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RESEARCH ARTICLE

# Effects of sodium nitroprusside (SNP) pretreatment on UV-B stress tolerance in lettuce (*Lactuca sativa* L.) seedlings

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**Abstract** Ultraviolet-B (UV-B) radiation is one of the most important abiotic stress factors that could influence plant growth, development, and productivity. Nitric oxide (NO) is an important plant growth regulator involved in a wide variety of physiological processes. In the present study, the possibility of enhancing UV-B stress tolerance of lettuce seedlings by the exogenous application of sodium nitroprusside (SNP) was investigated. UV-B radiation increased the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD) and total phenolic concentrations, antioxidant capacity, and expression of phenylalanine ammonia lyase (PAL) gene in seedlings, but the combination of SNP pretreatment and UV-B enhanced antioxidant enzyme activities, total phenolic concentrations, antioxidant capacity, and PAL gene expression even more. Moreover, UV-B radiation significantly inhibited chlorophylls, carotenoid, gibberellic acid (GA), and indole-3-acetic acid (IAA) contents and increased the contents of abscisic acid (ABA), salicylic acid (SA), malondialdehyde (MDA), hydrogen peroxide ($H_2O_2$), and superoxide radical ($O_2•^-$) in lettuce seedlings. When SNP pretreatment was combined with the UV-B radiation, we observed alleviated chlorophylls, carotenoid, GA, and IAA inhibition and decreased content of ABA, SA, MDA, $H_2O_2$, and $O_2•^-$ in comparison to non-pretreated stressed seedlings.

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## Introduction

Ultraviolet-B (UV-B) is one of the most detrimental environmental stresses that hinder plant growth and development. Several studies have shown deleterious effects of UV-B stress on plants via reduced photosynthesis and biomass production (Tossi et al. 2011, 2012; Zlatev et al. 2012; Hideg et al. 2013). On the other hand, UV-B not only inhibits the growth and development of plants, but also induces the overproduction of reactive oxygen species (ROS), including superoxide anion ($O_2•^-$) and hydrogen peroxide ($H_2O_2$) (Mittler 2002). The accumulation of ROS causes lipid peroxidation, which will ultimately lead to plant cell death. To mitigate the damage from ROS, plants have developed enzymatic antioxidant, such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT) and also possess non-enzymatic antioxidants that include reduced ascorbate, glutathione, α-tocopherol, and flavonoids.

Nitric oxide, a small highly diffusible bioactive molecule, is believed to play an important role in a wide range of physiological processes in plants, such as germination, iron homeostasis, mitochondrial functionality, fruit ripening, floral regulation, and programmed cell death (Khan et al. 2012; Xu et al. 2014). Many studies show that exogenous nitric oxide (NO) improves the growth and yield of a number of plants by enhancing growth and development of plant tissue (Mur et al. 2012). Furthermore, a number of studies indicated that NO in low concentration regulates key physiological processes associated with plant growth under various biotic and abiotic stresses, including UV (Tossi et al. 2011), low and high temperature (Song et al. 2006), salinity (Khan et al. 2012),

drought (Boogar et al. 2014), and heavy metals (He et al. 2012).

In the present study, lettuce was used as a model plant, since it is one of the most important dietary leafy vegetables which is primarily consumed worldwide fresh or fresh-cut (Putnam et al. 2000). In addition, lettuce is an important source of dietary antioxidants especially considering its high ROS scavenging activity (Ramos et al. 2011). It contains a number of nutritive and health-promoting compounds such as phenolic components; Vitamin A, C, and E; calcium; lutein; and dietary fiber (Ramos et al. 2011). Furthermore, it was also reported that lettuce was able to eliminate the stress factors such as drought or UV-B irradiation by activating genes responsible for phenylalanine ammonia lyase (PAL) biosynthesis (Oh et al. 2010) or triggering the synthesis of anthocyanin and other flavonoids (Tsormpatsidis et al. 2010).

The objective of this study was to investigate the interactive effect of UV-B radiation and exogenous sodium nitroprusside (SNP) (a nitric oxide donor) treatments on the ROS, photosynthetic pigments, antioxidant enzymes, total phenolic concentration, antioxidant capacity, some endogenous hormones, and soluble sugars of lettuce seedlings.

## Materials and methods

### Plant material

Lettuce seeds were supplied from the Agricultural Faculty of Atatürk University. They were sterilized with 5 % sodium hypochlorite for 15 min and rinsed thoroughly with distilled water, then germinated on moist filter paper in the dark at 25 °C for 3 days. Germinated seedlings were grown on soil/vermiculite (3:1, *v*/*v*) at 25 °C in an environment-controlled chamber at a light intensity of 120 μmol photons $m^{-2}$ $s^{-1}$ and a 14/10 h light/dark photoperiod.

