B stress exhibited significant decreases in P_N, c_i, g_s, and E by 30.4, 60.8, 16.2, and 52.5 %, respectively, in comparison with the control. When compared to the U treatment, the US, UN, and USN treatments significantly increased these parameters.

The U treatment significantly decreased F_v/F_m compared with the C treatment. However, the F_v/F_m under the US, UN and USN treatments significantly increased

Table 1. Growth parameters of dwarf polish wheat seedlings treated with UV-B, salicylic acid (SA), sodium nitropruside (SNP), and their combination. Means ± SDs ($n = 3$). Different letters indicate significant differences at $P < 0.05$. C - control, S - 0.5 mM SA, N - 0.5 mM SNP, SN - SA + SNP; U - UV-B, US - UV-B + SA, UN - UV-B + SNP, USN - UV-B + SA + SNP, R/S - root/shoot ratio.

Treatments	Plant height [cm]	Root length [cm]	Roots [g(d.m.) $plant^{-1}$]	Shoots [g(d.m.) $plant^{-1}$]	R/S ratio
C	57.05 ± 4.00 a	26.40 ± 3.23 ab	0.28 ± 0.01 a	1.73 ± 0.0 3a	0.16 ± 0.01 b
S	52.12 ± 1.20 ab	23.19 ± 1.02 b	0.19 ± 0.02 c	1.80 ± 0.09 a	0.11 ± 0.02 ef
N	55.38 ± 2.74 ab	29.18 ± 3.00 a	0.22 ± 0.01 b	1.75 ± 0.03 a	0.13 ± 0.05 d
SN	53.07 ± 2.03 ab	25.26 ± 2.07 ab	0.28 ± 0.02 a	1.54 ± 0.07 b	0.18 ± 0.00 a
U	49.06 ± 1.00 b	27.19 ± 3.04 ab	0.13 ± 0.01 e	1.32 ± 0.05 c	0.10 ± 0.02 f
US	54.18 ± 3.00 ab	23.32 ± 2.50 b	0.16 ± 0.01 c	1.39 ± 0.04 c	0.12 ± 0.03 de
UN	54.31 ± 2.03 ab	27.23 ± 2.95 ab	0.15 ± 0.01 de	1.40 ± 0.07 c	0.11 ± 0.01 e
USN	56.41 ± 7.05 a	30.38 ± 3.66 a	0.24 ± 0.02 b	1.61 ± 0.06 b	0.15 ± 0.02 c

Table 2. Photosynthetic pigments in leaves of dwarf polish wheat seedlings treated with UV-B, salicylic acid, sodium nitropruside, and their combination. Means ± SDs (n = 3). Different small letters indicate significant differences at $P < 0.05$. Chl - chlorophyll, Car - carotenoids. For treatment abbreviations see Table 1.

Treatments	Chl *a* [mg g^{-1}(f.m.)]	Chl *b* [mg g^{-1}(f.m.)]	Chl *a*/*b*	Car [mg g^{-1}(f.m.)]
C	2.12 ± 0.02 a	0.60 ± 0.03 a	3.53 ± 0.20 a	0.44 ± 0.02 a
S	2.02 ± 0.03 b	0.53 ± 0.02 b	3.84 ± 0.09 a	0.41 ± 0.00 b
N	2.06 ± 0.03 b	0.54 ± 0.02 b	3.84 ± 0.13 a	0.41 ± 0.00 b
SN	2.06 ± 0.02 b	0.55 ± 0.04 b	3.75 ± 0.31 a	0.41 ± 0.00 b
U	1.74 ± 0.04 d	0.46 ± 0.03 c	3.80 ± 0.15 a	0.35 ± 0.01 d
US	1.90 ± 0.03 c	0.53 ± 0.04 b	3.57 ± 0.22 a	0.38 ± 0.02 c
UN	1.90 ± 0.03 c	0.51 ± 0.02 b	3.75 ± 0.16 a	0.37 ± 0.02 c
USN	2.05 ± 0.03 b	0.53 ± 0.01 b	3.85 ± 0.07 a	0.42 ± 0.00 ab

Table 3. Photosynthesis and chlorophyll (Chl) fluorescence parameters of dwarf polish wheat seedlings treated with UV-B, salicylic acid, sodium nitropruside, and their combination. Means ± SDs (n = 3). Different small letters indicate significant differences at $P < 0.05$. P_N - net photosynthtic rate, g_s - stomatal conductance; c_i - intercellular CO_2 concentration; E - transpiration rate; F_v/F_m - variable to maximum fluorescence ratio. For treatment abbreviations see Table 1.

