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Analysis of interactions of nitric oxide and polyamine under cadmium stress in wheat

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Abstract: Wheat cultivars chosen for their cadmium (Cd) tolerance (Sönmez-2001) and sensitivity (Quality) were grown in Hoagland solution for 20 days and then they were treated with 100 µM 2-(4-carboxyphenyl)-4,4,5,5-tetramethylylimidazoline-1-oxyl (cPTIO), a nitric oxide (NO) scavenger, or with 100 µM sodium nitroprusside (SNP), a NO donor, with and without 9 mM Cd. NO, free polyamines, and Cd levels were analysed by using leaf and root samples taken 24 and 72 h after treatment. There was a significant increase in NO level in the Cd+SNP treatment in cv. Quality cultivars at 24 and 72 h. The NO level recorded in cv. Quality was higher in the Cd and Cd+SNP treatments at 24 h and in the SNP and Cd+SNP treatments at 72 h compared to cv. Sönmez-2001. Spermidine (Spd) had the highest amount of amine. In almost all treatments, the sensitive cultivar Quality included higher contents of Spd, putrescine, and spermine compared to cv. Sönmez-2001. Quality presented higher levels of Cd accumulation in root and leaf tissues in all treatments compared to Sönmez-2001.

Key words: *Triticum aestivum*, cadmium, nitric oxide, polyamine, SNP, cPTIO

1. Introduction

Cadmium (Cd), which is a highly toxic trace element, enters the environment mainly from industrial processes and phosphate fertilisers. It reaches high levels in agricultural soils and is easily assimilated by plants. Taken up excessively by plants, Cd induces various visible symptoms of phytotoxicity, such as leaf roll, chlorosis and necrosis, growth retardation, browning of root tips, and finally death (Tran and Popova, 2013).

Nitric oxide (NO) is a key signalling molecule that mediates various physiological functions and defence responses against abiotic and biotic stresses in plants. These functions include regulation of root development (Kolbert et al., 2008), senescence (Corpas et al., 2004), and adaptive responses to abiotic stresses like drought, heat, chilling, and high salinity, and defence responses against biotic stresses (Leitner et al., 2009; Wimalasekera et al., 2011). NO is also known as an intermediate signalling molecule in cytokinin, abscisic acid (Garcia-Mata and Lamattina, 2002), auxin (Kolbert et al., 2008), and polyamine (PA) (Tun et al., 2006) signalling. Sodium nitroprusside (SNP) is a "spontaneous" NO donor by releasing NO upon dissolution in aqueous solvents. 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) is a NO specific scavenger and widely used to abolish NO accumulation in plants.

Being nitrogenous growth regulators, PAs are present ubiquitously in all living cells, have low molecular weight, and are polycationic. The most common free PAs in plants are spermidine (Spd, a triamine), spermine (Spm, a tetramine), and their obligate precursor putrescine (Put, a diamine), which plays a pivotal role in the regulation of plant developmental and physiological processes (Kusano et al., 2007). Due to their polycationic nature at physiological pH levels, PAs are able to interact with proteins, nucleic acids, membrane phospholipids, and cell wall constituents by either activating or stabilising these molecules. PAs are related to plant response under different abiotic stress conditions including low pH (Young and Galston, 1983), temperature (Soumitra et al., 1987), salinity (Mutlu and Bozcuk, 2005), and heavy metal (Groppa et al., 2003, 2008). According to some researchers, free and bound PAs are regarded as possible indicators for stress tolerance in many species of plant such as rice, sunflower, and pea (Reggiani et al., 1994; Mutlu and Bozcuk, 2007).

In plants, both NO and PAs are multifunctional molecules implicated in numerous physiological and biological processes during plant development and stress

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response (Grün et al., 2006). PAs are biochemically related to NO through arginine, a common precursor in their biosynthetic routes, suggesting that alteration in PA homeostasis can affect NO bioavailability and vice versa (Filippou et al., 2013). Arginine decarboxylase (ADC) is a key enzyme responsible for PA biosynthesis and NO originates from two enzymatic sources in plants: nitrite-dependent and arginine-dependent sources.

