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Protective Role of Spermidine on two Tomato Cultivars against Cold-Induced Lipid Peroxidation by Enhancing Capacity of Anti-Oxidative System

Salwa A.Orabi[1], S.R. Salman[2], Faida A. Sharara[1] and S.D. Abou-Hussein[2]

[1]Botany Dept., and [2]Vegetable Research Dept., Agricultural & Biological Research Division, National Research Centre (NRC), 33 El-Buhouth St., 12622 Dokki, Giza, Egypt.

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ABSTRACT
Two pot experiments were conducted during two growing seasons 2015/2016 and 2016/2017 at the green house of the National Research Centre, Dokki, Giza, Egypt to study the effect of spermidine (Spd) on growth parameters, SPAD values, antioxidant enzymes, malondialdehyde (MDA), as well as fruits yield quantity and quality (total soluble solids (TSS) and ascorbic acid (AsA)) of two tomato cultivars (Strain B and Florida) grown under low temperature conditions. All treatments almostly caused significant increases in growth parameters and SPAD values of the two tomato cultivars relative to corresponding control plants. Moreover all Spd treatments caused marked decreases in MDA and methylglyoxal (MG) values accompanied by significant increases in APX, PAL and Gly I activities relative to control plants. All treatments almostly caused significant increases in fruits yield (g/plant) as well as TSS and AsA of fruit juices of the two tomato cultivars relative to control plants. It is worthy to mention that the highest recorded values were obtained in plants treated with 50 mg/L spermidine, these results hold true for both cultivars. Based on the obtained results, it could be concluded that the protection mechanism with Spd had helped the plants to increase their tolerance against low temperature stress, through mainly the decrease in membrane damage symptoms leading to intercellular osmotic adjustment.

Keywords**:** Low temperature, Antioxidant enzymes, Lipid peroxidation, spermidine, MG

Introduction
Low temperature (LT) is considered to be one of the major abiotic stresses, it causes physiological and metabolic disorder resulting in reduction of growth and productivity, reduced stomatal conductance, photosynthetic efficiency, changes in protein structure and enzyme activities (Prasad *et al.,* 1994). Under LT stress there will be inhibition of photochemistry efficiency leading to over generation of reactive oxygen species (ROS) (Orabi, 2004; Orabi *et al.,* 2010, 2014, 2017[a], 2018[a]; Abd El-Razek *et al.,* 2019) which may include singlet oxygen (1O2), superoxide anion (O2,΄), hydrogen peroxide (H2O2), and hydroxyl radical (OH,) (Gill and Tuteja, 2010). Under prolonged stress excess levels of ROS are produced and caused cell membrane lipid peroxidation (Ahmed *et al.,* 2009,2010; Hussein and Orabi, 2008; Hussin *et al.,* 2009;Mekki *et al.,* 2010; Abd El-Motty and Orabi, 2013; Abd Elhamid *et al.,* 2016; Orabi and Abdelhamid, 2016) beside protein denaturation, carbohydrate oxidation, photosynthetic pigment breakdown, impaired enzyme activity, damage to nucleic acids and programmed cell death (Bose *et al.,*2014).Therefore, plants need a delicate balance between ROS generation and scavenging(Mekki and Orabi, 2007; Orabi and Mekki, 2008; Kassab *et al.,* 2012; Orabi *et al.,* 2013, 2016 , 2017[b,c], 2018[b,c]).

Tomato (*Lycopersicon esculentum* L.) is a member of the Solanaceae family, it is considered as one of the most important vegetables grown in Egypt. It is used as a fresh vegetable beside its importance as a raw material for agricultural industry. Also it is a rich source of lycopene, vitamins and minerals. Lycopene is responsible for the characteristic deep red color of ripe tomato fruits and tomato products (Helyes *et al.,* 2009). Lycopene may help to counteract the harmful effects of substances called "free radicals" and different types of cancer, it is a key intermediate in the biosynthesis of many important carotenoids, such as beta-carotene and xanthophylls (DeStefani *et al.,* 2000). Tomato plants after ecxposure to low temperature will suffer from chilling injury as a result of exposure to low, but non-freezing temperatures (ca. >10˚C) (Raison and Lyons, 1986).

