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Exogenous Application of Spermidine Enhanced Tolerance of Pepper against *Phytophthora capsici* Stress

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Abstract

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The effect of exogenous spermidine – Spd (0.1 and 1 mM) on the relation between polyaminoxidase (PAO), diaminoxidase (DAO), H_2O_2, and malondialdehyde (MDA) in three cultivars of pepper (*Capsicum annum* L.) exhibiting different tolerance to *P. capsici* stress: KM-Hot (*P. capsici*-tolerant), PM-217 (*P. capsici*-resistant), and CM-334 (*P. capsici*-highly resistant) was investigated. The 0.1 mM Spd pre-treatment led to an increase in DAO activity on the third day in three pepper cultivars under the stress of *P. capsici*, 1 mM Spd + *P. capsici* led to an increase in DAO and PAO activities on the fifth day if compared to *P. capsici* treatment alone. *P. capsici* alone caused an increase in the amounts of H_2O_2 at all times in all cultivars and in the amounts of MDA on the third and fifth days in all cultivars. Conversely; under the stress of *P. capsici*, pre-application of 0.1 mM Spd at all times in KM-Hot and CM-334 cultivars decreased the amount of MDA and H_2O_2 and on the first and third days in PM-217 cultivar decreased the amount of MDA and H_2O_2. This data indicates that exogenous Spd application before inoculation decreases the plasma membrane injury by decreasing the level of H_2O_2 and regulating the activities of amine oxidase in both *P. capsici*-sensitive and *P. capsici*-resistant cultivars of peppers, so it may increase the tolerance of pepper cultivars against *P. capsici*.

Keywords: amine oxidase; oxidative stress; pepper; pathogen; polyamine

Phytophthora root rot is very difficult to control. No single method, currently available, provides adequate control against the disease. Researchers who carried out pepper breeding studies were not successful in obtaining a pepper culture that is resistant to all isolates of *P. capsici* (Palloix *et al*. 1988). While polygenic resistance and environmental changes play role in this failure, intensive research on obtaining the cultures used in the breeding programs is required. Therefore, in order to reduce the damage caused by *P. capsici* on peppers, there are many studies for the development of more stress-tolerant and resistant plants. Alternative approaches may increase the tolerance to *P. capsici* stress.

Currently, several areas need to be investigated. One is the investigation of a better tolerance at molecular and biochemical levels to environmental stresses (salinity, hyperosmosis, heat, chilling, drought, pH variation, UV, herbicide, hypoxia, environmental pollutants) in the presence of polyamines (PAs) which are found in a large class from bacteria to plants and animals (Alcazar *et al*. 2011; Gupta *et al.* 2013) and are the aliphatic cations with a biological activity (Hussain *et al.* 2011).

Spermidine (Spd), a member of polyamines, a group of phytohormone-like natural amine compounds, has been shown to play an essential role in stress tolerance in many important plants. *P. capsici* induced a considerable disturbance in several physiological processes inhibitory for growth including accumulation on hydrogen peroxide and an increase in lipid peroxidation. PAs, such as spermidine, putrescine (Put), and spermine (Spm), form another group of essential growth regulators in plants. As they have positive charge at physiological pH, negatively charged phenolic, proteins and phospholipids, due to their ability to conjugate with organic acids such as nucleic acids, their polycationic and antioxidant activities, are free radical scavengers and therefore they are believed to have an effect on plant toler-

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ance against biotic and abiotic stress. Therefore, the effect of stress on exogenous polyamine application has become an important subject of study. With the studies, it was reported that there is a correlation between stress–plant tolerance–PAs, although these studies have usually focused on the abiotic stress–plant interaction and the studies on the plant–biotic interaction have fallen behind (Rodriguez *et al.* 2008). The protective roles of exogenous PAs have been attributed to the reduction of abiotic stress-induced damages. These included ROS accumulation, lipid peroxidation, and membrane damage. Exogenous PAs (Spd and Spm) were reported to enhance the activity of antioxidative enzymes and reduce the MDA and H_2O_2 accumulation in salinity stress (Roychoudhury *et al.* 2011). Exogenous application of Spd was shown to modulate resistance against *Tobacco mosaic virus* (TMV) in tobacco and *Arabidopsis*. Previous research found that exogenous Spd treatment altered reactive oxygen species (ROS) level (Wan *et al.* 2007).