### SNP and UV-B treatment

Thirteen-day-old healthy seedlings were used in this experiment. When the second pair of leaves were fully expanded, seedlings were sprayed with 100 μM of SNP (Sigma Aldrich St. Lois, MO, USA) or $H_2O$. After 24 h, they were exposed to UV-B radiation for 18 h by using UV-B lamp (Philips TL100W/12) at an irradiance of 3.3 $Wm^{-2}$. After completion of UV-B treatment, leaves were immediately sampled for various analyses.

### Determination of lipid peroxidation

Lipid peroxidation was determined by the method of Heath and Packer (1968). This method is based on the determination of the content of malondialdehyde produced by thiobarbituric acid reacting substances (TBARS).

### Determination of hydrogen peroxide and superoxide radical

$H_2O_2$ concentration was determined according to Patterson et al. (1984). The assay was based on the absorbance change of the titanium peroxide complex at 415 nm. Absorbance values were quantified using standard curve generated from known concentrations of $H_2O_2$. The rate of superoxide production was measured by the method of Elstner and Heupel (1976).

### Determination of photosynthetic pigments

Fresh leaf tissues (0.1 g) were homogenized in chilled 80 % (*v*/*v*) acetone. The homogenate was centrifuged at 8800*g* for 10 min at 4 °C in dark. The absorbance of the acetone extract was measured at 663, 645, and 470 nm using a spectrometer (Shimadzu UV mini 1240). The contents of chlorophyll *a*, chlorophyll *b*, and total carotenoids were calculated according to Arnon (1949).

### Determination of antioxidant enzyme activity

To determine the activities of antioxidant enzymes, fresh leaves (0.5 g) were ground with a mortar and pestle under chilled conditions in the presence of phosphate buffer (0.1 M, pH 7.5) containing 0.5 mM EDTA. The homogenate was centrifuged at 12,000*g* for 10 min at 4 °C, and the resulting supernatant was used for the enzyme assay.

SOD activity was assayed using the method of Agarwal and Pandey (2004) that spectrophotometrically measures inhibition of the photochemical reduction of nitroblue tetrazolium at 560 nm.

POD activity was measured according to the method of Zhang and Kirkham (1994). The enzyme extract (20 mL) was added to the reaction mixture containing 20 mL guaiacol solution and 10 mL $H_2O_2$ solution in 3 mL of phosphate buffer solution (pH 7.0). The addition of enzyme extract started the reaction, and the increase in absorbance was recorded at 470 nm for 5 min. Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient (26.6 $mM^{-1}cm^{-1}$).

CAT activity was performed according to Qiu et al. (2011); the reaction mixture in a total volume of 2 ml contained 0.1 ml enzyme extract, 100 mM phosphate buffer (pH 7), 0.1 μM EDTA, and 0.1 % $H_2O_2$.

APX activity was determined according to Nakano and Asada (1981); the reaction mixture in a total volume of 3 ml consisted of 50 mM phosphate buffer (pH 7), 0.1 ml enzyme extract, 0.1 mM EDTA, 0.5 mM ascorbate, and 0.1 mM $H_2O_2$.

APX was assayed as a decrease in absorbance at 290 nm of ascorbate, and enzyme activity was quantified using the molar extinction coefficient for 2.8 $mM^{-1}$ $cm^{-1}$.

### Determination of total phenolic concentration and antioxidant capacity

The concentrations of phenolic compounds in the extracts of lettuce leaves were measured according to the Folin–Ciocalteu reagent method.

The antioxidant capacity of lettuce leaves was measured by the modified 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) or ABTS method (Miller and Rice-Evans 1996).

### PAL gene expression

Total RNA from the leaves of lettuce was isolated using RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. cDNA was synthesized using the RevertAidTM First Strand cDNA Synthesis Kit (Fermentas catalog no: K1622). The analysis of the PAL and γ-TMT gene expressions were carried out according to Lee et al. (2014) using lettuce-specific primers. The primers used for the PAL (forward: ACGAAATGGACCGTTACAG, reverse: TTCCCTCTCGATCATTTTGG) and γ-TMT (forward: TGTTGACGCAATACCACCAC, reverse: GCCATTG TCATCGGAGGAAC) were designed by Primer 3 software, referring to accessed sequencing in the gene bank. For normalization, the lettuce β-actin gene (forward: AGCAACTGGGATGACATGGA, reverse: GGGTTG AGAGGTGCCTCAGT) as endogenous control was used. Real-time PCR was performed with a Rotor-gene Q instrument using SYBR Green detection chemistry (Quantifast SYBR Green PCR Kit, Qiagen). The relative ratio of threshold cycle (ct) values between the endogenous control and the specific genes was calculated for each sample.