Corresponding Author: Salwa A. Orabi, Botany Dept., Agricultural & Biological Research Division, National Research Centre (NRC), 33 El-Buhouth St., 12622 Dokki, Giza, Egypt.
E:mail:- dr.salwaorabi@yahoo.com

The polyamines (PAs) are low-molecular-weight organic cations, they perform diverse biological functions (Kusano *et al.,* 2008). Putrescine (Put.), spermidine (Spd.), and spermine (Spm.) are the most common PAs. Their levels frequently increase during stress, they have essential roles in enhancing plant stress tolerance. Acting as molecular chaperones, PAs bind to negatively charged surfaces and protect membranes and biomolecules (Kusano *et al.,* 2008). PAs are considerd to be free-radical scavengers and activate the antioxidant enzyme machinery which help to reduce oxidative stress, membrane injury and electrolyte leakage (Kubi´ s *et al.,* 2014), acute application of PAs may cause endogenous PAs catabolism in the apoplast, which is responsible for ROS-induced oxidative damage (Di Tomaso *et al.,* 1989). Polyamines (PAs) are aliphatic polycation compounds ubiquitously found in living organisms. PAs are usually considered to be as a new class of plant growth regulators that because their fundamental roles in physiological processes, such as protein synthesis, cell-cycle regulation, ion-channel regulation, free-radical scavenging, gene expression (Takahashi and Kakehi, 2010). Under low temperature they have protective function in LT affected plants. The polycationic PAs can combine with cellular membrane and help to avoid intracellular ice formation. PAs enhance cellular antioxidant capacity, and decrease the peroxidation of unsaturated fatty acids during LT stress. PAs improve membrane stabilization and increase the fluidity (Alcázar *et al.,* 2010).

Materials and Methods

Two pot experiments were carried out in the greenhouse of the National Research Centre, Giza, Egypt during two successive growth seasons (2015/2016 and 2016/2017) to study the effect of foliar application of different concentrations of spermidine (25, 50 and 100 mg/L) on growth, fruit yield quantity and quality of two tomato cultivars (Strain B and Florida) grown under low temperature conditions. Seedlings (one true leaf stage) were transplanted carefully in 40 cm diameter pots (2seedlings /pot) at last week of November during two successive seasons. During this experiment, plants are subjected to low temperature where level of night temperature drops several times below 10◦C. Each pot filled with 13kg of a mixture of loam clay soil and sand soil at the ratio of 1:1 (w/w) mixed with 6.7g of super phosphate and 3.25g of ammonium nitrate. The plants were supplied with water according to their requirements which was governed by climatic conditions. Each pot received 3.25g ammonium nitrate weekly for a period of 4 weeks. The pots were arranged in complete randomized block design with three replicates for each treatment. The replicates were represented by five pots. The treatments were three levels of spermidine (Spd1:25mg/L, Spd2:50mg/L, Spd3:100mg/L) which applied exogenously on plants after 15 and 30 days from transplanting, while the control plants were sprayed with distilled water.

1. Growth Criteria Determination:

Random samples of plants were collected at 75 days after transplanting from each treatment to determine some growth parameters (plant height, leaves number / plant, fresh and dry weights of leaves and root/plant) as well as determination of antioxidant enzymes, total cholorophyll (SPAD values), methylglyoxal and malondialdehyde levels. At harvest, tomato fruits were collected weekly and total yield was calculated as g/plant. Fruit quality i.e. total soluble solids (TSS) and vitamin c of fruit juice were determined.