PAs are catabolised by one or more diaminoxidase (DAO) (EC 1.4.3.6) and polyaminoxidase (PAO) (EC 1.5.3.11). In plants, different roles of DAO and PAO have been reported in cell growth development and defence responses leading to disease resistance (Cona *et al.* 2006). Increased PAO levels were observed in incompatible interaction between barley and powdery mildew, chickpea and *Asochyta rabiei*, and increased DAO activity was shown in systemic protection to powdery mildew (Walters 2003). In addition, in TMV-resistant tobacco, increased activity of PAO was observed (Yoda *et al.* 2003). As a result of the oxidation of Spm by PAO, 1,3-aminopropylpyrroline is produced and H_2O_2 is released (Cona *et al.* 2006). H_2O_2, the reaction product of DAO and PAO, is found to be involved in signalling in programmed cell death and lignification (Rybkowska & Borucki 2014), cross linking of protein and polysaccharides and have a direct antimicrobial effect, although high H_2O_2 accumulation during stress causes a toxic effect. Despite this, the contribution of polyamine mechanism in plant adaptation to stress is still a subject of research.

Therefore, the studies on PAs and stress tolerance are at an interesting stage and a series of intensive studies were initiated in order to understand the functions of these simple molecules. To understand especially the role of PAs during plant growth in normal and stressful conditions, the experimental data obtained as a result of the analysis to be conducted appears to be important. Some striking evidences of exogenous application of PA to counteract environment stresses are expected to promote its extended application to other plant species.

The effects of exogenous Spd on changes of DAO and PAO activity have not been revealed in peppers exposed to *P. capsici* stress. The objectives of this study are to determine whether the acquired *P. capsici* stress tolerance induced by exogenous Spd is associated with the changes in DAO and PAO activity, the amount of H_2O_2 and MDA. Such information will help further understand the effects of plant tolerance to *P. capsici* stress and gain more insight into the possible mechanisms of the enhanced *P. capsici* stress tolerance induced by exogenous Spd.

MATERIAL AND METHODS

Plant material. Seeds of three *Capsicum annuum* cultivars – one susceptible KM-Hot (Kahramanmaraş-Hot), one resistant PM-217, and one high resistant CM-334 (Criollo de morelos) were used. The plants were maintained in a growth chamber under controlled environmental conditions (25 ± 2°C and a 16-h light, 8-h dark photoperiod). Seedlings were harvested when they reached the 6–7 leaf phase.

P. capsici-22 zoospore inoculation and Spd treatment. *P. capsici*-22 (obtained from the fungal culture collection of Ankara University, Faculty of Agriculture, Ankara, Turkey) was grown on V_8 agar plates at 25°C in the dark (Jones 1975). Zoospores were produced from mycelium (Ward & Stoessl 1974). The concentration of zoospores was then adjusted to 10^4 per ml using a haemocytometer (Harrigan & McCance 1966).

Seedlings with 6–7 leaves grown in greenhouse conditions were collected, then their roots were washed with tap water and disinfected by keeping inside 0.75% sodium hypochlorite for 1–2 minutes. Later, it was irrigated with sterile distilled water with 1–2 drops of tween 20 per l inside. Root straits were aligned in a way so that every five seedling made a bunch and they were tied in bunches by wrapping with an aluminium folio at 3–4 cm above the root. 1–2 cm was cut from the root tips with a sharp knife. As an aside, 3 sterile glass bottles with a capacity of 500 ml containing 400 ml of sterile full Hoagland solution for each treatment were prepared. Six bunches were put in each glass bottle with a wide mouth so that each bottle contained 30 seedlings.

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Bunches inside the bottle with an opening just wide enough for plant bunches were supported with the cotton wrapped around them and incubated for 3 days in a plant breeding chamber adjusted to 22 ± 3°C, 60% humidity, and 14 h of a light period to adapt to changing environmental conditions (Koç *et al.* 2011).

Before inoculation, 0.1 and 1 mM Spd treatments were performed by superficial spraying onto pepper seedlings. Distilled water treatment was performed in the control groups. Inoculation procedure (Koç *et al.* 2011) was done 72 h after treatment. Under the same conditions, random samples were taken on the first, third and fifth days according to the random block design model. Leaves from the plants taken were separated and immediately frozen in liquid nitrogen. Later, they were put inside the plastic bags, labelled and kept at –70°C until analysis.