Most studies, on the other hand, have focused on the effects of Cd on plant growth and development, membrane lipid peroxidation, and antioxidant system (Anjum et al., 2011). There have been a limited number of studies conducted on the changes in PA metabolism under Cd stress (Tang et al., 2009; Yang et al., 2010). Although further confirmation of PA-induced NO production is necessary, the observations reported by Tun et al. (2006) could imply the presence of an unknown enzyme responsible for direct conversion of PAs to NO. To our knowledge, little information is present about the ameliorative effects of SNP on Cd toxicity of two wheat cultivars that are different in terms of Cd tolerance. Therefore, in this study, the effects of SNP and cPTIO on the contents of PAs, NO, and Cd in wheat seedlings under Cd stress were investigated. Additionally, possible relations between PA, NO, and Cd content as well as protection and defence mechanisms of plants under Cd stress were determined.

2. Materials and methods

2.1. Plant material, growth conditions, and treatments

The cultivars of wheat used in the study were obtained from Trakya Agricultural Research Institute and various firms. Prestudies were carried out with 12 cultivars: Cömert, Çeşit1252, Kızıltan, Esperia, Sönmez-2001, Ekiz, Aldane, Konya, Quality, Müfit Bey, Ahmet Ağa, and Selimiye, which are *Triticum aestivum* L. (wheat) cultivars. While the most tolerant cultivar was *Triticum aestivum* L. cv. Sönmez-2001, the most sensitive cultivar was *Triticum aestivum* L. cv. Quality.

Culture media including different concentrations (5 µM–16 mM) of Cd were prepared by adding $CdSO_4$ to ½ N Hoagland culture solutions (Hoagland and Arnon, 1938) in this study. The greatest difference between cultivars was determined in 9 mM $CdSO_4$ concentration, and afterwards this concentration was used in the study. Solution groups used in the study were Hoagland (Control), Hoagland + 100 µM SNP (SNP), Hoagland + 100 µM cPTIO (cPTIO), 9 mM $CdSO_4$ (Cd), 9 mM $CdSO_4$ + 100 µM SNP (Cd+SNP), and 9 mM $CdSO_4$ + 100 µM cPTIO (Cd+cPTIO).

2.2. Analyses

Plants, which were kept for growing in climate chamber for 20 days, were applied with Hoagland culture solution at specific intervals. On day 21, the solution groups were applied to 20-day-old wheat and then they grew for 3 more days. Leaf and root samples, which were taken at 24 and 72 h for necessary analyses, were kept in a deep freeze. After the experiments were carried out on 3 replicates, 9 samples were taken from each culture group to conduct all analyses.

2.3. Determination of Cd content

Leaf and root samples taken for Cd analyses were kept at 70 °C for 24 h and dried. Samples were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES; Varian-725-ES) (Rodríguez-Llorente et al., 2010).

2.4. Determination of NO content

NO levels were determined in leaf tissues by using a NO assay kit (ENZO Life Sciences, Catalogue no. ADI-917-020). One gram of plant tissue was weighed and homogenised in 4 mL of sodium phosphate (pH 7.2) buffer according to Garcia-Mata and Lamattina (2001).

2.5. Determination of PA contents

Free PA was determined by using the method of Smith and Davies (1985) with modifications. PAs were assayed by high performance liquid chromatography. Samples were injected into a fixed 20-µL loop for loading onto a reversed phase C_{18} column. Samples were eluated from the column with programmed water:methanol (v/v) solvent gradient, ranging from 60% to 95% at a flow rate of 1 mL/min. Elution was completed in 30 min (Smith and Davies, 1985). Retention times of the different dansylated PAs were as follows: 15 min Put, 22 min Spd, and 29 min Spm. The fluorescence detector was set to an excitation wavelength of 365 nm and an emission wavelength of 510 nm for dansyl PAs. Areas and retention times of eluent peaks were recorded by an attached integrator.

2.6. Statistical analysis

Statistical variance analysis of the results was performed and compared using least-significant differences (LSD) at 5% level. The experiments were performed with three replicates.

3. Results

3.1. NO level in leaf tissue of cv. Sönmez-2001 and cv. Quality

Lower NO levels were measured in leaf samples of both cv. Sönmez-2001 and cv. Quality treated with cPTIO and Cd+cPTIO after 24 h and 72 h of treatment compared to the control values ($P < 0.05$) (Figures 1 and 2). However, higher NO content was found in samples of cv. Quality treated with Cd+SNP at 24 h and 72 h. The differences were significant ($P < 0.05$). The NO level detected in cv. Quality was higher compared to cv. Sönmez-2001 in 24 h samples with Cd and Cd+SNP treatments, and in 72 h samples with SNP and Cd+SNP treatment.