2. Biochemical Constituents Determination:

Extraction of the antioxidant enzyme ascorbate peroxidase (APX) was that of Mukherjee and Choudhuri (1983). The activity of APX (EC1.11.1.11) was determined according to Nakano and Asada (1981). One unit of APX was defined as the amount of enzyme that breaks down 1µ mol of ascorbate per min. The extraction and assay of phenylalanine ammonia lyase (PAL, EC 4.3.1.5) was estimated according to the method adopted by Beaudoin-Egan and Thrope (1985). The activity of PAL is defined as the amount of enzyme forming 1 mmol of transcinnamic acid from the substrate phenylalanine per min. Glyoxalase I (Gly I, EC: 4.4.1.5) activity was measured following Hasanuzzaman *et al.,* (2011). Chlorophyll was determined using chlorophyll meter (Model: TYS-A, Zhe Jang Top Instrument Co. LTD., Hangzhou, China). Methylglyoxal Level (Mg) was measured after the formation of the product N-α-acetyl-*S*-(1-hydroxy-2-oxo-prop-1-yl) cysteine following (Wild *et al.,* 2012). Lipid peroxidation was determined by measuring Malondialdehyde (MDA) content as described by Dhindsa *et al.,* (1982).

Ascorbic acid (AsA) was determined using phenol indophenols dye method (A.O.A.C., 1990) Total soluble solids (TSS) were determined using a portable refractometer (Brixstix BX 100 Hs; Technique Corporation, Livermore, CA).

Statistical Analysis:

The data obtained were subjected to standard analysis of variance procedure according to Snedecor and Cochran (1980). The values of L.S.D. were calculated whenever F values were significant at 5% level.

Results and Discussions

1. Effect on vegetative growth:

Data recorded on vegetative growth traits *i.e.* plant height, leaves number /plant, fresh and dry weights of leaves and root /plant as affected by spermidine. Florida cultivar was characterized by higher significant increases in these growth parameters than those of Strain B cultivar under all treatments (Table 1). Spd at 50mg/L caused the highest significant increases in the growth parameters of the two tomato cultivars followed by 100mg/L. Parallel to these results of spermidine treatments as one of the PAs. Abd El Wahed and Gamal El Din (2004) mentioned that spermidine, stimulated vegetative growth characters of chamomile plants. Also, putrescine enhanced the growth (fresh and dry weight) of *mentha pipeprita* (Youssef *et al.,* 2002). Putrescine significantly promoted vegetative growth characters in addition to significant increases in dry matter contents of geranium occurred in the two cuttings (Ayad *et al.,* 2010) and Jojoba plants (Taha *et al.,* 2015). Moreover, Abd El- Monem (2007) found that foliar application of various concentrations of putrescine at 30 DAS induced a marked significant reduction in shoot length when the samples were harvested at 75 days old plants as compared with similar treatments sprayed at 60 DAS. In addition, Orabi *et al.,* (2015) reported that spermine treatments significantly promoted plant height, fresh and dry mass (g/plant) of *Cymbopogon citratus* L., especially 100 mg/l spermine which mostly recorded the highest increments in total nitrogen, total phosphorous and total potassium, mainly in the second cut. Polyamines play an important role in flower initiation and development, fruit setting and ripening and they are essential when plants are exposed to stress (Pathak *et al.,* 2014).

Table 1: Effect of spermidine on some growth parameters of two tomato cultivars grown under low temperature conditions

Treatments	**Plant height(cm)**		**Number of leaves**		**Leaves fresh weight (g)**	
	Strain B **cv.**	**Florida cv.**	Strain B **cv.**	**Florida cv.**	Strain B **cv.**	**Florida cv.**
Control	43.00	52.69	16.33	20.00	44.57	60.21
SPd1 25mg/L	50.33	56.50	20.67	26.33	51.46	65.14
SPd2 50mg/L	55.67	61.00	25.33	31.67	59.11	74.22
SPd3 100mg/L	53.33	57.67	22.67	29.33	56.09	71.31
L.S.D 5%	4.58		2.70		7.01	

Table 1: Cont.

Treatments	**Leaves dry weight (g)**		**Root fresh weight (g)**		**Root dry weight (g)**	
	Strain B **cv.**	**Florida cv.**	Strain B **cv.**	**Florida cv.**	Strain B **cv.**	**Florida cv.**
Control	6.01	7.68	18.40	21.90	6.76	7.88
SPd1 25mg/L	7.12	8.63	20.16	25.67	7.96	9.99
SPd2 50mg/L	8.31	10.22	24.81	27.42	10.04	12.64
SPd3 100mg/L	7.88	9.88	24.09	26.00	9.93	11.97
L.S.D 5%	0.96		2.38		0.87	

Effect on biochemical traits:

Florida cultivar was characterized by higher activititis of APX, PAL and Gly I than Strain B cultivar either in treated or untreated plants (Table 2). All SPd treatments mostly led to significant increases in all studied antioxidant enzymes (APX, PAL & Gly I). Meanwhile, significant decrements were obtained in MG and MDA with most of the applied treatments.