### Determination of phytohormones

Extraction and purification of phytohormones were done by some modifications of the methods of Kuraishi et al. (1991) and Battal and Tileklioglu (2001). First, methanol (80 % and −40 °C) was added to fresh leaf samples (Davies 1995). Material was homogenized for 10 min with Ultra Turrax and incubated for 24 h in the dark. The final samples were filtered through Whatman No. 1 filter paper, and the supernatants were filtered again through a 0.45-μm pore filter (Cutting 1991). Then, the supernatants were dried at 35 °C using an evaporator pump. Powder supernatants were dissolved in 0.1 M $KH_2PO_4$ (pH 8.0) and centrifuged at 3600*g* for 1 h at 4 °C for the separation of fatty acids (Palni et al. 1983). One gram of polyvinylpyrrolidone (PVPP) was added to the supernatant to remove the phenolic and colored materials (Chen 1991; HernandezMinana 1991; Qamaruddin 1996). It was filtered with Whatman No. 1 paper to remove the PVPP (Cheikh and Jones 1994). On the other hand, for further specific separation, a Sep-Pak C-18 (Waters) cartridge was used. Adsorbed hormones were transferred to vials using 80 % methanol and analyzed by high-performance liquid chromatography (HPLC) using a Zorbax Eclipse-AAA C-18 column (Agilent 1200 HPLC) and by absorbance at 265 nm in a UV detector. Flow speed was set to 1.2 mL $min^{-1}$ at a column temperature of 25 °C. For the determination of phytohormones (abscisic acid (ABA), gibberellic acid (GA), and indole-3-acetic acid (IAA)), 13 % acetonitrile (pH 4.98) was used as the mobile phase.

### Determination of contents of soluble sugars

The soluble sugar contents of plants were determined according to the method of Rosa et al. (2004) with some modifications. For this purpose, the plant tissue was powdered with a mortar. Soluble sugars were extracted from 0.7 g of powdered tissue by homogenization in 2 mL of 80 % (*v*/*v*) ethanol. The homogenate was heated in water bath at 75 °C for 10 min and was centrifuged at 5000*g* for 10 min. It was cooled and precipitate was reextracted using 2 mL of 80 % (*v*/*v*) ethanol and centrifuged again. Under a hot air stream, the supernatant was evaporated and the residue was suspended in distilled water. The final material was subjected to the desalination procedure and applied to determine individual (glucose, fructose, sucrose, and maltose) soluble sugars.

### Statistical analysis

All data presented are mean values. Each value was presented as the mean±SE with a minimum of three experiments. Data were subjected to a one-way analysis ANOVA procedure, and significant differences among treatments were determined by Duncan's multiple range test ($p<0.05$). All statistical analyses were conducted using SPSS version 20.0 (SPSS Inc./IBM Corp.).

## Results

### MDA and ROS

UV-B radiation significantly increased malondialdehyde (MDA) and $H_2O_2$ contents and the rate of $O_2^{\bullet -}$ production in lettuce seedlings over the control (Fig. 1). SNP pretreatment did not significantly affect MDA content and the rate of $O_2^{\bullet -}$ production (Fig. 1a, b). But SNP pretreatment significantly affects $H_2O_2$ content (Fig. 1c). The combination of UV-B and SNP induced an important decrease in MDA (24.5 %)

