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PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN LEAVES OF RADISH (*RAPHANUS SATIVUS* L. CHERRY BELLA) SEEDLINGS TREATED WITH SODIUM NITROPRUSSIDE (SNP)

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Abstract

In the present study, SNP (0, 50, 100, 200, and 300 µM) was administered hydroponically to 10 –day-old seedlings of radish (*Raphanus sativus* L. cherry bella) through roots for 5 days and physiological and biochemical effects on leaves were examined. It was determined that compared to control; leaves had loss of pigment (chl a+b and carotenoid), soluble protein content was declined and MDA and GSSG contents were increased. In addition, GSH content and SOD activity was decreased whereas CAT activity was increased. While % rates of fatty acids in the leaf were declined and exceptionally decreased for palmitic, linoleic and palmitoleic acid, it showed an increase and an exceptional increase for stearic and linolenic acid. Generally, high doses of SNP significantly triggered the toxicity.

Key words: Sodium nitroprusside, *Raphanus sativus* L., Toxic stress.

Introduction

Sodium nitroprusside (SNP) is often used as a donor of Nitric oxide (NO) is a free radical that can be easily diffused from cell membranes (Stöhr and Ullrich, 2002). NO is also described as physiological messenger molecule with a half-life of few seconds within the cell (Graziano & Lamattina, 2005; Baudouin & Hancock, 2014). NO plays important roles in various plant physiological processes, including seed germination and dormancy, growth and development of plant tissue, plant cell maturation and senescence, flowering, hormone responses, stomatal closure and programmed cell death (Kong *et al.*, 2016).

The role of NO has been widely discussed in recent years. In a study conducted by Yan *et al.*, (2016) on wheat seedlings, 50 µM of SNP treatment decreased root growth, root/shoot ratio, and contents of chlorophyll a, chlorophyll b, and carotenoids and increased MDA and proline contents and GR activity, compared to the control. Singh & Shah (2014) demonstrated in rice seedlings, that SNP treatment increased lipid peroxidation, CAT and SOD activity. Total protein content was decreased as SNP concentration was increased, but an increase was observed at 700 µM of SNP (Kerkütlüoğlu, 2007). In a study in which soy bean seedlings were treated with SNP (0.01, 1 and 100 µM), chlorophyll and carotenoid contents were decreased with the increasing concentration (Aytamka, 2005). Yin *et al.*, (2012) conducted a study on sweet potato (*Ipomoea batatas Lam* cv. Xinxiang) plant and reported that 24 hours of SNP treatment elevated both SOD and CAT activity in roots, but decreased in case of ongoing treatment.

Oxidative stress occurs when reactive oxygen species that are harmful for cells or kill cells form in plant tissue (Chen *et al.*, 2016). SNP was administered after pea seeds were exposed to Cd and GSH, SOD, and CAT activities were found to decrease in roots and leaves of seedlings (Nahar *et al.*, 2016; Yu *et al.*, 2013). In a study conducted on chickpea (*Cicer arietinum* L.) plant it was reported that SNP treatment protected the plant by significantly decreasing ion leakage and lipid peroxidation levels induced by Cd toxicity (Kumari *et al.*, 2010). The effects of SNP (250 µM) on Cd (750 µM) toxicity were evaluated on *Cynodon dactylon* (dog's tooth grass) seedlings. Compared to seedlings treated with SNP, fresh weight and GSH content was decreased but MDA content was increased for seedlings treated with SNP+Cd (Shi *et al.*, 2014). In another study, the levels of soluble protein, proline, total phenol, GSH and GSH/GSSG were increased evidently in the presence of SNP under chilling stress (Dong *et al.*, 2018). Since there are no studies in the literature that examine SNP and fatty acids together, we had to address this issue through salicylic acid (SA) which is also a signal molecule for plants. Especially 250 µM of SA considerably influenced fatty acid content in leaves of sunflower (Moradkhani *et al.*, 2012). Similar results were obtained for some weeds, as well (Popova *et al.*, 2012).

In the present study, physiological and biochemical effects in leaves of radish seedlings treated with SNP were examined.

Material and Methods

Seedlings growth and experimental design: To grow seedlings of radish (*Raphanus sativus* L. cherry bella) which was the experimental material of the present study, uniform seeds were chosen and soaked in tap water at 23-25^{o}C in darkness for 6 hours. At the end of this period, seedlings were left for germination at 23-25^{o}C in darkness for 3 days by aligning them in the germination boxes with lids where they can breathe. The seedlings with equal radicula length were chosen and transferred between filter papers soaked with nutrient solution contained by 150 ml of glass beakers prepared previously. Seedlings were allowed to grow in growth chamber for 10 days. Seedlings growing homogenously were treated with different test solutions of SNP (0, 50, 100, 200, and 300 µM) via hydroponic method for 5 days. Leaves of seedlings were harvested following the treatments. Contents of pigment (chl a+b and carotenoid), soluble protein, MDA, GSH, and GSSG, activities of SOD and CAT, and fatty acid contents in these leaves were analyzed.