Treatments	P_N [μmol m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]	c_i [μmol mol^{-1}]	E [mmol m^{-2} s^{-1}]	F_v/F_m
C	26.41 ± 0.44 a	0.51 ± 0.02 c	299.15 ± 6.68 d	3.94 ± 0.04 b	0.75 ± 0.01 a
S	20.92 ± 0.96 b	0.44 ± 0.10 d	309.38 ± 4.88 c	3.64 ± 0.10 c	0.71 ± 0.01 c
N	20.15 ± 0.33 bc	0.43 ± 0.02 d	307.69 ± 3.03 c	3.44 ± 0.08 c	0.69 ± 0.01 d
SN	20.90 ± 0.92 b	0.54 ± 0.20 b	321.91 ± 1.96 ab	4.12 ± 0.11 b	0.69 ± 0.01 d
U	18.37 ± 0.37 d	0.20 ± 0.12 f	250.56 ± 2.45 e	1.87 ± 0.13 e	0.66 ± 0.01 e
US	18.60 ± 0.18 d	0.36 ± 0.06 e	304.58 ± 2.44 cd	3.02 ± 0.16 d	0.68 ± 0.01 d
UN	19.22 ± 0.28 cd	0.44 ± 0.10 d	316.31 ± 2.09 b	3.56 ± 0.14 c	0.68 ± 0.01 d
USN	25.58 ± 0.40 a	0.72 ± 0.01 a	324.62 ± 3.12 a	5.11 ± 0.11 a	0.73 ± 0.01 b

when compared to the U treatment (Table 3, Fig. 1).

No significant difference in proline content was observed among the S, SN, and C treatments, whereas the N treatment significantly increased proline content compared with the controls (Fig. 2). Compared with the control, proline content significantly increased under the UV-B stress. When SA, SNP, and their combination were applied, proline content decreased approximately by 24.6, 10.3, and 46.3 %, respectively, in the plants under the UV-B stress. Additionally, MDA content under the UV-B stress was higher than in the controls (Fig. 2). There was no significant difference in MDA between the US and UN treatments, but MDA significantly decreased in the USN treatment. Hydrogen peroxide (Fig. 3*A*) and $O_2^{\bullet-}$ (Fig. 3*B*) were over-produced as indicated by the scattered brown polymerization products and dark blue spots, respectively, in leaves of the UV-B-treated plants. Compared with the plants under the UV-B stress, both H_2O_2 and $O_2^{\bullet-}$ were

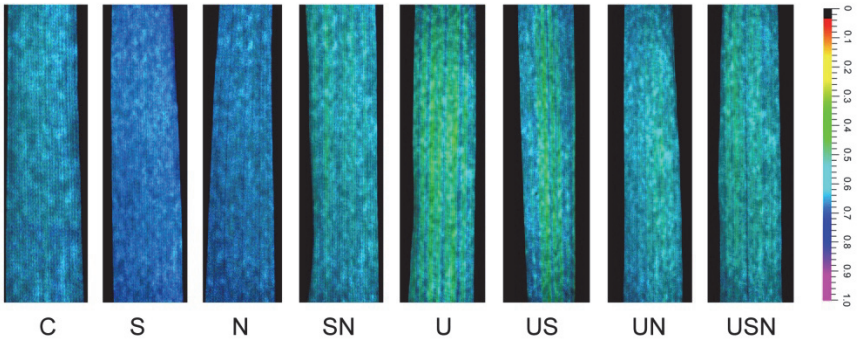

Fig. 1. Representative fluorescence images of maximum efficiency of photosystem II - variable to maximum fluorescence ratio F_v/F_m in leaves of dwarf polish wheat seedlings treated with UV-B, salicylic acid, sodium nitropruside, and their combination. For treatment abbreviations see Table 1.

considerably diminished in the US, UN, and especially USN treatments. The UV-B stress changed activities of antioxidant enzymes (Fig. 4). Compared with the C treatment, the UV-B stress significantly increased activities of APX, POD, SOD, and GR by 73.2, 66.9, 23.0, and 53.3 %, respectively. Application of SA, SNP, or their combination led to a further increase in activities of APX, POD, SOD and GR. Moreover, the combined treatment induced higher increases in activities of SOD, APX, and GR than SA or SNP alone under the UV-B stress.