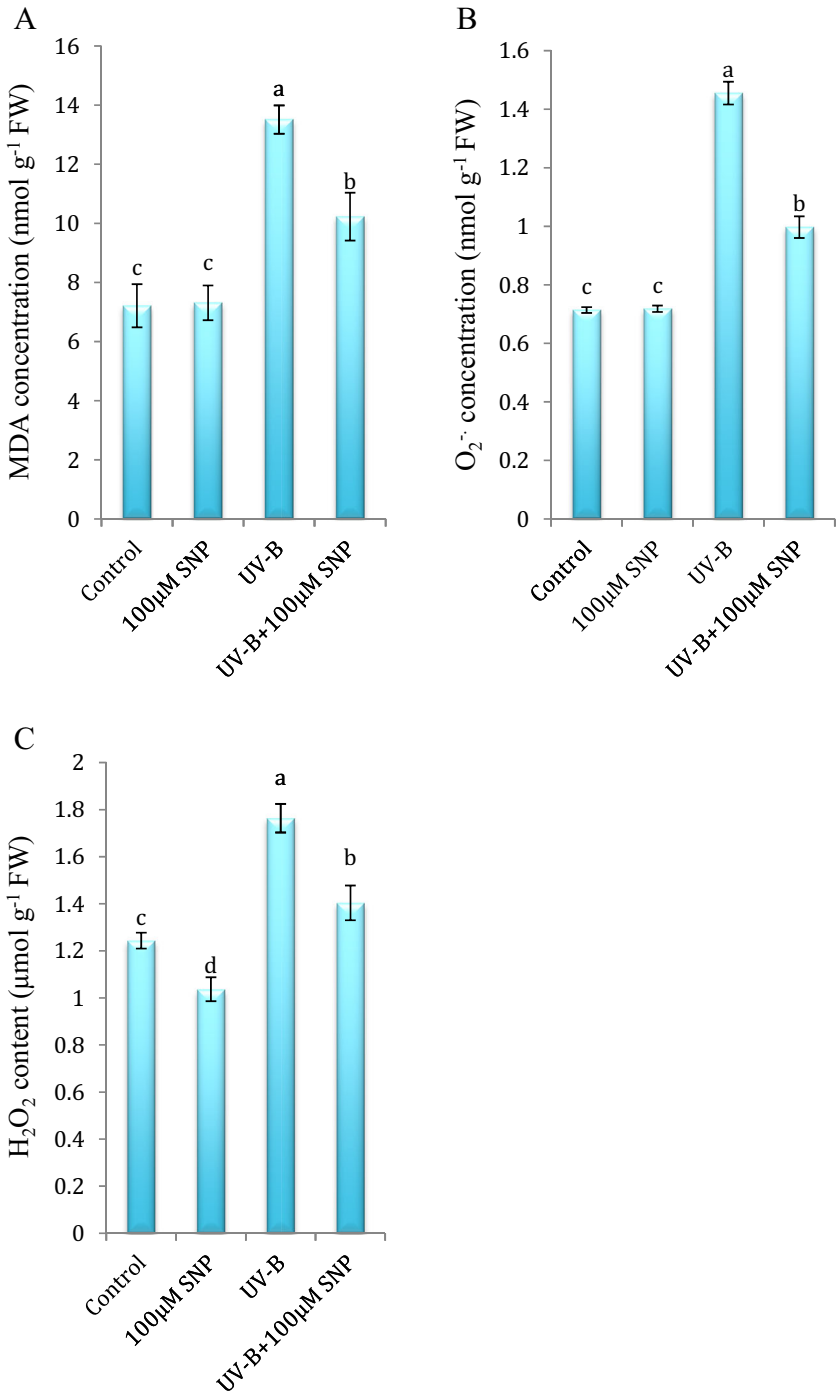


**Fig. 1** Effect of SNP pretreatment on **a** MDA, **b** $O_2^{\bullet -}$, and **c** $H_2O_2$ content in lettuce seedlings exposed to 3.3 $Wm^{-2}$ UV-B for 18 h. *Bars* represent a mean±standard error of three independent experiments. *Different letters* indicate significant differences between treatments ($p<0.05$)

and $H_2O_2$ (20.5 %) contents and the rate of $O_2^{\bullet -}$ production (31.8 %) compared with UV-B treatment alone.

### Antioxidant enzyme activities

As shown in Fig. 2a, SOD activity in leaves increased markedly when plants were subjected to UV-B. Application of 100 μM SNP alone had no statistically important effect on SOD activity. On the other hand, SNP pretreatment further activated activity SOD under UV-B radiation.

As compared with plants grown in control, POD activity under UV-B radiation was remarkably higher (Fig. 2b). Exogenously applied SNP further activated POD under UV-B radiation.

Exposure to UV-B increased CAT activity as well. Compared with the UV-B-only treatment, CAT activity under UV-B radiation with SNP increased notably and significantly (Fig. 2c).

Similarly, APX activity in leaves increased under UV-B radiation. SNP pretreatment significantly enhanced the APX activity under UV-B stress (Fig. 2d).