Disease severity and necrosis length. The root inoculation test was performed as described by Palloix *et al.* (1988). Inoculation was done 72 h after Spd pre-treatment and seedlings were incubated in a plant breeding chamber adjusted to 22 ± 3°C, 60% humidity, and 14 h of a light period. Ten seedlings were used for each treatment (*P. capsici*, 0.1 mM Spd + *P. capsici,* and 1 mM Spd + *P. capsici*) (for each pepper cultivar), then necrosis length and disease severity were measured during 5 days in each seedling. Lesion development was expressed as necrosis length (mm). The disease severity was rated based on a 0–5 scale (Kim *et al*. 1989). Scale values 0–3 were accepted as resistant and 3–5 as sensitive (0: no visible disease symptoms, 1: leaves slightly with brownish lesions beginning to appear on stems, 2: 30–50% of entire plant diseased, 3: 50–70% of entire plant diseased, 4: 70–90% of entire plant diseased, 5: plant dead).

DAO (EC 1.4.3.6) and PAO (EC 1.5.3.11) activity. DAO (EC 1.4.3.6) and PAO (EC 1.5.3.11) activities were estimated spectrophotometrically using a method based on the colourimetric assay of Δ^1-pyrroline using Put (for DAO) or Spd (for PAO) as substrates (Holmstedt *et al.* 1961). Enzyme activity was expressed in pmol Δ^1-pyrroline/min/g FW using an extinction coefficient of 1.86×10^3 mol^{-1} cm^{-1}.

Determination of H_2O_2 content. H_2O_2 content in leaves was determined in accordance with Velikova *et al.* (2000). The content of H_2O_2 was calculated by comparison with a standard calibration curve previously made using different concentrations of H_2O_2.

Malondialdehyde determination. The malondialdehyde (MDA) concentration in pepper leaves was determined by the thiobarbituric acid (TBA) reaction in accordance with the method of Heath and Packer (1968). The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Statistical method. All the features were analyzed by a three-factor (3 × 3 × 4) analysis of variance. Conformity of the data to normal distribution was tested by the Kolmogorov-Smirnov test and the homogeneity of variances was controlled by the Levene test. As a result of analysis of variance, the Duncan Multiple Range Test for the determination of different mean values at 5% significance was used. Introductory statistics related to the features were calculated and the results of the Duncan test are expressed in letters next to the mean ± standard error. Data presented are mean values ± standard error measures for three replicates (n = 3). Analyses of variance were done by Minitab 16, Duncan tests were performed using MSTAT package programs. Statistical significance is indicated by appropriate letters within the tables.

RESULTS

Reaction experiments demonstrated by KM-Hot, PM-217, and CM-334 pepper cultivars exposure to *P. capsici* and Spd + *P. capsici* were performed under controlled conditions. The most significant differences were determined on the fifth day in terms of disease severity and necrosis length. It was observed that the disease agent displayed faster progress in KM-Hot pepper cultivars and most of the seedlings were damaged on the fifth day after inoculation, although Spd pre-application reduced the severity of the disease (Table 1). When all three pepper genotypes were compared in terms of necrosis length, on the fifth day following infection with the *P. capsici* treatment, the difference in necrosis length was significant for all three cultivars ($P < 0.05$). The highest necrosis length was determined in the KM-Hot genotype, and the difference between necrosis lengths was significant for all three cultivars, although Spd + *P. capsici* treatments in pepper seedlings reduced the necrosis length if compared to *P. capsici* alone ($P < 0.05$) (Table 2).

Maximum DAO activity in the leaves of KM-Hot seedlings were observed on the first and fifth days of treatment in 1 mM Spd + *P. capsici* treatment ($P < 0.05$) (Table 3). Maximum enzyme activity was detected on the third day in 0.1 mM Spd + *P. capsici* and 1 mM Spd +

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Table 1. Disease scale values (Kim *et al.* 1989) of pepper seedlings pre-treated by Spd and inoculated by *P. capsici* (10^4 zoospores/ml) from root necks on the fifth day

Scale	KM-Hot			PM-217			CM-334		
	P. capsici	0.1 mM Spd + *P. capsici*	1 mM Spd + *P. capsici*	*P. capsici*	0.1 mM Spd + *P. capsici*	1 mM Spd + *P. capsici*	*P. capsici*	0.1 mM Spd + *P. capsici*	1 mM Spd + *P. capsici*
0	–	–	–	2	3	4	2	4	3
1	–	–	–	3*	3*	2*	4*	2*	2*
2	–	–	–	2*	2*	2*	1*	2*	3*
3	–	3*	2*	1*	1*	1*	1*	2*	2*
4	4*	3*	4*	1*	1*	1*	2*	–	–
5	6*	4*	4*	1*	–	–	–	–	–
Average	4.6	4.1	4.2	1.9	1.4	1.3	1.7	1.2	1.4

Average = Σ (pepper seedlings number × scale values)/total pepper seedlings number; *diseased seedlings

P. capsici treatments and Spd + *P. capsici* increased the enzyme activity compared to both the control and *P. capsici* alone ($P < 0.05$). 1 mM Spd + *P. capsici* treatment at all times in the leaves of KM-Hot seedlings increased the PAO enzyme activity compared to both the control and *P. capsici* alone ($P < 0.05$). The highest enzyme activity was detected on the fifth day in 1 mM Spd + *P. capsici* treatment (Table 4).