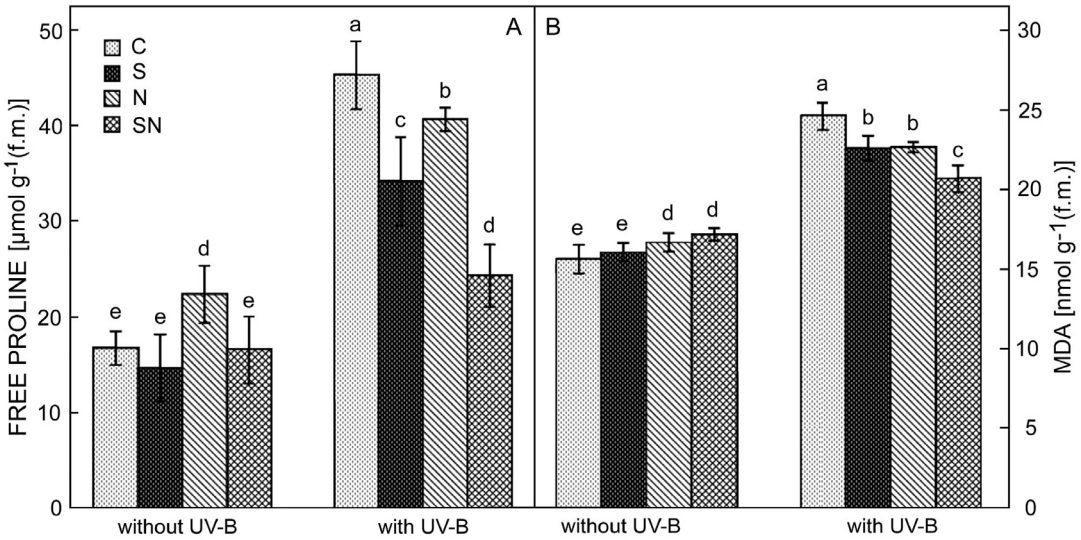

Fig. 2. Free proline and malondialdehyde (MDA) content in dwarf polish wheat seedlings treated with UV-B, salicylic acid, (SA), sodium nitropruside (SNP), and their combination. C - control, S - 0.5 mM SA; N - 0.5 mM SNP, SN - 0.5 SA + SNP. Means ± SDs (n = 3). Different small letters indicate significant differences at $P < 0.05$.

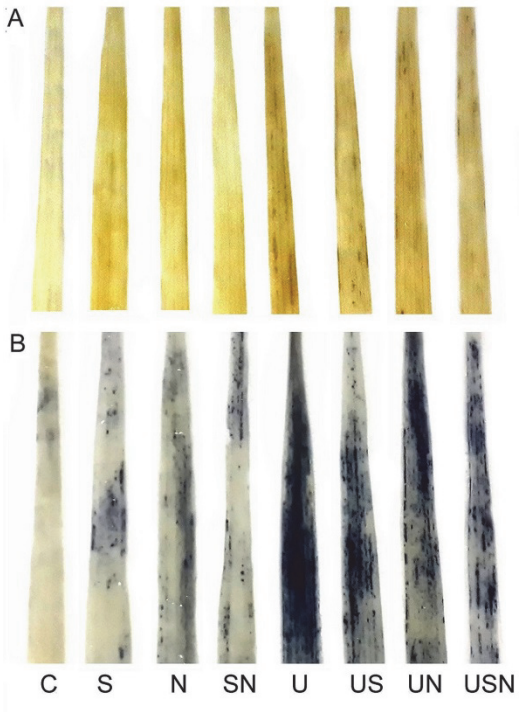

Fig. 3. Histochemical detection of H_2O_2 with 3,3-dimethoxybenzidine (*A*) and of $O_2^{\bullet-}$ with nitroblue tetrazolium chloride (*B*) in leaves of polish wheat seedlings treated with UV-B, salicylic acid, sodium nitropruside, and their combination. For treatment abbreviations see Table 1.

Discussion

It is generally accepted that enhanced UV-B is detrimental to plants by reducing growth and biomass production (De la Rosa *et al*. 2003) and affecting root/shoot ratio (Yang *et al*. 2005, Xu *et al*. 2010). Moreover, a change in biomass accumulation is a reliable measure to assess plant sensitivity to UV-B stress (Smith *et al*. 2000). In the present study, a reduction in dry mass of both shoots and roots was observed in dwarf polish wheat grown under the UV-B stress (Table 1). A higher concentration of SA or SNP has a negative effect on growth of roots of maize seedlings (Gouvea *et al*. 1997) and chamomile plants (Kováčik *et al*. 2009). In our study, we found that SA or SNP significantly decreased root dry mass, however, the treatments with SA + SNP increased dry mass of shoots and plant height under the UV-B stress (Table 1). These results are in agreement with growth stimulation by SA in mung bean seedlings (Singh *et al*. 2014) and alleviation of UV-B stress by NO in wheat (Fu *et al*. 2013). It is possible that under the UV-B stress, the better performance of the plants treated with SA, SNP, or SA + SNP was mainly associated with higher activities of antioxidant enzymes and direct ROS scavenging capacities.