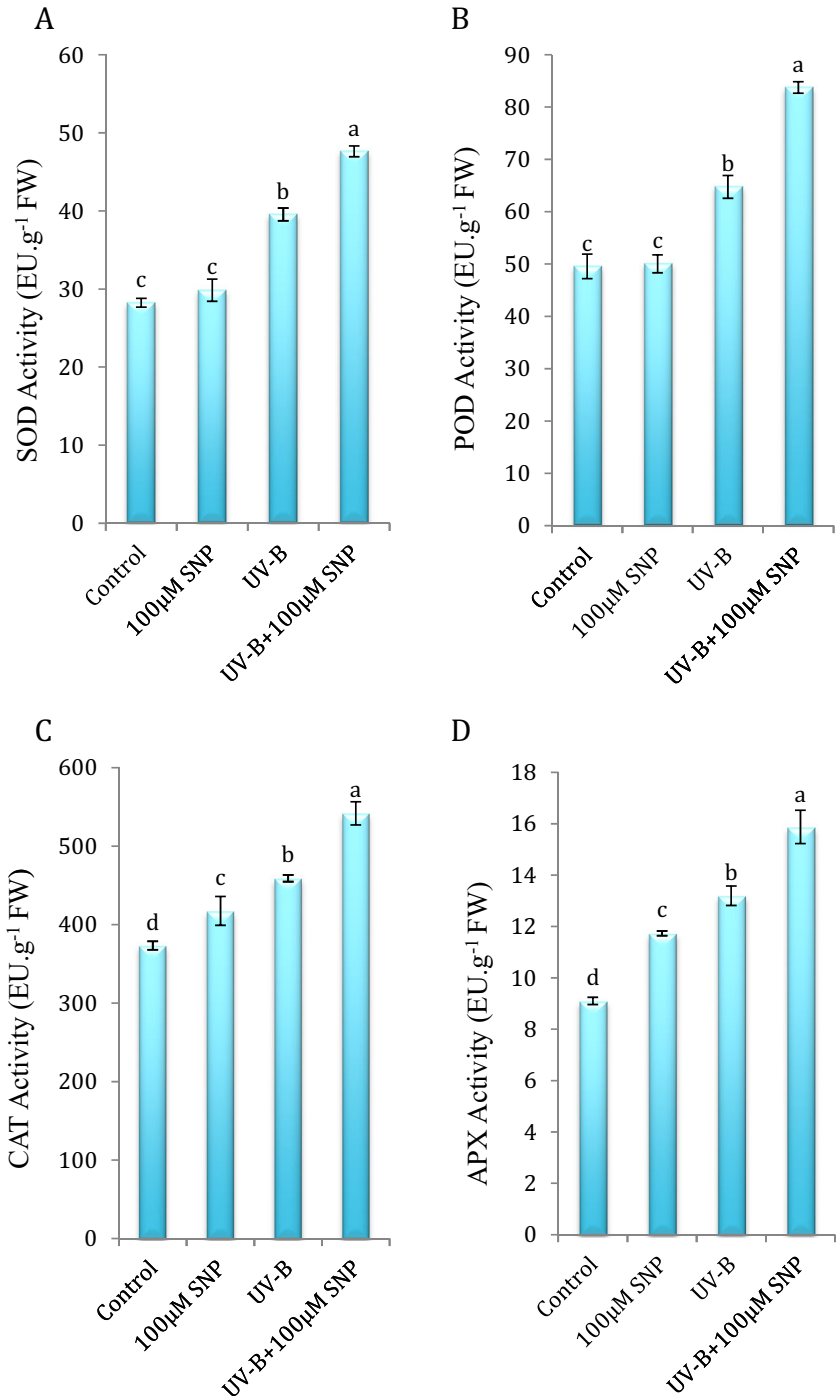


**Fig. 2** Effect of SNP pretreatment on **a** SOD, **b** POD, **c** CAT, **d** APX, and activities in lettuce seedlings exposed to 3.3 $Wm^{-2}$ UV-B for 18 h. *Bars* represent a mean±standard error of three independent experiments. *Different letters* indicate significant differences between treatments ($p<0.05$)

### Photosynthetic pigments

Compared with the control, under UV-B radiation alone, Chl *a*, Chl *b*, and Car contents were reduced by 27, 21, and 8 %, respectively (Table 1). However, Chl *a*, Chl *b*, and Car contents in leaves treated with SNP increased by 7, 20, and 1 %, respectively. Under the combination of UV-B and SNP, Chl *a*, Chl *b*, and Car contents were more than those in treatment with UV-B alone.

### Total phenolic concentration, antioxidant capacity, and PAL gene expression

The changes in the total phenolic concentration and antioxidant capacity in lettuce seedlings exposed to UV-B, SNP, and UV-B+SNP were measured and shown in Table 1. As can be seen in Table 1, total phenolic concentrations and antioxidant capacity were significantly affected by SNP, UV-B, and UV-B+SNP. UV-B alone and in combination with SNP promoted

**Table 1** Effect of SNP pretreatment on chlorophylls and carotenoid content, total phenolic concentrations, and antioxidant capacity in lettuce seedlings exposed to 3.3 $Wm^{-2}$ UV-B for 18 h

| | Total phenolic concentrations (mg GAE $g^{-1}$ FW) | Antioxidant capacity (μmol TEAC $g^{-1}$ FW) | Chl *a* (mg/g fr wt) | Chl *b* (mg/g fr wt) | Car (mg/g fr wt) |
|---|---|---|---|---|---|
| Control | 0.90±0.04$^{c}$ | 37.9±2.27$^{c}$ | 2.77±0.08$^{ab}$ | 0.94±0.01$^{b}$ | 1.37±0.03$^{ab}$ |
| 100 μM SNP | 0.94±0.02$^{c}$ | 40.8±1.21$^{c}$ | 2.97±0.04$^{a}$ | 1.13±0.02$^{a}$ | 1.39±0.05$^{ab}$ |
| UV-B | 1.35±0.05$^{b}$ | 51.0±1.8$^{5b}$ | 2.02±0.09$^{c}$ | 0.74±0.02$^{c}$ | 1.26±0.03$^{b}$ |
| UV-B+100 μM SNP | 1.62±0.03$^{a}$ | 58.7±1.13$^{a}$ | 2.58±0.06$^{b}$ | 0.95±0.06$^{b}$ | 1.43±0.04$^{a}$ |

Each value is the mean±standard error of three independent experiments. Different superscript letters indicate significant differences between treatments ($p<0.05$)

both total phenolic concentrations and antioxidant capacity significantly ($P<0.05$); however, SNP alone had no statistically significant influence on these parameters compared to the control. Total phenolic concentrations and antioxidant capacity in the combined treatment of UV-B and SNP were about 20 and 13 % more than those in UV-B treatment alone, respectively.