Biochemical analysis: Chlorophyll and carotenoid contents ($mg.g^{-1}$.FW) in leaves of seedlings treated with SNP were analyzed according to the method of Arnon (1949). Total chlorophyll and carotenoid contents were analyzed using the absorption values according to Witham *et al.,* (1971). Protein (1g) was extracted in leaves in accordance with Larson and Beevers (1965). Extracts were stored in separate test tubes. Total protein content ($mg.g^{-1}$) was measured with the method of Lowry *et al.,* (1951). The level of lipid peroxidation in the leaf was quantified by determining MDA content based on the method of Yilmaz *et al.,* (2009). GSH and GSSG contents in leaf extracts were determined according to the method of Yilmaz *et al.,* (2009). SOD activity (Mourente, 1999) and CAT activity (Aebi, 1984) were analyzed in liquid portions of plant extracts. Fatty acids were analyzed in solid parts of leaf extracts. Fatty acids were analyzed in gas chromatography (Christie, 1990; Hara and Radin, 1978). Results were determined in terms of weight % of total. Three replicates were maintained for each treatment. All physiological analyses were replicated three times for each treatment. In each analysis, 1 g of leaf tissue was used.

Statistical analyses: One-way ANOVA (SPSS 15.0 Evaluation Version Production Mode Facility) was used to analyze the results. The difference between the treatments was considered as significant at the value of $p \leq 0.05$. Duncan's test was performed to compare the means. In all these parameters, the data which were not statistically significant were not considered ($p > 0.05$) (Tables 1, 2).

Results

Variations in chlorophyll (a+b) and carotenoid contents: Compared to the control, SNP treatment led to decreases of 15.04%, 24.77 %, and 33.62 % in chlorophyll a+b contents in leaves of radish seedlings for 100 µM, 200 µM and 300 µM concentrations; respectively. For carotenoid content, decreases of 16.32%, 26.53%, and 30.61% were determined for 100 µM, 200 µM, and 300 µM concentrations, respectively ($p \leq 0.05$) (Table 1).

Variations in protein and malondialdehyde contents: It was found that there were an increase of 18.25 % and a decrease of 64.29% in protein content respectively for 50 µM and 300 µM concentrations, and an increase of 116.85% and a decrease of 21.45% in MDA content respectively for 200 µM and 300 µM concentrations in leaves of radish seedlings treated with SNP, compared to the control ($p \leq 0.05$) (Table 1).

Variations in reduced glutathione (GSH) and oxidized glutathione (GSSG) contents: Compared to control; a decrease of 40.40% for 300 µM concentration concerning the effect over GSH and a decrease of 73.01% for 300 µM concentration concerning the effect over GSSG were determined in leaves of radish seedlings treated with SNP ($p \leq 0.05$) (Table 1).

Variations in superoxide dismutase (SOD) and catalase (CAT) contents: Compared to control, 100 µM, 200 µM, and 300 µM concentrations caused the decreases of 23.31%, 28.53%, and 40.14%, respectively in SOD activities and 50 µM, 100 µM, 200 µM, and 300 µM concentrations caused the increases of 25.39%, 62.92%, 64.39%, and 70.33% in CAT activities in leaves of radish seedlings treated with SNP ($p \leq 0,05$) (Table 2).

Variations in fatty acid contents: According to our data we found the following; increase of 124% in 50 µM concentration for fatty acid 16:0, a decrease of 69.94% in 300 µM concentration for fatty acid 16:1, increases of 82.35% and 61.34% in 200 µM and 300 µM concentrations for fatty acid 18:0 respectively, decreases of 80.71% and 64.47% in 200 µM and 300 µM concentrations for fatty acid 18:2, and an increase of 39.15% in 100 µM concentration for fatty acid 18:3 in the leaves of seedlings treated with SNP, compared to control ($p \leq 0.05$) (Table 2).

Table 1. Variations of photosynthetic pigment, protein, MDA, GSH and GSSG in leaves of radish seedlings treated with SNP.

Groups	Chl a+b ($mg.g^{-1}$.FW)	Carotenoid ($mg.g^{-1}$.FW)	Protein ($mg.g^{-1}$)	MDA ($nmol.g^{-1}$)	GSH ($\mu g.g^{-1}$)	GSSG ($\mu g.g^{-1}$)
Control	1.13 ± 0.01	0.49 ± 0.01	2.52 ± 1.53	2.61 ± 0.22	15.27 ± 3.63	14.49 ± 7.25
50 µM	1.17 ± 0.05	0.46 ± 0.02	2.98 ± 1.33*	2.66 ± 0.18	15.50 ± 7.75	13.17 ± 6.59
100 µM	0.96 ± 0.01*	0.41 ± 0.02*	0.87 ± 0.39	3.03 ± 0.72	17.17 ± 6.59	9.68 ± 4.84
200 µM	0.85 ± 0.01*	0.36 ± 0.02*	0.95 ± 0.43	5.66 ± 0.67*	16.86 ± 5.43	6.97 ± 4.48
300 µM	0.75 ± 0.02*	0.34 ± 0.02*	0.90 ± 0.4*	2.05 ± 0.15*	9.1 ± 1.25*	3.91 ± 0.25*

*: Compared to the control; Significant at probability levels of $p \leq 0.05$. Mean of the data ± SE (n: 3)

Table 2. Variations in SOD and CAT enzyme activity and fatty acids in leaves of radish seedlings treated with SNP.