Under the UV-B stress, significant decreases in Chl *a*, Chl *b*, and Car content (Table 2) were observed in our study. It has been well documented that ROS produced under stress conditions cause pigment degradation (Anjum *et al*. 2011). Application of SA scavenges ROS and then inhibits Chl degradation under abiotic stress (Sheng *et al*. 2015). In addition to SA, NO is believed to be a signaling molecule and it enhances antioxidant defense ability in plants exposed to UV-B radiation (An *et al*. 2005). Correia *et al*. (2005) reported that an enhanced UV-B reduces

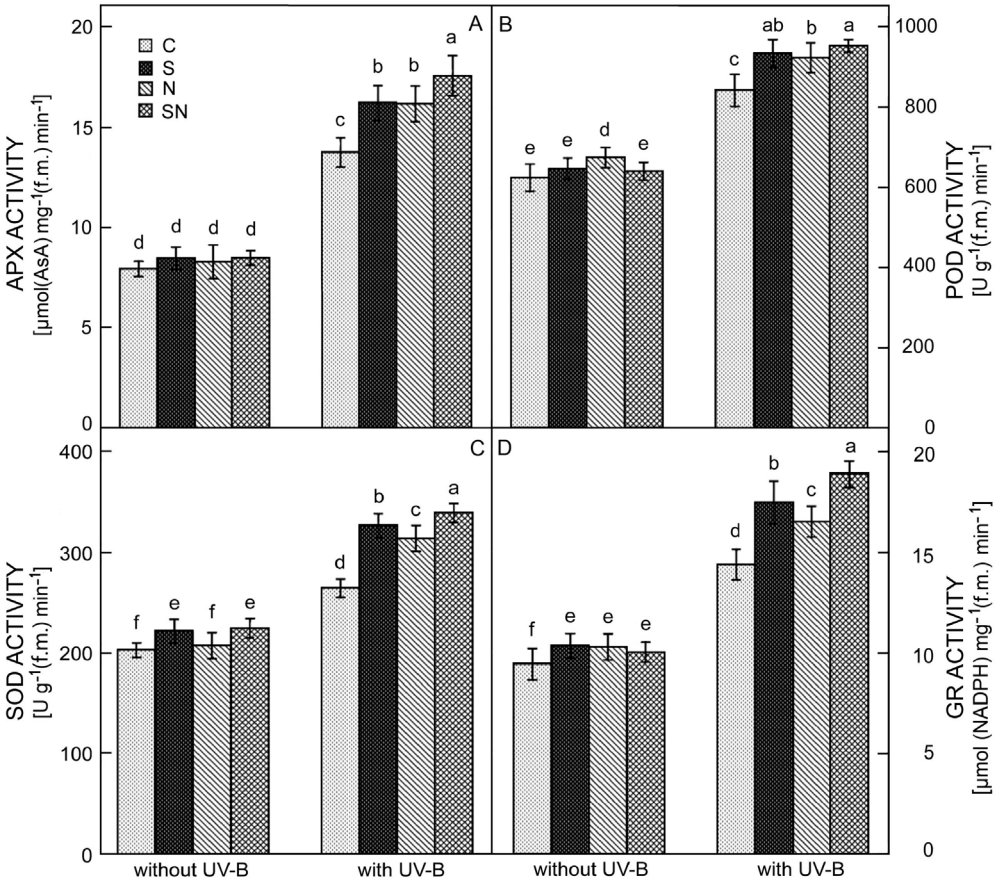

Fig. 4. Activities of ascorbate peroxidase (APX), peroxidase (POD), superoxide dismutase (SOD), and glutathione reductase (GR) in leaves of gwarf polish wheat seedlings treated with UV-B, salicylic acid (SA), sodium nitropruside (SNP), and their combination. C - control, S - 0.5 mM SA, N - 0.5 mM SNP, SN - SA + SNP. Means ± SDs (n = 3). Different small letters indicate significant differences at $P < 0.05$.