On the first and third days of treatment, 0.1 mM Spd + *P. capsici* increased the DAO enzyme activity compared to the control and *P. capsici* treatment alone in the leaves of PM-217 seedlings ($P < 0.05$). On the fifth day, 1 mM Spd + *P. capsici* increased the DAO enzyme activity compared to both the control and *P. capsici* treatment alone ($P < 0.05$) (Table 3). The highest PAO activity was determined in *P. capsici* treatment alone in the leaves of PM-217 seedlings on the first and third days of treatment ($P < 0.05$), although Spd pre-treatments before inoculation in the leaves of PM-217 cultivar were not effective on the first and third days of treatment compared to the *P. capsici* treatment alone ($P < 0.05$). On the fifth day, 0.1 mM Spd + *P. capsici* and 1 mM Spd + *P. capsici* increased the PAO enzyme activity compared to both the control and *P. capsici* treatment alone ($P < 0.05$) (Table 4). Maximum increase in enzyme on the fifth day was detected in 1 mM Spd + *P. capsici* treatment ($P < 0.05$) (Table 4).

Table 2. Necrosis length of pepper seedlings after exposure to *P. capsici* and exogenous pre-application of Spd + *P. capsici* on the fifth day ($P < 0.05$)

Cultivars	Treatments	Necrosis length (mm) ($\bar{x} \pm s_{\bar{x}}$)
KM-Hot	*P. capsici*	35.6 ± 0.954 Aa
	0.1 mM Spd + *P. capsici*	26.18 ± 1.01 Ba
	1 mM Spd + *P. capsici*	27.24 ± 0.676 Ba
PM-217	*P. capsici*	27.222 ± 0.385 Ab
	0.1 mM Spd + *P. capsici*	21.511 ± 0.547 Bb
	1 mM Spd + *P. capsici*	18.444 ± 0.581 Cb
CM-334	*P. capsici*	8.76 ± 1.51 Ac
	0.1 mM Spd + *P. capsici*	2.711 ± 0.038 Bc
	1 mM Spd + *P. capsici*	3.444 ± 0.022 Bc

Capital letters represent application differences in the same cultivar; lowercase letters represent differences in cultivars for the same application

On the first and third days of treatment, 0.1 mM Spd + *P. capsici* treatment and 1 mM Spd + *P. capsici* treatment on the fifth day increased the DAO activity compared to both the control and *P. capsici* alone ($P < 0.05$) in the leaves of CM-334 seedlings. Maximum enzyme activity was detected on the third day in 0.1 mM Spd + *P. capsici*. Maximum DAO activity was detected on the fifth day in the treatment of 1 mM Spd + *P. capsici* ($P < 0.05$) (Table 3) although Spd pre-treatment before inoculation in the leaves of CM-334 cultivar was not effective on the first and third days of treatment. Spd + *P. capsici* treatment in the leaves of CM-334 cultivar was effective on the first day of treatment and the highest PAO activity was determined in 0.1 mM Spd + *P. capsici* treatment and it has increased the activity of PAO enzyme compared to *P. capsici* treatment alone ($P < 0.05$) (Table 4). The highest PAO activity was defined on the fifth day in 1 mM Spd + *P. capsici* and *P. capsici* treatment alone ($P < 0.05$) (Table 4).

At all times of treatment, 0.1 mM Spd + *P. capsici* decreased the amount of H_2O_2 both in the control

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Table 3. DAO activity in leaves of pepper seedlings after exposure to *P. capsici* and exogenous pre-application of Spd + *P. capsici* ($P < 0.05$)