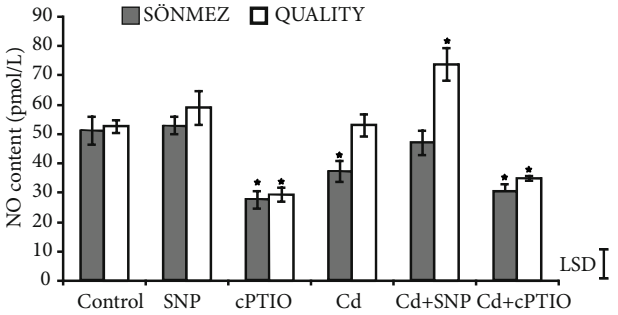

Figure 1. NO levels at 24 h in cv. Sönmez-2001 and cv. Quality. Asterisk indicates significant differences compared with control ($P < 0.05$). Vertical bars represent standard error (SE) of the mean (n = 3).

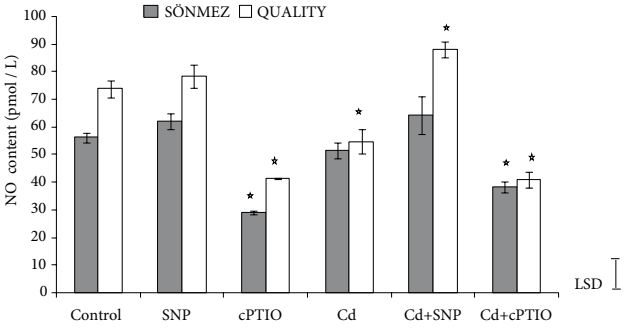

Figure 2. NO levels at 72 h in cv. Sönmez-2001 and cv. Quality. Asterisk indicates significant differences compared with control ($P < 0.05$). Vertical bars represent standard error (SE) of the mean (n = 3).

3.2. PA content in leaf tissue of cv. Sönmez-2001 and cv. Quality

Tables 1 and 2 contain the results of PA levels (µg/g FW) in 24 h and 72 h leaf samples of wheat cultivars on day 21 in terms of SNP, cPTIO, Cd, Cd+SNP, and Cd+cPTIO treatments.

3.2.1. Spd content in leaf tissue of cv. Sönmez-2001 and cv. Quality

There was a significant decrease in the 24 h Spd content of cv. Sönmez-2001 with Cd+SNP treatment ($P < 0.05$). Spd content decreased in all treatments compared to the control at 72 h ($P < 0.05$). There was a decrease in the 24 h Spd content of cv. Quality in all treatments ($P < 0.05$). The increase in the Cd treatment and the decrease in the cPTIO treatment at 72 h were significant ($P < 0.05$).

It was determined that cv. Quality included higher content of Spd compared to cv. Sönmez-2001. Content of Spd was highest in the control group in almost all treatments ($P < 0.05$).

3.2.2. Put content in leaf tissue of cv. Sönmez-2001 and cv. Quality

There was a significant decrease in the 24 h Put content of cv. Sönmez-2001 with Cd, Cd+SNP, and Cd+cPTIO treatment ($P < 0.05$). Put content increased only in the

Table 1. Polyamine content in 24 and 72 h leaf tissues of cv. Sönmez-2001 (µg/g FW). Data are expressed as mean ± SE of three replicates.

Cultivar	Polyamines	Treatment	24 h	72 h
SÖNMEZ-2001	Spm LSD 5% 43.63	Control	39.30 ± 8.30	93.60 ± 12.40
		SNP	66.30 ± 11.70	58.80 ± 10.80
		cPTIO	157.80 ± 20.40	31.50 ± 1.50
		Cd	45.90 ± 2.10	55.80 ± 4.80
		Cd+SNP	37.50 ± 1.50	81.90 ± 17.30
		Cd+cPTIO	70.2 ± 14.40	63.30 ± 10.50
	Spd LSD 5% 119.77	Control	396.92 ± 33.32	835.20 ± 72.37
		SNP	336.01 ± 30.80	245.40 ± 27.40
		cPTIO	455.10 ± 32.10	356.70 ± 52.30
		Cd	273.90 ± 24.30	455.70 ± 15.30
		Cd+SNP	83.40 ± 13.80	663.90 ± 72.10
		Cd+cPTIO	310.92 ± 26.28	527.10 ± 40.90
	Put LSD 5% 23.22	Control	28.31 ± 3.47	2.58 ± 0.06
		SNP	13.37 ± 0.47	28.74 ± 3.89
		cPTIO	23.95 ± 2.17	1.52 ± 0.03
		Cd	3.33 ± 0.47	1.59 ± 0.03
		Cd+SNP	1.58 ± 0.01	6.60 ± 1.82
		Cd+cPTIO	1.05 ± 0.04	3.51 ± 0.65