P_N, g_s, and E; and increases c_i of maize. In our study, the UV-B treatment resulted in a significant decrease of all photosynthetic parameters. The decreases in P_N, g_s, c_i, E, F_v/F_m, and pigment content of the dwarf polish wheat seedlings exposed to the UV-B stress may have been due to damage to chloroplasts. Compared with the controls, the S and N treatments decreased P_N, g_s, E, and F_v/F_m, but increased c_i (Table 3). This implies that the reductions in

photosynthesis were probably nonstomatal. Salicylic acid reduces the capacity for CO_2 fixation independently of diffusion limitations (Pancheva *et al*. 1996). Nitric oxide has been found to decrease activity of enzymes that regulate photosynthesis in wheat (Tu *et al*. 2002) and also to reduce F_v/F_m in potato leaves (Yang *et al*. 2004). When SA or NO are applied to plants exposed to stress conditions, P_N, g_s, and E in *Brassica juncea* are significantly ameliorated (Fariduddin *et al*. 2003). In our experiments, the application of SA, SNP, or their combination increased P_N, g_s, c_i, E, and F_v/F_m of the wheat plants under the UV-B stress (Table 3, Fig. 1). These results indicate that SA, SNP, and especially their combination might improve photosynthesis in plants exposed to UV-B stress.

The dwarf polish wheat seedlings responded to the UV-B stress by accumulation of proline (Fig. 2). Proline accumulated in shoots of rice, mustard, and mung bean seedlings exposed to UV-B irradiation could protect plant cells against peroxidation (Saradhi *et al*. 1995). It is also known that proline acts as compatible solute, plasma membrane protector, and hydroxyl radical scavenger. The observed decrease in proline content under the SA, SNP, and SA + SNP treatments suggests a partial relief from the UV-B stress. Malondialdehyde, as the final product of lipid peroxidation, is widely used as indicator of oxidative stress (Tayebimeigooni *et al*. 2012). The UV-B stress significantly increased MDA content in our study (Fig. 2) indicating oxidative damage. Content of MDA in the presence of SA, SNP or SA + SNP under the UV-B stress was much lower than under the UV-B stress alone indicating reduction in lipid peroxidation. Therefore, SA, SNP or SA + SNP contribute to amelioration of UV-B stress. The results are in agreement with other studies (Alexieva *et al*. 2001, Yao and Liu 2007).

The UV-B radiation increases ROS production in plants (Santos *et al*. 2004). Previous studies have shown that SA is direct scavenger that protects plants from oxidative damage (Li *et al*. 2014, Singh *et al*. 2014, Sheng *et al*. 2015). Additionally, NO protects plants from oxidative damage by maintaining cellular redox homeostasis and promoting transformation of $O_2^{\bullet-}$ to H_2O_2 and O_2 and also by enhancing activities of enzymes scavenging H_2O_2 (Zheng *et al*. 2009). NO is involved in various UV-B signaling pathways leading to protective mechanisms (Beligni and Lamattina 1999). In our study, the excess UV-B radiation caused a dramatic increase of H_2O_2 and $O_2^{\bullet-}$ accumulation in leaves of dwarf polish wheat (Fig. 3). We also found that SA and SNP increased accumulation of H_2O_2 and $O_2^{\bullet-}$ compared with the control. However, SA, SNP, and especially SA + SNP significantly counteracted the increase in H_2O_2 and $O_2^{\bullet-}$ accumulation induced by the UV-B (Fig. 3).

A number of enzymatic antioxidants are produced in plants in response to abiotic stresses. They save them from oxidative damage caused by ROS (Ashraf 2009). The UV-B radiation promotes ROS formation significantly and exerts oxidative stress to plants (Chen *et al*. 2003). Activities of SOD, APX, POD, and GR show that they significantly increased under the UV-B stress (Fig. 4). Similar results were documented by Yao and Liu (2007). Application of SA, SNP, and their combination further enhanced activities of SOD, APX, POD, and GR in the plant leaves under the UV-B stress (Fig. 4). In fact, many previous studies demonstrated that NO can effectively protect plants from UV-B destruction mostly by enhanced activities of antioxidant enzymes (Santa-Cruz *et al*. 2010) and also SA induces an increase in antioxidant activity under multiple stresses (Wang *et al*. 2011, Saruhan *et al*. 2012, Sheng *et al*. 2015).