Cultivars	Treatments	DAO activity (Δ^1-pyrroline pmol/g FW) ($\bar{x} \pm s_{\bar{x}}$)		
		Day 1	Day 3	Day 5
KM-Hot	Control	1349.8 ± 73.9 Bb2	1427.4 ± 36.5 Cb2	1801.1 ± 80.8 Ca1
	P. capsici	1167.2 ± 26.7 Cb3	1458.9 ± 15.9 Cc2	3073.2 ± 26 Aa1
	0.1 mM Spd + *P. capsici*	1517.8 ± 40.5 Bb2	3509.5 ± 44.6 Aa1	2292.7 ± 4.09 Ba2
	1 mM Spd + *P. capsici*	2098.7 ± 38.5 Aa2	3035.7 ± 23.7 Ba1	3208.3 ± 67.1 Aa1
PM-217	Control	1516.7 ± 43.1 Bab3	1882.4 ± 91.9 Ca1	1704 ± 56.8 Ba2
	P. capsici	1012.3 ± 48.6 Cb3	2525.9 ± 38.6 Ba1	1538.5 ± 71.9 Bc2
	0.1 mM Spd + *P. capsici*	1962.7 ± 42 Aa2	3105 ± 191 Ab1	897.4 ± 15.9 Cc3
	1 mM Spd + *P. capsici*	1060.7 ± 47.5 Cb2	2366.1 ± 32.9 Bb1	2656.1 ± 40.2 Ac1
CM-334	Control	1587.7 ± 26.4 Ca2	1830.6 ± 20.3 Ba1	1707.1 ± 76.3 Ca1
	P. capsici	2680.1 ± 74.9 Aa2	2227.5 ± 65.3 Ab3	2532 ± 133 Bb1
	0.1 mM Spd + *P. capsici*	1930.4 ± 30.6 Ba2	2310.4 ± 38.1 Ac1	1381 ± 57.1 Db3
	1 mM Spd + *P. capsici*	877.2 ± 15.4 Dc3	1257.7 ± 22 Cc2	2872.7 ± 61.2 Ab1

Capital letters represent application differences in the same cultivar; lowercase letters represent differences in cultivars for the same application; numbers represent differences in days for the same cultivar and the same application

and *P. capsici* treatment alone in the leaves of KM-Hot seedlings ($P < 0.05$). On the fifth day of treatment, 0.1 mM Spd + *P. capsici* decreased and 1 mM Spd + *P. capsici* increased the amount of H_2O_2 compared to both the control and *P. capsici* treatment alone ($P < 0.05$) (Table 5). 0.1 mM Spd + *P. capsici* pre-treatment was effective on the third and fifth days in the leaves of CM-334 seedlings and 1 mM Spd + *P. capsici* on the third and fifth days very slightly increased the amount of H_2O_2 ($P < 0.05$). Spd + *P. capsici* pre-treatment before inoculation was effective on the third day of treatments in the leaves of PM-217 seedlings, Spd + *P. capsici* decreased the amount of H_2O_2 compared to *P. capsici* treatment alone ($P < 0.05$). While 0.1 mM Spd + *P. capsici* increased the amount of H_2O_2 on the fifth day, 1 mM Spd + *P. capsici* treatment decreased the amount of H_2O_2 compared to *P. capsici* treatment alone ($P < 0.05$) (Table 5).

Table 4. PAO activity in leaves of pepper seedlings after exposure to exogenous pre-application of Spd + *P. capsici* ($P < 0.05$)

Cultivars	Treatments	PAO activity (Δ^1-pyrroline pmol/g FW) ($\bar{x} \pm s_{\bar{x}}$)		
		Day 1	Day 3	Day 5
KM-Hot	Control	1386.2 ± 18.1 Aa1	1169.6 ± 5.92 Dc2	1048.8 ± 16.8 Dc3
	P. capsici	954.6 ± 20.2 Cc3	1765.1 ± 17.5 Bb2	2527.3 ± 65.3 Ba1
	0.1 mM Spd + *P. capsici*	1291.1 ± 8.5 Bc3	1563.2 ± 1.51 Cb1	1459.6 ± 53.8 Cb2
	1 mM Spd + *P. capsici*	1496.2 ± 16.1 Aa3	1976.6 ± 13.8 Aa2	2772.6 ± 40.5 Aa1
PM-217	Control	1147.8 ± 62.9 Cb3	1259.9 ± 38.3 Db2	1408.7 ± 34.6 Db1
	P. capsici	3593.6 ± 2.53 Aa1	3368.3 ± 44.9 Aa2	1640.1 ± 0.261 Cc3
	0.1 mM Spd + *P. capsici*	1702.9 ± 8.83 Ba3	2705.4 ± 24.6 Ba1	1962.7 ± 76.2 Ba2
	1 mM Spd + *P. capsici*	991.15 ± 5.38 Db3	1534.2 ± 2.21 Cb2	2711.0 ± 3.36 Aa1
CM-334	Control	1312.7 ± 3.73 Ca2	1742.2 ± 17.5 Aa1	1725.7 ± 3.0 Ba1
	P. capsici	1428.2 ± 4.06 Bb2	980.61 ± 7.02 Dc3	2398.9 ± 24.1 Ab1
	0.1 mM Spd + *P. capsici*	1497.4 ± 31.4 Ab1	1497.6 ± 24.6 Bc1	1345.1 ± 34.9 Cc2
	1 mM Spd + *P. capsici*	928.3 ± 10.3 Db3	1246.2 ± 36.7 Cc2	2424.2 ± 18.1 Ac1