Table 2. Polyamine content in 24 and 72 h leaf tissues of cv. Quality (μg/g FW). Data are expressed as mean ± SE of three replicates.

Cultivar	Polyamines	Treatment	24 h	72 h
QUALITY	Spm LSD 5% 43.63	Control	116.10 ± 21.50	141.99 ± 9.90
		SNP	72.60 ± 4.21	148.50 ± 9.55
		cPTIO	52.88 ± 7.80	131.10 ± 11.77
		Cd	151.80 ± 12.60	215.77 ± 6.33
		Cd+SNP	40.81 ± 8.46	197.42 ± 12.00
		Cd+cPTIO	123.90 ± 4.67	181.22 ± 6.05
	Spd LSD 5% 119.77	Control	1035.00 ± 87.06	798.01 ± 46.05
		SNP	408.61 ± 59.40	738.02 ± 44.55
		cPTIO	295.85 ± 34.20	526.88 ± 72.24
		Cd	464.72 ± 5.12	918.55 ± 78.22
		Cd+SNP	378.95 ± 14.10	693.88 ± 51.00
		Cd+cPTIO	548.44 ± 6.60	777.75 ± 81.01
	Put LSD 5% 23.22	Control	160.22 ± 14.27	48.84 ± 3.24
		SNP	63.21 ± 6.03	51.45 ± 1.89
		cPTIO	100.51 ± 11.40	4.80 ± 0.01
		Cd	92.58 ± 18.80	167.88 ± 1.32
		Cd+SNP	32.37 ± 2.79	117.96 ± 12.30
		Cd+cPTIO	65.04 ± 8.44	130.26 ± 0.84

SNP treatment ($P < 0.05$) at 72 h ($P < 0.05$). Put content at 24 h decreased generally in the treatment groups in cv. Sönmez-2001 and cv. Quality ($P < 0.05$). There were significant increases in the Cd, Cd+SNP, and Cd+cPTIO groups at 72 h in cv. Quality ($P > 0.05$).

It was determined that cv. Quality had higher content of Put compared to cv. Sönmez-2001 in all treatment groups ($P < 0.05$).

3.2.3. Spm content in leaf tissue of cv. Sönmez-2001 and cv. Quality

The 24 h Spm content in cv. Sönmez-2001 decreased in the SNP cPTIO and Cd+cPTIO groups ($P < 0.05$). At 72 h, Spm content decreased compared to the control; however, the decrease detected was significant only in the cPTIO treatments compared to the control ($P < 0.05$). The 24 h Spm content in cv. Quality decreased in the SNP, cPTIO, and Cd+SNP treatments ($P < 0.05$); on the other hand, the increase detected in the Cd treatment was significant (Table 2). There was an increase only in the Cd and Cd+SNP treatments at 72 h in this cultivar, as well ($P < 0.05$).

As seen in Tables 1 and 2, Spm content determined in cv. Quality at 72 h was higher than that in cv. Sönmez-2001 in all treatments, which was distinctive in the Cd treatment ($P < 0.05$).

3.3. Cd accumulation in leaf tissue of cv. Sönmez-2001 and cv. Quality

Cd levels assayed in leaf tissues taken from cv. Sönmez-2001 and cv. Quality at 24 and 72 h depending on Cd, Cd+SNP, and Cd+cPTIO treatments are shown in Figures 3 and 4.

At 24 and 72 h of Cd treatment in leaf tissues of cv. Sönmez-2001 and cv. Quality, especially the Cd+SNP and Cd+cPTIO treatments caused a decrease in Cd accumulation compared to the Cd treatment ($P < 0.05$).

There was a higher level of Cd accumulation in cv. Quality in the 24 and 72 h leaf tissues in all treatments compared to cv. Sönmez-2001 ($P < 0.05$). This accumulation was 2.74 times greater with Cd treatment, 2.86 times greater with Cd+SNP treatment, and 3.22 times greater with Cd+cPTIO treatment in the 24 h leaf tissues of cv. Quality.