In conclusion, the UV-B stress was harmful to dwarf polish wheat. The effects of the UV-B mainly resulted from the accumulation of ROS. Salicylic acid, SNP, and SA + SNP significantly ameliorated the adverse effects of the UV-B stress by the enhanced activities of the antioxidative enzymes and by promotion of photosynthesis. The combination of SA + SNP was better than SA or SNP alone.

References

Alexieva, V., Sergiev, I., Mapelli, S., Karanov, E.: The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. - Plant Cell Environ. **24**: 1337-1344, 2001.

An, L.Z., Liu, Y.H., Zhang, M.X.: Effect of nitric oxide on growth of maize seedling leaves in the presence or absence of ultraviolet-B radiation. - J. Plant Physiol. **162**: 317-326, 2005.

Anjum, S.A., Xie, X.Y., L. Wang, L.C., Saleem, M.F., Man, C., Wang, L.: Morphological, physiological and biochemical responses of plants to drought stress. - Afr. J. agr. Res. **6**: 2026-2032, 2011.

Antoniou, C., Filippou, P., Mylona, P., Fasoula, D., Ioannides, I., Polidoros, A., Fotopoulos, V.: Developmental stage and concentration-specific sodium nitroprusside application results in nitrate reductase regulation and the modification of nitrate metabolism in leaves of *Medicago truncatula* plants. - Plant Signal Behav. **8**: e25479, 2014.

Ashraf, M.: Biotechnological approach of improving plant salt tolerance using antioxidants as markers. - Biotechnol. Adv. **27**: 84-93, 2009.

Bandurska, H., Ciéslak, M.: The interactive effect of water deficit and UV-B radiation on salicylic acid accumulation in barley roots and leaves. - Environ. exp. Bot. **94**: 9-18, 2013.

Bates, L.S., Waldern, S.P., Teare, I.D.: Rapid determination of free proline for water-stress studies. - Plant Soil **39**: 205-207, 1973.

Beauchamp, C., Fridovich, I.: Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. - Anal. Biochem. **44**: 276-287, 1971.

Beligni, M.V., Lamattina, L.: Nitric oxide counteracts cytotoxic processes mediated by reactive oxygen species in plant tissues. - Planta **28**: 337-344, 1999.

Boveris, A.D., Galatro, A., Puntarulo, S.: Effect of nitric oxide and plant antioxidants on microsomal content of lipid radicals. - Biol. Res. **33**: 159-165, 2000.

Caldwell, M.M.: Solar ultraviolet radiation and the growth and development of higher plants. - In: Giese, A.C. (ed.): Phytophysiology. Pp. 131-177. Academic Press, New York 1971.

Caldwell, M.M., Bornman, J.F., Ballare, C.L., Flint, S.D., Kulandaivelu, G.: Terrestrial ecosystems, increased solar ultraviolet radiation and interactions with other climatic factors. - Photochem. Photobiol. Sci. **6**: 252-266, 2007.

Chen, K., Feng, H., Zhang, M., Wang, X.: Nitric oxide alleviates oxidative damage in the green alga *Chlorella pyrenoidosa* caused by UV-B radiation. - Folia microbiol. **48**: 389-393, 2003.

Correia, C.M., Pereira, J.M.M., Coutinho, J.F., Björn, L.O., Torres-Pereira, J.M.: Ultraviolet-B radiation and nitrogen affect the photosynthesis of maize: a Mediterranean field study. - Eur. J. Agron. **22**: 337-347, 2005.

De La Rosa, T.M., Aphalo, P.J., Lehto, T.: Effects of ultraviolet-B radiation on growth, mycorrhizas and mineral nutrition of silver birch (*Betula pendula* Roth) seedlings grown in low-nutrient conditions. - Global Change Biol. **9**: 65-73, 2003.

Fariduddin, Q., Hayat, S., Ahmad, A.: Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity, and seed yield in *Brassica juncea*. - Photosynthetica **41**: 281-284, 2003.

Flint, S.D., Caldwell M.M.: A biological spectral weighting function for ozone depletion research with higher plants. - Physiol. Plant. **117**: 137-144, 2003a.

Flint, S.D., Caldwell, M.M.: Field testing of UV biological spectral weighting functions for higher plants. - Physiol Plant. **117**: 145-153, 2003b.

Foyer, C.H., Halliwell, B.: The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. - Planta **133**: 21-25, 1976.

Fu, X.L., Hai, L.T., Li, L.G., Rong, H.: Effects of exogenous nitric oxide on seedling growth, reactive oxygen species and photosynthetic characteristic of wheat under UV-B stress.- Chin. Agr. Sci. Bull. **29**: 43-50, 2013.