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Table 5. H_2O_2 content in leaves of pepper seedlings after exposure to *P. capsici* and exogenous pre-application of Spd + *P. capsici* ($P < 0.05$)

Cultivars	Treatments	H_2O_2 (µmol/g FW) ($\bar{x} \pm s_{\bar{x}}$)		
		Day 1	Day 3	Day 5
KM-Hot	Control	20.358 ± 0.517 Ba2	26.04 ± 1.04 Aa1	26.899 ± 0.202 Aa1
	P. capsici	25.006 ± 0.259 Aa2	28.724 ± 0.95 Aa1	27.33 ± 1.27 Ab1
	0.1 mM Spd + *P. capsici*	19.4 ± 1.24 Bb1	20.358 ± 0.517 Bb1	19.86 ± 0.207 Bb1
	1 mM Spd + *P. capsici*	24.01 ± 0.674 Ab2	27.96 ± 1.05 Aa1	27.29 ± 1.21 Ab1
PM-217	Control	21.264 ± 0.469 Ba2	23.379 ± 0.166 Ab1	25.338 ± 0.46 Da1
	P. capsici	22.981 ± 0.176 Ba2	24.874 ± 0.295 Ab2	43.13 ± 3.34 Ba1
	0.1 mM Spd + *P. capsici*	21.78 ± 0.34 Bab2	19.86 ± 0.207 Bb2	47.118 ± 0.818 Aa1
	1 mM Spd + *P. capsici*	32.44 ± 1.78 Aa1	17.171 ± 0.697 Cc3	29.612 ± 0.634 Ca2
CM-334	Control	17.702 ± 0.295 Bb1	19.13 ± 0.604 Cc1	19.229 ± 0.347 Cb1
	P. capsici	17.927 ± 0.179 Bb3	24.731 ± 0.552 Ab2	26.234 ± 0.983 Ab1
	0.1 mM Spd + *P. capsici*	22.98 ± 1.35 Aa1	23.299 ± 0.318 Ba1	18.963 ± 0.304 Cb2
	1 mM Spd + *P. capsici*	15.212 ± 0.272 Cc2	25.006 ± 0.383 Ab1	26.317 ± 0.233 Ab1

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On the first, third, and fifth days of treatments, 0.1 mM Spd + *P. capsici* decreased the amount of MDA compared to *P. capsici* treatment alone in the leaves of KM-Hot seedlings ($P < 0.05$) (Table 6). Conversely, 1 mM Spd + *P. capsici* treatment increased the amount of MDA compared to other treatments ($P < 0.05$) (Table 6). 0.1 mM Spd pre-treatment before inoculation was effective at all times of treatments in the leaves of CM-334 seedlings, 0.1 mM Spd + *P. capsici* decreased the amount of MDA compared to *P. capsici* treatment alone ($P < 0.05$) (Table 6). 0.1 mM + *P. capsici* and 1 mM Spd + *P. capsici* on the first and third days in the leaves of PM-217 seedlings decreased the amount of MDA compared to *P. capsici* treatment alone ($P < 0.05$). Conversely, only 1 mM Spd + *P. capsici* on the fifth day of treatment de-

Table 6. MDA content in leaves of pepper seedlings after exposure to exogenous pre-application of Spd + *P. capsici* ($P < 0.05$)