3.4. Cd accumulation in root tissue of cv. Sönmez-2001 and cv. Quality

Cd levels assayed in root tissues taken at 24 and 72 h from cv. Sönmez-2001 and cv. Quality depending on Cd, Cd+SNP, and Cd+cPTIO treatments are presented in Figures 5 and 6.

SNP and cPTIO decreased the accumulation of Cd in leaves, especially in the Cd+SNP and Cd+cPTIO groups in 24 and 72 h root tissues of cv. Sönmez-2001 and cv. Quality, which was similar to the result of leaf tissues ($P < 0.05$).

In 24 and 72 h root tissues, cv. Quality presented higher levels of Cd accumulation compared to Sönmez-2001 in all treatment groups ($P < 0.05$). This accumulation was 1.84 times greater with Cd treatment, 1.48 times greater with Cd+SNP treatment, and 1.93 times greater with Cd+cPTIO treatment in the 24 h root tissues of cv. Quality

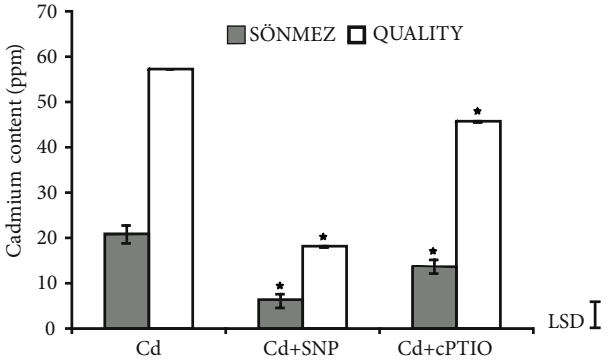

Figure 3. Cadmium levels (ppm) in 24 h leaf tissue of cv. Sönmez-2001 and cv. Quality. Asterisk indicates significant differences compared with cadmium treatments ($P < 0.05$). Vertical bars represent standard error (SE) of the mean (n = 3).

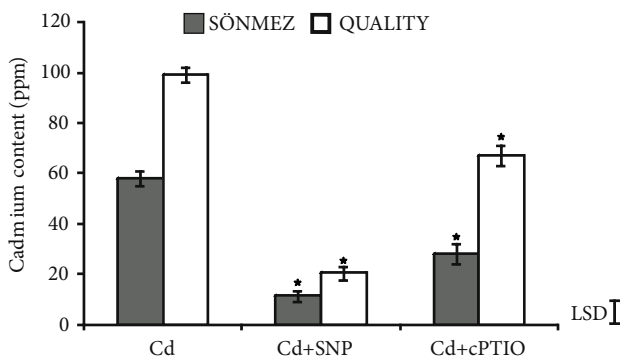

Figure 4. Cadmium levels (ppm) in 72 h leaf tissue of cv. Sönmez-2001 and cv. Quality. Asterisk indicates significant differences compared with cadmium treatments ($P < 0.05$). Vertical bars represent standard error (SE) of the mean (n = 3).

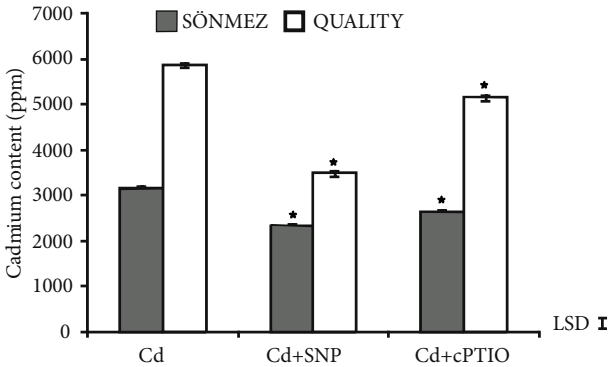

Figure 5. Cadmium content (ppm) in 24 h root tissue of cv. Sönmez-2001 and cv. Quality. Asterisk indicates significant differences compared with cadmium treatments ($P < 0.05$). Vertical bars represent standard error (SE) of the mean (n = 3).