Gouvea, C., Souza, J.F., Magalhaes, A.C.N.: NO-releasing substances that induce growth elongation in maize root segments. - Plant Growth Regul. **21**: 183-187, 1997.

Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. - Arch Biochem. Biophys. **125**: 189-198, 1968.

Kováčik, J., Grúz, J., Bačkor, M., Stmad, M., Repčák, M.: Salicylic acid-induced changes to growth and phenolic metabolism in *Matricaria chamomilla* plants. - Plant Cell Rep. **28**: 135-143, 2009.

Jamali, B., Eshghi, S., Tafazoli, E.: Vegetative and reproductive growth of strawberry plants cv. 'Pajaro' affected by salicylic acid and nickel. - J. agr. Sci. Technol. **13**: 95-904, 2011.

Larkindale, J., Knight, M.: Protection against heat stress induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene, and salicylic acid. - Plant Physiol. **128**: 682-695, 2002.

Laspina, N.V., Groppa, M.D., Tomaro, M.L., Benavides, M.P.: Nitric oxide protects sunflower leaves against Cd-induced oxidative stress. - Plant Sci. **169**: 323-330, 2005.

Lichtenthaler, H.K.: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. - Methods Enzymol. **148**: 350-382, 1987.

Li, X.M., Ma, L.J., Bu, N., Li, Y.Y., Zhang, L.H.: Effects of salicylic acid pre-treatment on cadmium and/or UV-B stress in soybean seedlings. - Biol. Plant. **58**: 195-199, 2014.

Modolo, L.V., Cunha, F.Q., Braga, M.R., Salgado, I.: Nitric oxide synthase mediate phytoalexin accumulation in soybean cotyledons in response to the *Diaporthe phaseolorum* f. sp. *meridionalis* elicitor. - Plant Physiol. **130**: 1288-1297, 2002.

Mutlu, S., Atici, O., Nalbantoglu, B.: Effects of salicylic acid and salinity on apoplastic antioxidant enzymes in two wheat cultivars differing in salt tolerance. - Biol. Plant. **53**: 334-338, 2009.

Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. - Plant Cell Physiol. **22**: 867-880, 1981.

Neil, S.J., Desikan R., Clarke, A., Hancock, J.T.: Nitric oxide is a novel component of abscisic acid signalling in stomatal guard cells. - Plant Physiol. **128**: 13-16, 2002.

Nickel, R.S., Cunningham, B.A.: Improved peroxidase assay method using ieuco 2,3,6-trichloroindophenol and application to comparative measurements of peroxidase catalysis. - Anal Biochem. **27**: 292-299, 1969.

Pagnussat, G.C., Simontacchi, M., Puntarulo, S., Lamattina, L.: Nitric oxide is required for root organogenesis. - Plant Physiol. **129**: 954-956, 2002.

Pancheva, T.V., Popova, L.P., Uzunova, A.N.: Effects of salicylic acid on growth and photosynthesis in barley plants. - J. Plant Physiol. **149**: 57-63, 1996.

Prochazkova, D., Sairam, R.K., Srivastava, G.C., Singh, D.V.: Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. - Plant Sci. **161**: 765-771, 2001.

Ren, J., Yao, Y., Yang, Y., Korpelainen, H., Junttila, O., Li, C.: Growth and physiological responses to supplemental UV-B radiation of two contrasting poplar species. - Tree Physiol. **26**: 665-672, 2006.

Rozema, J., Van de Staaij, J., Björn, L.O., Caldwell, M.: UV-B as an environmental factor in plant life: stress and regulation. - Trends Ecol. Evol. **12**: 22-28, 1997.

Santa-Cruz, D.M., Pacienza, N.A., Polizio, A.H., Balestrasse, K.B., Tomaro, M.L., Yannarelli, G.G.: Nitric oxide synthase-like dependent NO production enhances heme oxygenase up-regulation in ultraviolet-B-irradiated soybean plants. - Phytochemistry **71**: 1700-1707, 2010.

Santos, I., Fidalgo, F., Almeida, J.M., Salema, R: Biochemical and ultrastructural changes in leaves of potato plants grown under supplementary UV-B radiation. - Plant Sci. **167**: 925-935, 2004.

Saradhi, P.P., Alia, S.A., Prasad, K.V., Arora, S.: Proline accumulates in plants exposed to UV radiation and protects them against UV induced peroxidation. - Biochem. biophys. Res. Commun. **209**: 1-5, 1995.