Groups	SOD (unit/g)	CAT (µg/g)	Fatty Acid Content (wt % of total)				
			16:0	16:1	18:0	18:2	18:3
Control	10.34 ± 0.61	394.72 ± 5.28	0.91 ± 0.40	1.73 ± 0.77	1.19 ± 0.53	1.97 ± 0.88	1.58 ± 1.15
50 µM	9.26 ± 0.73	494.95 ± 6.05*	2.04 ± 0.31*	0.95 ± 0.43	1.62 ± 0.73	1.98 ± 0.89	3.49 ± 0.80
100 µM	7.93 ± 0.01*	643.06 ± 12.50*	0.66 ± 0.30	1.75 ± 0.78	0.99 ± 0.44	1.54 ± 0.69	3.59 ± 0.60*
200 µM	7.39 ± 0.18*	648.89 ± 10.0*	0.90 ± 0.40	1.18 ± 0.53	2.17 ± 0.20*	0.38 ± 0.17*	2.62 ± 0.17
300 µM	6.19 ± 0.18*	672.34 ± 5.67*	0.52 ± 0.23	0.52 ± 0.23*	1.92 ± 0.08*	0.70 ± 0.31*	2.71 ± 0.21

*: Compared to the control; Significant at probability levels of $p \leq 0.05$. Mean of the data ± SE (n: 3).

Discussion

Because we did not find any report on SNP in radish, therefore, we tried to discuss the parameters mostly through indirect publications. It was determined that as concentration of SNP increased, breakdown of photosynthetic pigments increased; in other words, chlorophyll a+b contents (Filippou *et al.,* 2013; Ziogas *et al.,* 2015; Sheokand *et al.,* 2008; Yan *et al.,* 2016) and carotenoid content decreased (Yu *et al.,* 2013; Yan *et al.,* 2016) in leaves of radish seedlings. It was reported that NO repressed ATP synthesis and electron transport in chloroplasts reversibly and NO produced by nitrate reductase (NR) enzyme inhibited the photosynthesis (Aytamka, 2005).

Other detoxification mechanisms that plants have developed to cope with abiotic stress (including metals) are linked to some plant growth regulators such as NO, jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) (Khan and Khan, 2014; Iqbal *et al.,* 2015; Liu *et al.,* 2015). In leaves of radish seedlings, SNP treatment with 300 µM concentration was found to cause the highest decrease in protein contents (Kerkütoğlu, 2007; Filippou *et al.,* 2013; Finkel, 2012) and the highest increase at 200 µM concentration on MDA content (Kumari *et al.,* 2010; Zhao-Duan *et al.,* 2009; Singh *et al.,* 2008; Ziogas *et al.,* 2015; Sun *et al.,* 2007; Sheokand *et al.,* 2008; Yan *et al.,* 2016). Impaired permeability of membrane occurring in cells was reported to lead to protein and lipid breakdown and later the death of cell (Selçukcan and Cevahir, 2008). As concentration increased, GSH and GSSG contents of radish seedlings decreased (Shi *et al.,* 2014; Nahar *et al.,* 2016; Sun *et al.,* 2007; Sheokand *et al.,* 2008; Kaur & Bhatla, 2016).

Nitric oxide (NO) is a small signaling molecule that has cytoprotective roles in plants. SNP treatment led to the highest decrease in SOD activity (Silveria *et al.,* 2015; Nahar *et al.,* 2016; Graziano and Lamattina, 2005; Sun *et al.,* 2007; Sheokand *et al.,* 2008; Yin et al., 2012) and the highest increase in CAT activity (Sun *et al.,* 2007; Kazemi *et al.,* 2010; Kumari *et al.,* 2010; Yin *et al.,* 2012; Yan *et al.,* 2016) in 300 µM concentration in leaves of radish seedlings. NO decreased accumulation of H_2O_2 and $O_2^{\cdot-}$. Over-production of reactive oxygen species caused oxidative damage such as peroxidation of membrane lipids, protein oxidation, enzyme inhibition, DNA and RNA damage (Sun *et al.,* 2007). The highest decrease was observed in 300 µM concentration in fatty acids 16:0 and 16:1, the highest increase in 200 µM concentration in fatty acid 18:0, the highest decrease in 200 µM concentration in fatty acid 18:2, and the highest increase in 100 µM concentration in fatty acid 18:3 in leaves of radish seedlings treated with SNP (Moradkhani *et al.,* 2012; Popova *et al.,* 2012). In conclusion, high doses of SNP solely triggered toxic effect, therefore antioxidant responses.

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