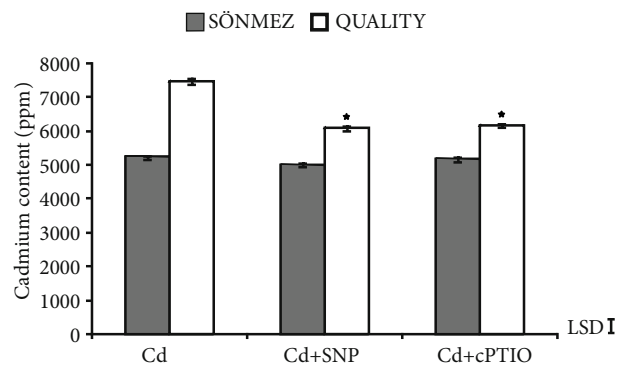

Figure 6. Cadmium content (ppm) in 72 h root tissue of cv. Sönmez-2001 and cv. Quality. Asterisk indicates significant differences compared with cadmium treatments ($P < 0.05$). Vertical bars represent standard error (SE) of the mean (n = 3).

compared to cv. Sönmez-2001. Cd accumulation in the 24 and 72 h root tissues was higher than that in leaf tissues ($P < 0.05$). Comparing the 72 h root tissues with leaf tissues in cv. Quality, there was 75.28 times greater Cd accumulation in the Cd treatment, 293.91 times greater in Cd+SNP, and 91.76 times greater in Cd+cPTIO.

4. Discussion

It was determined that NO level decreased in terms of Cd treatment under specific conditions with cPTIO and Cd+cPTIO treatments in 24 and 72 h samples of cv. Sönmez-2001 and cv. Quality. Similarly, there have been studies reporting that Cd stress causes a decrease in NO level in *Pisum sativum* L. (Rodríguez-Serrano et al., 2006) and *Oryza sativa* L. (Xiong et al., 2009). On the other hand, contrary to our results, there have also been studies that report an increase in NO levels in crop plants due to exposure to Cd (Mahmood et al., 2009).

The data of this study are compatible with the literature stating that SNP applied together with Cd increases NO level and provides protection against heavy metal stress and also it may have a curative effect (Gill et al., 2013). In recent years, various studies using NO donors have shown that NO provides protection against heavy metal toxicity (Xiong et al., 2009; Arasimowicz-Jelonek et al., 2011). Since SNP caused a higher rate of NO in the sensitive Quality compared to the tolerant cultivar, it was thought that SNP had a protective effect in response to heavy metal stress.

Numerous studies have demonstrated that the protective effect of NO against abiotic stresses is closely related to the NO-mediated reduction of ROS in plants (Xiong et al., 2009). Under these conditions, NO appears to serve as an antioxidant agent that is able to scavenge ROS to protect plant cells from ROS damage. NO may also function as a signalling molecule and indirectly mediate

ROS levels in the cascade of events leading to alterations in antioxidative gene expression (Leshem, 1996). Wang and Yang (2005) reported that exogenous NO reduced Al toxicity in the roots of *Cassia tora* L. by acting as an antioxidant to protect the plant against Al-induced oxidative stress.

While there has been an insufficient number of studies on the relation between heavy metal stress and PA/NO (Zhang et al., 2011) and there has been a limited number of studies on changes in PA levels in plants exposed to Cd stress, the studies have been mostly about exogenous PA and NO treatment (Groppa et al., 2008; Tang et al., 2009; Yang et al., 2011).

Application of especially Cd and Cd+SNP led to a significant increase in 72 h Spm, Spd, and Put levels in cv. Quality (Table 2). Groppa et al. (2003) reported that Put could be accumulated in wheat leaf discs under Cd stress, which was mediated by a simultaneous enhancement of ADC and ODC (ornithine decarboxylase) activities and an inhibition of DAO activity. This result was similar to our results obtained in the sensitive wheat cultivar.

The 24 h Spm level increased in SNP, cPTIO, and Cd+cPTIO groups in cv. Sönmez-2001. In cv. Quality, Spm level increased at 24 h with Cd treatment and at 72 h with Cd and Cd+SNP treatments. Yang et al. (2010) determined a decrease in Spd and Spm levels and an increase in Put levels of *Potamogeton crispus* L. species on which the Cd was applied. Jiang et al. (2012) reported an increase in Put level and a decrease in Spm and Spd levels depending on Cd treatment on *Malus hupehensis* Rehd. roots left under Cd stress.