Cultivars	Treatments	MDA (nmol/g FW) ($\bar{x} \pm s_{\bar{x}}$)		
		Day 1	Day 3	Day 5
KM-Hot	Control	16.91 ± 2.31 Bb2	21.205 ± 0.48 Bb1	25.56 ± 2.16 Ba1
	P. capsici	16.32 ± 0.991 Bb2	32.06 ± 1.12 Ab1	29.302 ± 0.871 Ab1
	0.1 mM Spd + *P. capsici*	12.917 ± 0.046 Cb2	23.15 ± 1.38 Bb1	27.24 ± 1.19 Bb1
	1 mM Spd + *P. capsici*	31.2 ± 3.61 Aa1	31.69 ± 2.61 Aa1	34.66 ± 4.58 Aa1
PM-217	Control	30.889 ± 0.592 Aa1	29.69 ± 1.85 Ba1	26.164 ± 0.60 Ca1
	P. capsici	30.36 ± 1.61 Aa3	39.165 ± 0.535 Aa2	54.89 ± 5.82 ABa1
	0.1 mM Spd + *P. capsici*	25.31 ± 2.59 Ba3	33.89 ± 3.65 ABa2	60.26 ± 5.82 Aa1
	1 mM Spd + *P. capsici*	30.44 ± 3.47 Aa2	31.954 ± 0.93 ABa2	33.99 ± 2.56 Ba1
CM-334	Control	27.41 ± 2.4 Ab1	23.4 ± 1.09 Bb1	24.93 ± 1.99 Ba1
	P. capsici	27.27 ± 1.5 Aa2	30.59 ± 1.86 Ab1	32.125 ± 0.885 Ab1
	0.1 mM Spd + *P. capsici*	26.714 ± 0.628 Aa1	26.043 ± 0.27 ABb1	10.509 ± 0.371 Cc2
	1 mM Spd + *P. capsici*	24.48 ± 2.48 Bb2	21.77 ± 4.96 Bb2	32.79 ± 2.23 Ab1

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creased the amount of MDA compared to *P. capsici* treatment alone ($P < 0.05$) (Table 6).

DISCUSSION

DAO and PAO activities play an important role in increasing the stress-resistance of plants. Data from studies in recent years has shown that DAO and PAO play a role in the PA catabolism and the products formed as a result of PA degradation are required in many important physiological events (Kongkiattikajorn 2009).

PA oxidation plays an essential role during PA signal transduction. Importantly, the activities of PAO and DAO enzymes are increased upon pathogen infection (Moschou *et al.* 2009). In tobacco, oxidation induces the hypersensitive response (HR) during TMV infection and this is essential for defence against the bacterium *P. syringae* pv. *tabaci* and the oomycete *Phytophthora parasitica* var. *nicotianae* (Yoda *et al*. 2003; Moschou *et al*. 2009) because PAO and DAO activities result in the production of H_2O_2, a process that contributes to stimulate host cell death. With the induction of HR, it has been demonstrated that the oxidation of PAs is important to strengthen the cell wall during pathogen attack (Angelini *et al*. 2010). Yoda *et al.* (2003, 2006) reported that H_2O_2 was produced as a result of polyamine catabolism in tobacco plants exposed to biotic stress (TMV acts as a signalling molecule that stimulates the defence genes). Paschalidis and Roubelakis-Angelatis (2005) reported that a programmed cell death occurs as a result of the increase in PAO level and the accompanying increase in H_2O_2 amount. The fact that being a product of DAO and PAO reaction, having a role in the generation of hypersensitive response considered as the form of programmed cell death and having role in the lignification during normal growth and stress response (Walters 2003) confirms these results. The present study indicated that *P. capsici* stress generally increased DAO and PAO activities in pepper leaves. In addition, an increase in the amount of H_2O_2 was detected in all pepper cultivars after *P. capsici* infection. H_2O_2 production might be related to PAO and DAO activation in the infected cultivars. Bestwik *et al.* (1997) found low accumulation of H_2O_2 in the tissues 48 h after the inoculation with *Botrytis cinerea*. This may indicate that the potential pathogen cannot activate the defence mechanisms if it is not recognised quickly by the plant. The plant defence system operates at lower levels in the early phases of infection because tissue injury is also low. *Pseudomonas syringae* pv. *tabaci* treatment increased the H_2O_2 content and PAO activity in the wild-type tobacco (*Nicotiana tabacum* cv. Xanthi) plants (Moschou *et al.* 2009). DAO and PAO activity were observed during HR induction in powdery mildew resistant varieties of barley at 3 days after inoculation (Cowley & Walters 2002). In our study, it was determined that the amount of H_2O_2 is low in KM-Hot, CM-334, and PM-217 cultivars on the first day following the *P. capsici* infection, when compared to the third and fifth day. Large amounts of peroxide were found and high DAO and PAO activities on days 3 and 5 following the infection generally. H_2O_2 produced by PAO and DAO could act as a signalling agent in the defence responses of plant–pathogen interactions. Localised H_2O_2 production is reported to be important for a variety of plant responses like protein cross-linking, callose deposition, and accumulation of phenolic compounds in fungal–plant interactions (Walters 2003). H_2O_2, the reaction product of DAO and PAO, may stimulate the synthesis of lignin (Cona *et al.* 2003), which can prevent fungi from entering the cell.