However scientists working on polyamines found that polyamines mostly increased the activities of the antioxidant enzymes such as SOD, CAT, POX, APX, GR and PAL in plants under stress (Nasibi *et al.,* 2011; Shallan *et al.,* 2012; Orabi *et al.,* 2015,2016,2017[b],2020). It was also reported that application of polyamines could effectively scavenge O_2^- generated in both enzymatic and chemical cell-free systems and could inhibit the production of O_2^- by the superoxide-dependent conversion of l-amino-cyclo-propane-1-carboxylic acid and that led to ethylene (Bors *et al.,* 1989). Polyamines could act as free radical scavenger (Nayyar and Chender, 2004) leading to obvious reduction in MDA contents (Orabi and Abou Hussein 2019[a,b])

Increase of MG toxicity in LT affected plants was reversed by Spd treatment (Nahar *et al.,* 2015) working on mung bean, that because the enhanced activities of the glyoxalase enzymes and increased GSH level after Spd pretreatment leading to reduction of lipid peroxidation represented by MDA.
APXs enzymes involved in scavenging H_2O_2 in water-water and AsA-GSH cycles using AsA as the substrate, to catalyz the transfer of electrons from AsA to H_2O_2, to produc DHA and water (Pang and Wang 2010) . Phenylalanine ammonia-lyase (PAL) catalyzes the transformation by deamination of L-phenylalanine into trans-cinnamic acid, which is the prime intermediary in the biosynthesis of phenolics (Levine *et al.,* 1994).

Methylglyoxal (MG) is a highly cytotoxic compound that causes protein and DNA degradation. MG disrupts the antioxidant machinery and act as mediator for O•− 2 generation, causing an oxidative burst (Yadav *et al.,* 2005). Gly I, participates to catalyze the detoxification reaction of MG to D-lactate. Gly I converts MG to SLG utilizing GSH (Yadav *et al.,* 2005).

Under environmental stresses including LT stress reduction of chl biosynthesis and rapid degradation of chl are common occurrence (Mohanty *et al.,* 2006). Seedlings pretreated with Spd recovered the reduction of total chl (a + b) by increasing its content under LT stress (Nahar *el al.*, 2015). PAs prevent stress-induced loss of protein and enzymes, and protects chloroplasts and photosynthetic pigments (Graziano *et al.,* 2002) resulting in reduction of the lipid peroxidation.

Table 2: Effect of spermidine on Spad values, some antioxidant enzyme activities, methylglyoxal (MG) and malondialdehyde (MDA) levels of two tomato cultivars grown under low temperature conditions

Treatments	**Total chlorophyll (Spad units)**		**APX**		**PAL**	
			(µ mole /g fresh weight)			
	Strain B **cv.**	**Florida cv.**	Strain B **cv.**	**Florida cv.**	Strain B **cv.**	**Florida cv.**
Control	33.30	35.50	5.11	6.23	28.34	32.13
SPd1 25mg/L	35.20	36.90	5.78	7.33	32.11	34.50
SPd2 50mg/L	37.00	39.30	7.71	9.20	34.30	38.02
SPd3 100mg/L	36.00	38.30	6.88	8.44	33.18	35.49
L.S.D 5%	1.76		0.42		3.01	

Table 2: cont.