In our study, it could be asserted that the decrease in PA level apart from Cd treatment could be related to the fact that PA synthesis was restricted by NO. While L-arginine transforms into NO with nitric oxide synthase (NOS) metabolically, it transforms into Put through ornithine decarboxylase (ODC) enzyme over ornithine with arginase enzyme and also transforms into Put through agmatine. The fact that Put was the minimum level of amine supported our result (Tables 1 and 2). In leaf tissues, there were significant increases in Spm and Spd levels and a decrease in Put level. This gives the impression that Put synthesised in leaf tissue probably transformed into Spd and Spm. The results of the study conducted by Rabiti et al. (1989) using labelled Put supported the result of our study. Fan et al. (2013) stated that exogenous NO treatment increased Spm level in cucumber. NO restricted transformation of methionine into ethylene, while accelerating its transformation into Spm. It was reported that the decrease in Spm was associated with either Spd accelerating transformation to Spm or the decrease in Spd synthesis.

On the other hand, it was determined that amine types were higher in Quality compared to Sönmez-2001, which was more distinctive at 72 h. This difference for Cd treatments at 72 h was 2.02 times greater for Spd, 105.28 times greater for Put, and 3.87 times greater for Spm (Tables 1 and 2). Parallel to these results, Katiyar and Dubey (1990) compared salt sensitive and salt tolerant cultures and determined that PA concentration was higher in sensitive cultures even in the absence of salt. Langerbartels et al. (1991) reported an increased content of PAs bound to various phenolic compounds of the apoplast in ozone-treated tobacco plants, which may protect cells by being scavengers for extracellular oxygen radicals. Therefore, in this study, it makes us think that more PA accumulation on cv. Quality (a sensitive cultivar) leaves is a criterion that can be used in determination of sensitive cultivars. It was found that data about changes in PA levels in sensitive and tolerant cultivars of the same plant material could differ (Basu and Ghosh, 1991; Lefevre et al., 2001).

In 24 and 72 h root and leaf tissues, cv. Quality had higher levels of Cd accumulation compared to Sönmez-2001 in all treatments. Our results showed that there was greater Cd accumulation in root tissue compared to leaf tissue, which was in parallel with results of previous studies (Öztürk et al., 2003; Doğanlar and Yürekli, 2009). Zhang et al. (2002) determined that wheat genotypes showed significant differences in the sense of reaction towards Cd in different tissues against addition of Cd.

Lower Cd accumulation was observed in 24 and 72 h root and leaf tissues of cv. Sönmez-2001 and cv. Quality, especially in the Cd+SNP and Cd+cPTIO groups, compared to the Cd treatment group. The fact that there was a small amount of Cd in the Cd+SNP group demonstrated that the adaptation to the stress environment in plants was potentiated after SNP treatment, and thus SNP could ameliorate Cd toxicity.

When we analysed the research as a whole, it was determined that while NO level and Cd accumulation decreased depending on cPTIO treatment, there was an increase in PA levels, which is known to a play role in the reaction against stress. Considering that living beings including higher plants react with various mechanisms, it was thought that as other molecules increased, cPTIO caused a decrease in NO level that was much greater in the sensitive wheat cultivar.

The low Cd accumulation in the root and leaf tissues of the cultivar chosen as Cd tolerant shows that this cultivar will be less exposed to the negative effects of heavy metal stress. It is thought that cv. Sönmez-2001 can be used in phytoremediation technology since it has resistance against Cd amount accumulated in the tissue despite heavy metal stress.

5. Conclusion

Several biotic and abiotic stresses where PAs are also involved may use NO as a mediator. When the findings of tolerant and sensitive cultivars were analysed comparatively, an increase in NO level was determined together with NO donor SNP treatment. When cPTIO was applied, NO formation decreased but PA synthesis increased, and we could assert that PA may have stress protective effects against the negative effects of Cd and consequently more Cd accumulation in the roots of sensitive cultivars in particular may decrease. Moreover, it is thought that the PA and NO levels obtained in this study might contribute to the presentation of the PAs and NO relation since they use L-arginine as common precursor and would be useful in further studies. As a result of our study, cv. Sönmez-2001 was evaluated as an important cultivar since it had high Cd tolerance and low Cd accumulation.

Future studies should evaluate whether a sensitive cultivar is a hyperaccumulator plant or not by analysing phytochelatin levels, antioxidant enzyme activities, and antioxidant molecules.

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