The effect of SNP, UV-B, and UV-B+SNP on the PAL mRNA abundance was shown in Fig. 3. When compared with control, SNP application caused a small increment in PAL (not significant) but the applications of UV-B and UV-B+SNP increased the PAL mRNA abundance significantly ($p<0.05$). The most effective application on PAL expression was UV-B+SNP.

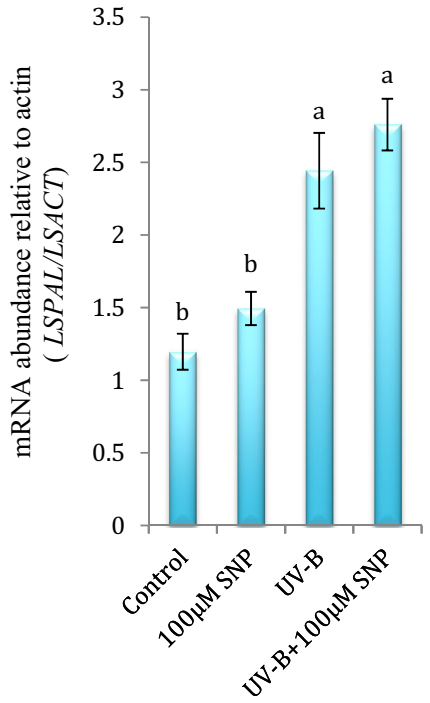


**Fig. 3** Effect of SNP pretreatment on PAL mRNA gene expression in lettuce seedlings exposed to 3.3 $Wm^{-2}$ UV-B for 18 h. *Bars* represent a mean±standard error of three independent experiments. *Different letters* indicate significant differences between treatments ($p<0.05$)

### Phytohormones

In order to investigate the changes of hormonal contents under UV-B stress with SNP or not, the effects of SNP on the ABA, GA, IAA, and salicylic acid (SA) contents of lettuce seedlings were examined (Table 2). The hormone content of lettuce seedlings was affected by UV-B and SNP treatments. The results indicated that UV-B induced ABA and SA contents of seedlings. Compared to control, ABA and SA contents of lettuce seedlings were increased by 25.1 and 30.41 % under UV-B stress, respectively (Table 2). SNP pretreatment decreased ABA and SA contents. Through SNP treatment, ABA and SA contents were decreased by 16.5 and 18.5 % in lettuce than only UV-B-treated plants. As shown in Table 2, UV-B decreased GA and IAA contents in lettuce seedlings compared to control. SNP increased GA and IAA contents of lettuce seedlings under UV-B stress. Application of SNP alone resulted in the significant increase of GA and IAA contents.

### Soluble sugars

Soluble sugar (glucose, fructose, and sucrose) concentrations were significantly affected by UV-B and UV-B+SNP (Table 3). Compared with control, SNP treatment alone had no significant effect of soluble sugars. On the other hand, UV-B enhanced the content of glucose, fructose, and sucrose concentrations by 52.2, 39.3, and 18.3 %, respectively. The combination of SNP and UV-B led to an increase in glucose fructose and sucrose concentrations of about 69.1, 69.6, and 32.2 % respectively, even higher than that of the UV-B alone.

## Discussion

Lettuce is one of the most consumed vegetables in the world either fresh or produced. However, lettuce plants are also sensitive to different adverse environmental conditions including UV-B that provoke a significant reduction in growth and development, and results of the current study further confirmed

**Table 2** Effect of SNP pretreatment on some phytohormones in lettuce seedlings exposed to 3.3 $Wm^{-2}$ UV-B for 18 h

| Plant hormone | ABA (ng/μl) | GA (ng/μl) | IAA (ng/μl) | SA (ng/μl) |
|---|---|---|---|---|
| Control | 0.131±0.02$^{ab}$ | 106.9±2.50$^{a}$ | 2.61±0.17$^{a}$ | 8.58±0.74$^{ab}$ |
| 100 μM SNP | 0.118±0.01$^{b}$ | 112.0±2.54$^{a}$ | 2.87±0.20$^{a}$ | 7.68±0.80$^{b}$ |
| UV-B | 0.164±0.01$^{a}$ | 88.03±2.14$^{b}$ | 2.02±0.13$^{b}$ | 11.19±1.30$^{a}$ |
| UV-B+100 μM SNP | 0.137±0.01$^{ab}$ | 101.20±1.73$^{ab}$ | 2.33±0.13$^{ab}$ | 9.13±0.63$^{ab}$ |

Each value is the mean±standard error of three independent experiments. Different superscript letters indicate significant differences between treatments ($p<0.05$)

the negative effect of UV-B treatments on lettuce metabolism. On the other hand, data presented in this study indicated that SNP application through foliar spray protected lettuce seedlings from the damaging effects of UV-B stress.