Treatments	**Gly I**		**MG**		**MDA**	
	(µ mole /g fresh weight)					
	Strain B **cv.**	**Florida cv.**	Strain B **cv.**	**Florida cv.**	Strain B **cv.**	**Florida cv.**
Control	3.11	5.20	70.31	53.24	10.68	9.10
SPd1 25mg/L	5.49	6.96	55.24	40.62	7.95	6.37
SPd2 50mg/L	7.18	8.41	42.61	31.29	6.88	4.90
SPd3 100mg/L	6.31	7.86	30.83	21.14	7.20	5.11
L.S.D 5%	0.59		2.29		0.69	

Effect on fruits yield and fruits quality:

Florida cultivar was characterized by almostly significant increases in fruits yield (g/plant) and fruits quality than Strain B cultivar under all treatments (Table 3). SPd treatment at 50mg/L caused the highest significant increase in the yield, TSS and AsA of both tomato cultivars followed by SPd treatment at 100mg/L

These increments in yield components due to spermidine treatments may be attributed to the increase in growth rate. In this regard, Davis (1995) reported that polyamines play a critical role in different biological processes, including cell division, growth, somatic embryogenesis, floral initiation, development of flowers and fruits.
Our obtained data of spermidine treatments agreed with those reported by El-Tohamy et al (2012) who found that the higher level of Putrescine significantly increased yield of Cape gooseberry. Putrescine up to 100mg L-1 significantly increased the chickpea yield criteria (number of branches, pods and seeds/ plant, seed and straw yield/ plant and/ fed and biological yield/ fed (Amin *et al.,* 2013). Orabi *et al.,* (2017[b],2020)recorded yield increments in yield traits by using putracine on orange trees and spermine on canola plants respectively.

Fruits of florida cultivar were characterized by significant increases in the TSS and AsA than fruits of Strain B cultivar (Table 3). TSS values associated with taste and had significant indication for improvement in yield quality as reported by Vural *et al.,* (2000). Alsokari (2011) stated that, spermine treatment increased total soluble sugars, polysaccharides and carbohydrates contents of Vigna sinensis. Additionally, Li *et al.,* (2014), under water stress found that white clover plants treated with spermidine exhibited more accumulated organic solutes including soluble sugar, reducing sugar, betaine and free proline. Under new reclaimed soil conditions putrescine treatments recorded higher levels of TSS in Valencia orange trees leaves (Orabi *et al.,* 2017[b]). Nahar *et al.* (2015) mentioned that exogenous Spd pretreatment reduced the oxidative stress by decreasing H2O2 content and lipid peroxidation where One of the primary indicators of LT stress was oxidative damage. Polyamine increased AsA content, decreased DHA levels, increased AsA/DHA ratio, increased GSH levels, decreased GSSG levels, and increased GSH/GSSG ratios in grapevine leaf tissues (Ikbal *et al.,* 2014). Ascorbate is a vital water-soluble antioxidant of plant cell reacting with a range of ROS including H_2O_2, O_2,´ and 1O2. AsA also scavenges OH, at diffusion-controlled rates (Smirnoff, 2005). By MDHAR and DHAR AsA is regenerated from DHA, electron donors here are NADPH and GSH, respectively (Gill and Tuteja, 2010; Orabi and El-Noemani 2015). Its correlation with oxidative stress protection of photosynthetic apparatus, enhancement of PSII quantum yield in grapevine plants that improved photosynthesis and plant growth (Ikbal *et al.,* 2014).

Table 3: Effect of spermidine on fruit yield, total soluble solids and ascorbic acid of two tomato cultivars grown under low temperature conditions

Treatments	Fruit yield g/plant		Total soluble solids%		AsA mg/100ml	
	Strain B cv.	Florida cv.	Strain B cv.	Florida cv.	Strain B cv.	Florida cv.
Control	391.80	430.50	4.18	4.49	31.05	37.41
SPd1 25mg/L	565.22	622.14	4.76	4.93	33.87	39.11
SPd2 50mg/L	722.00	835.24	5.11	5.88	41.56	47.77
SPd3 100mg/L	589.32	716.96	4.97	5.16	35.63	42.38
L.S.D 5%	64.30		0.34		6.53	

Conclusion:

It could be concluded that when tomato plants grown under low temperature and foliar treated with Spd after transplanting, could alleviate the harmful impacts of low temperature through the enhancement of their protective parameters, such as antioxidant enzymes activity and AsA to play vital roles in reducing Mg toxicity and lipid peroxidation. Reduction of ROS by SPd were manifested on the levels of biomembranes and biomolecules protection leading finally to reduction of oxidative damage in tomato plants under cold stress.

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