Saruhan, N., Saglam, A., Kadioglu, A.: Salicylic acid pretreatment induces drought tolerance and delays leaf rolling by inducing antioxidant systems in maize genotypes. - Acta Physiol. Plant. **34**: 97-106, 2012.

Sheng H., Zeng, J., Yan, F., Wang, X., Wang, Y., Kang, H., Zhou, Y.: Effect of exogenous salicylic acid on manganese toxicity, mineral nutrients translocation and antioxidative system in polish wheat (*Triticum polonicum* L.). - Acta Physiol. Plant. **37**: 1-11, 2015.

Singh, V.P., Kumar, J., Singh, S., Prasad, S.M.: Dimethoate modifies enhanced UV-B effects on growth, photosynthesis and oxidative stress in mung bean (*Vigna radiata* L.) seedlings: Implication of salicylic acid. - Pestic. Biochem. Physiol. **116**: 13-23, 2014.

Smith, J.L., David, J.B., Bannister, P.: Shoot dry weight chlorophyll and UV-B-absorbing compounds as indicators of plant's sensitivity to UV-B radiation. - Ann Bot. **86**: 1057-1063, 2000.

Strid, A., Chow, W.S., Anderson, J.M.: UV-B damage and

protection at the molecular level in plants. - Photosynth. Res. **39**: 475-489, 1994.

Tanou, G., Filippou, P., Belghazi, M., Job, D., Diamantidis, G., Fotopoulos, V.: Oxidative and nitrosative-based signaling and associated post-translational modifications orchestrate the acclimation of citrus plants to salinity stress. - Plant J. **72**: 585-599, 2012.

Tayebimeigooni, A., Awang, Y., Mahmood, M., Selamat, A., Wahab, Z.: Leaf water status, proline content, lipid peroxidation and accumulation of hydrogen peroxide in salinized Chinese kale (*Brassica alboglabra*). - J. Food Agr. Environ. **10**: 371-374, 2012.

Tu, J., Shen, W.B., Xu, L.: Regulation of nitric oxide on the aging process of wheat leaves. - Acta bot. Sin. **45**: 1052-1062, 2002.

Wang, C., Zhang, S., Wang, P., Hou, J., Qian, J., Ao, Y., Li, L.: Salicylic acid involved in the regulation of nutrient elements uptake and oxidative stress in *Vallisneria natans* (Lour.) Hara under Pb stress. - Chemosphere **84**: 136-142, 2011.

Waseem, M., Athar, H.U.R., Ashraf, M.: Effect of salicylic acid applied through rooting medium on drought tolerance of wheat. - Pakistan J. Bot. **38**: 1127-1136, 2006.

Wiwart, M., Suchowilska, E., Kandler, W., Sulyok, M., Groenwald, P., Krska, R.: Can polish wheat (*Triticum polonicum* L.) be an interesting gene source for breeding wheat cultivars with increased resistance to *Fusarium* head blight? - Genet. Resour. Crop Evol. **60**: 2359-2373, 2013.

Xu, X., Zhao, H., Zhang, X., Hänninen, H., Korpelainen, H., Li, C.: Different growth sensitivity to enhanced UV-B radiation between male and female *Populus cathayana*. - Tree Physiol. **30**: 1489-1498, 2010.

Yang, J. D., Zhao, H. L., Zhang, T. H., Yun, J. F., Liang, S.C.: Effects of exogenous nitric oxide on photochemical activity of photosystem II in potato leaf tissue under non-stress condition. - Acta bot. sin. **46**: 1009-1014, 2004.

Yang, Y.Q., Yao, Y.A., Xu, G., Li, C.Y.: Growth and physiological responses to drought and elevated ultraviolet-B in two contrasting populations of *Hippophae Rhamnoides*. - Physiol. Plant. **124**: 431-440, 2005.

Yao, X., Liu, Q.: Changes in photosynthesis and antioxidant defenses of *Picea asperata* seedlings to enhanced ultraviolet-B and to nitrogen supply. - Physiol. Plant. **129**: 364-374, 2007.

Yu, J., Tang, X.X., Zhang, P.Y., Tian, J.Y., Cai, H.J.: Effects of CO_2 enrichment on photosynthesis, lipid peroxidation and activities of antioxidative enzymes of *Platymonas subcordiformis* subjected to UV-B radiation stress. - Acta bot. sin. **46**: 682-690, 2004.

Zheng, C., Jiang, D., Liu, F., Dai, T., Liu, W., Jing, Q., Cao, W.: Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. - Environ. exp. Bot. **67**: 222-227, 2009.