Stress conditions cause the overproduction of ROS, which damage lipid membranes and increase MDA contents (Perveen et al. 2013). In the present study, UV-B stress not only increased the levels of $O_2•^-$ and $H_2O_2$, but it also increased the MDA contents in lettuce seedlings. Pretreatment with exogenous 100 μM SNP decreases the levels of $O_2•^-$, $H_2O_2$, and MDA in high-light-stressed tall fescue leaves (Xu et al. 2014). Similarly, in the present study, SNP pretreatment reduced the MDA content under UV-B stress, which is in accordance with the reduced levels of $O_2•^-$ and $H_2O_2$ in the SNP-pretreated UV-B-stressed seedlings (Fig. 1). These results suggest that pretreatment with exogenous SNP alleviates UV-B stress by reducing the accumulation of $O_2•^-$ and $H_2O_2$ and reducing MDA levels in lettuce seedlings.

To protect against oxidative stress, plants evolutionally developed enzymatic and non-enzymatic ROS scavenging systems. Non-enzymatic compounds include reduced forms of ascorbate and glutathione, as well as tocopherol, flavonoids, and alkaloids, etc. Enzymatic scavenging mechanism includes SOD, POD, CAT, and APX, etc. Among antioxidant enzymes, SOD detoxifies superoxide radicals ($O_2•^-$) by forming $H_2O_2$, which is harmful to the chloroplast, nucleic acids, and proteins. $H_2O_2$ can be eliminated by POD, CAT, and APX. Present experiments demonstrated that UV-B stress increased remarkably SOD, POD, CAT, and APX activities in lettuce leaves when it was applied alone. Furthermore, it was determined that co-application of SNP pretreatment and UV-B gave rise to more increments in the activities of these enzymes compared with using UV-B alone. This finding should not be so surprising because of the fact that SNP can induce the expression of some antioxidant genes and enhance the activities of antioxidant enzymes such as SOD, POD, CAT, and APX (Fan et al. 2013; Siddiqui et al. 2011). Several reports indicated that SNP application resulted in the enhancement of antioxidant enzymes' activity under various stresses (Siddiqui et al. 2011). Khan et al. (2012) and Xu et al. (2014) showed that SNP treatment increased antioxidant enzyme activities in mustard leaves and tall fescue leaves under stress conditions. Santa-Cruz et al. (2010) reported that SNP-enhanced antioxidant enzyme activities play an important role in UV-B tolerance.

It is well documented that the plants exposed to stressful environments such as UV-B resulted in decreased chlorophyll concentration thereby leading to overall retarded growth (Zlatev et al. 2012). In the present study, lettuce seedlings treated with UV-B exhibited a significant reduction in chlorophyll content (Table 1). Under UV-B condition, chlorophyll content reduction might be due to instability of protein complexes and destruction of chlorophyll by increased activity of chlorophyll-degrading enzyme chlorophyllase. However, the results presented here show that foliar application of 100 μM SNP to lettuce seedlings led to a significant increase in Chl *a*, Chl *b*, and Car concentration under UV-B stress. Tossi et al. (2011) reported that exogenous 100 μM SNP significantly increased the total chlorophylls content in maize under UV-B stress, and Santa-Cruz et al. (2010) also reported that exogenous 1.2 mM SNP significantly increased the leaf chlorophyll concentration in soybean under UV-B stress, which further supported the present results that exogenous SNP treatment significantly increased Chl *a*, Chl *b*, and Car concentration in lettuce seedlings under UV-B stress. These results

**Table 3** Effect of SNP pretreatment on glucose, fructose, and sucrose concentrations in lettuce seedlings exposed to 3.3 $Wm^{-2}$ UV-B for 18 h

| Soluble sugar | Glucose (nmol $g^{-1}$ FW) | Fructose (nmol $g^{-1}$ FW) | Sucrose (nmol $g^{-1}$ FW) |
|---|---|---|---|
| Control | 982±56.0$^{b}$ | 94±5.3$^{c}$ | 2926±71.6$^{c}$ |
| 100 μM SNP | 1196±96.7$^{b}$ | 110±6.0$^{c}$ | 3038±58.1$^{c}$ |
| UV-B | 1495±53.1$^{a}$ | 131±5.6$^{b}$ | 3462±61.2$^{b}$ |
| UV-B+100 μM SNP | 1666±65.6$^{a}$ | 159±4.7$^{a}$ | 3871±66.5$^{a}$ |

Each value is the mean±standard error of three independent experiments. Different superscript letters indicate significant differences between treatments ($p<0.05$)

suggested that exogenous SNP treatment could alleviate the negative effect of UV-B stress that allows plants to increase their tolerance to unfavorable conditions.