Spd may serve two functions in plant stress tolerance; one as a direct stress-protecting compound and the other as a stress signalling regulator. Exogenous Spd treatment caused a substantial reduction in high ROS amount and thereby reduced the oxidative stress under stress. This positive effect of exogenous Spd may be related to its antioxidant properties (Kubis 2005). Rea *et al.* (2004) determined in their study that high amounts of H_2O_2 are decreased in the transgenic *Zea mays* and *Pisum sativum* in the presence of exogenous substrates (Spd and Put). The expression of PAO activity from maize in tobacco cells is sufficient to induce programmed cell death when PAs are exogenously added (Rea *et al*. 2004). Hu *et al.* (2012) reported that the exogenous Spd pre-treatment increases the PAO and DAO activity in two tomato species under salinity-alkalinity stress and they asserted that these results increase the tolerance of tomato plants exposed to salinity-alkalinity stress of the exogenous Spd. In our study, 0.1 mM Spd pre-treatment led to an increase in DAO activity on the third day in three cultivars under the stress of *P. capsici*, 1 mM Spd + *P. capsici* led to an increase in DAO and PAO activities on the fifth day compared to *P. capsici* treatment alone. Therefore, 0.1 mM Spd + *P. capsici* application was effective on

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the third day of treatments in the leaves of pepper seedlings, whereas 1 mM Spd + *P. capsici* was effective on the fifth day of treatment. Although H_2O_2 induces defence-related genes and defence responses at low concentrations, it causes cell damage and cell death in high concentrations. In this study, 0.1 mM Spd + *P. capsici* treatment caused a decrease in the amount of H_2O_2 produced on the third day in all cultivars. 1 mM Spd + *P. capsici* treatment did not affect the amount of H_2O_2 produced on the fifth day in KM-Hot and CM-334 cultivars, whereas in PM-217 cultivar it caused a decrease. This may be due to the internal protection mechanism of the cell and antioxidant properties of Spd. It may have caused a decrease in the amount of H_2O_2 because the PAs like Spd are also accepted as scavengers of free radicals (Paschalidis & Roubelakis 2005). Therefore, they may decrease the MDA content.

Membrane lipid peroxidation occurs as a result of the generation of reactive oxygen species like H_2O_2 (Xu *et al.* 2011). Intracellular accumulation of H_2O_2 causes a lipid peroxidation in the membrane and so the content of MDA increases in *P. capsici* treatment. Studies have shown that PAs act directly as a scavenger of free radicals against the oxidative injury in the plants or bind to antioxidant enzymes to break up the free radicals (Roychoudhury *et al.* 2011). In our study, MDA as the indicator of oxidative stress showed a significant increase in all genotypes on the third day following the *P. capsici* infection alone. Conversely, it was detected that 0.1 mM Spd + *P. capsici* treatment in all pepper cultivars on the third day decreased the amount of MDA and H_2O_2. These results indicate that 0.1 mM Spd pre-treatment before *P. capsici* infection reduces the plasma membrane injury by decreasing the level of ROS and therefore it may increase the tolerance of pepper genotypes to *P. capsici*. 1 mM Spd + *P. capsici* treatment caused a very slight increase in the amount of MDA in KM-Hot and CM-334 cultivars on the fifth day. This may block the plant defence system (antioxidative) in KM-Hot and CM-334 cultivars on the fifth day. 1 mM Spd + *P. capsici* treatment decreased MDA and H_2O_2 accumulation in PM-217 cultivar on the fifth day. Moreover, it was shown in this study that Spd + *P. capsici* treatments in pepper seedlings reduced the necrosis length and the severity of disease compared to *P. capsici* alone generally. These results showed that Spd has the potential to scavenge free radicals and alleviate pathogen stress.

The present study showed that tolerance to *P. capsici* can be regulated by the treatment of exogenous Spd at a proper concentration. The response to *P. capsici* differs among the cultivars and different effects of Spd applied exogenously at two different concentrations during stress may be related to the different genotypes of species. This is supported by studies conducted on different genotypes of the same species and species susceptible to or resistant against biotic stress. Despite this, many physiological and biochemical investigations including the defence system are required for a better understanding of the effect of exogenous Spd treatment on the yield, quality, and disease severity of pepper under the stress of *P. capsici*. Therefore, our efforts and project study on this issue continue.

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