Reddy et al. (2004) have reported that higher plants exhibit a unique capability to synthesize non-enzymatic secondary metabolites including phenolic compounds. Phenolic compounds have an antioxidative role in scavenging ROS. On the other hand, the synthesis and release of phenolic compounds are induced by various biotic and abiotic stress factors (Oh et al. 2009). Table 1 shows that total phenolic concentrations were significantly increased under UV-B stress compared to their corresponding controls. The foliar spraying of SNP concentration resulted in significant increases in total phenolic concentration compared to the control. Moreover, lettuce leaves treated with an application of SNP under UV-B stress showed significant increases in phenolic concentrations compared to controls. In this regard, it can be speculated that phenolic contents protect cells from potential oxidative damage, increase the stability of cell membranes, and mitigate UV-B stress injuries. Besides, the accumulation of phenolic compounds and other antioxidants in response to abiotic stress would be attributed to the activation of phenylalanine ammonia lyase (PAL) (Rivero et al. 2001; Oh et al. 2009). PALs, which are involved in phenylpropanoid pathway and led to accumulation of phenolic compounds in plants, were stimulated by UV radiation (Caldwell and Britz 2006; Oh et al. 2009). Figure 3 shows that an increased mRNA level of PAL was observed in lettuce leaves in response to UV-B stress. This response was consistent with the higher accumulation of phenolic matters in lettuce leaves. The results obtained in this study clearly suggest that UV-B stress can activate key genes involved in the biosynthesis of secondary metabolites in lettuce. Our results have also shown that SNP and UV-B applications increased the antioxidant capacity of lettuce, and these results may be related with the increasing of phenolic compounds. Thus, phenolic compounds and some secondary metabolites are largely responsible for antioxidant capacity in plant tissues (Larson 1988).

Phytohormones play critical roles in regulating plant responses to stress (Yang et al. 2013). Under the effect of UV-B, the endogenous growth hormones GA and IAA content decreased, while ABA and SA content increased. The results appeared that UV-B stress led to sharp changes in the balance of endogenous hormones which associated with the accumulation of ABA and SA and decrease in the level of GA and IAA. These results are in a good agreement with those of Peng and Zhou (2009) who showed that treatment of soybean with UV-B caused changes in ABA, GA, and IAA; similar results were obtained by Yang et al. (2004) working on tomato. GA and IAA contents were decreased by UV-B radiation due to photooxidation free radical damage, reinforcing harm from free radical induced by UV-B stress. In addition to this, the decrease of GA and IAA contents in lettuce seedlings under UV-B stress is likely associated with its low UV-B tolerance. On the other hand, synthesis of ABA and SA were affected by stress conditions. Li et al. (2010) reported that UV-B radiation damage chlorophyll and cell membrane structure, cause a decreasing in Mg-ATPase activity of membranes and pH in chloroplast, and this phenomenon may cause an increase in ABA content. The increases in ABA and SA contents help to improve the stress tolerance in plants. As seen from Table 2, there were no significant increases in the amounts of GA and IAA when SNP was applied alone. In contrast, the amounts of ABA and SA were found to be significantly decreased in SNP-pretreated plants as compared with untreated ones. Data presented in Table 2 also clearly show that although combined UV-B and SNP treatment resulted in noteworthy increases in GA and IAA contents, it significantly decreased ABA and SA contents, as compared with the UV-B treatment alone. The similar findings were also shown in the previous report of He et al. (2012) who demonstrated that SNP increased in GA and IAA but decreased in ABA contents in rye in response to Al stress. These results indicate that SNP can ameliorate UV-B stress by increasing the secretion of GA and IAA and decreasing of ABA and SA.

In the present study, low level of SNP application significantly enhanced soluble sugar contents in lettuce leaves (Table 3). Our results also showed that SNP induced the accumulation of glucose, fructose, and sucrose under UV-B stress, which were much higher than under UV-B stress alone in lettuce leaves. It is well known that soluble sugars have an essential role in plant metabolism. They act as typical osmoprotectants and stabilize cellular membranes, maintain turgor. They are also signal molecules in sugar sensing and signaling system. It has been also reported that there is a relation between sugar accumulation and ROS balance (Couee et al. 2006). The present results also suggest that the protective effect of ALA on UV-B stress might be related to its regulative roles on soluble sugar levels in lettuce leaves.

In conclusion, the present study revealed that SNP pretreatment alleviate the negative effect of UV-B radiation through reducing MDA and ROS, improving antioxidant system, and regulating hormonal balance.

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