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Role of Ascorbic Acid in Alleviating Air Pollutants in Eggplant Seedlings

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The measuring values of growth parameters of *Solanum melongena* were highly affected by the different stresses of O_3. The plant height was highly significant affected (P<0.0001) by ascorbic acid (AA) (32.66±8.08 cm/plant) followed by the control treatment (29.00±5.19 cm/plant) and the lowest highest was found by the plants grown in the second power plant (SPP II) (12.00±2.00 cm/plant). Moreover, the growth parameters were positively highly affected P< 0.001) by treatment of AA. The introducing of AA with the plants grown in the second industrial city (SIC II) which has an O_3 with a concentration of 136.66 pbb, didn't significantly enhanced the growth parameters. The growing plants planted in SPP II (exposed to approximately 92 ppb) and enhanced by introducing of AA (SPP II+AA), exhibited the higher amounts of Chl a (27.45± 1.03mg g^{-1} FW), Chl b (10.43±1.05 mg g^{-1} FW), T Chl (47.35±3.41 mg g^{-1} FW) and Caro (9.46±1.35 mg g-1 FW). On the other hand, the Pro content was affected by SIC II (8.69±0.61 g/100 g^{-1} Fw), SIC II+AA (8.07±0.27 g/100 g^{-1} Fw) and SPP II (8.92±2.08 g/100 g^{-1} Fw). The different parameters of anatomical features or stomatal parameters and vessels diameters of *S. melongena* grown in the locations with high level of O_3 in comparisons with the control plants were significantly affected (P<0.0001).

Key words: Eggplant *(Solanum melongena L.)*, Ascorbic acid, Ozone, Anatomical, Biochemical, Physiological, air pollution.

Ozone is highly reactive, unstable and does not accumulate in plant tissue. Ozone enters the leaf primarily through the stomata where it reacts with cellular components. The organic radicals and various reactive forms of oxygen that are generated through O_3 decomposition damage proteins and membranes leading to impaired physiological function and cell death (US EPA, 2006). The current ozone ground level is found to be 45 ppb in unpolluted areas of the world (Narsto 2000; Vingarzan 2004) and has been hypothesized to increase globally by 1 to 2% per year (Olszyk *et al.* 2001). Also, the peak episodes, reaching 150 ppb in the most polluted sites and occasionally up to 250 ppb (Sandermann 1998; Vahala *et al.* 2003), are predicted to increase in both duration and frequency (Meehl 2007), in spite of control measures for ambient ozone containment. In many parts of the world, ozone concentrations were found to be higher in going from urban areas to peri-urban areas (Agrawal *et al*., 2003). De Temmerman *et al.* (2004) observed that there are some plants can be used as bio-indicators because they show characteristic responses when exposed to O_3 and initiated when the O_3 enters the leaf through stomata and reacts with the intercellular water forming reactive oxygen species (ROS).

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Ascorbic acid (AA) is a vitamin which used for protecting plants from ROS which associated with environmental stress and ozone exposure (Conklin and Barth, 2004; Burkey *et al*., 2006). It have been reported that the effects of O_3 on plants are varies duration of ozone exposure (Pasqualini *et al*. 2003), *i.e.*, the root production appears particularly susceptible to O_3 exposure (Cooley and Manning, 1987; Grantz *et al*., 2006). The decreasing in the yield and quality of Bahia grass (*Paspalum notatum*) and sericea lespedeza (*Lespedeza cuneata*) exposed to O_3 was of sufficient magnitude to have nutritional implications in their utilization by mammalian herbivores (Krupa *et al*. 2004). Many studies have been shown the effect of ozone exposure on the anatomical parameters (stomatal density, leaf thickness, intercellular spaces, substomatal chambers); *i.e.*, Pedroso and Alves (2008) showed that the sensitive cultivar of *Nicotiana tabacum* L. (Bel-W3) showed lower stomatal density on the abaxial leaf surface when compared to the tolerant cultivar (Bel-B) and slightly prominent stomata on both leaf surfaces when exposed to ozone and no stomatal conductance differences of the two tobacco cultivars when exposed to ozone (Pasqualini *et al*. 2002). However, observed that there was no significant difference between the stomatal density of the upper epidermis between the two clones *Populus deltoides x maximowiczii*, sensitive and *Populus xeuramericana*, tolerant Giacomo *et al*. (2010). The proportion of forbs increased from 23.4 to 36.2%, grasses decreased from 67.6 to 60.5%, and legumes decreased from 8.9 to 3.3% of biomass harvested from an extensively managed, semi-natural pasture that had been fumigated for five consecutive growing seasons with 1.5 × ambient O_3 concentrations (Lin *et al*. (2007). Previously, the *Arabidopsis thaliana* mutants and pairs of O_3-sensitive and tolerant plant lines suggest that many detrimental effects of O_3 are initiated and mediated by increased reactive oxygen species formation along with changes in plant hormone levels (US EPA, 2006). However, there were differences between control and O_3-enriched plots in forage concentrations of cell-wall constituents and in nutritive quality. In contrast, the negative effects of elevated O_3 on nutritive quality resulting from altered leaf chemistry in individual plant species were reported (Lin *et al*. 2007). In O_3-tolerant genotypes, either the oxidative burst is suppressed (Schraudner *et al*. 1998) or oxidative damage is highly localized (Koch *et al*. 2000), thereby restricting the extent of foliar lesions. Ozone impairs growth primarily by inhibiting photosynthesis and perhaps translocation processes, which limit availability of photosynthate needed for biomass production (Fiscus *et al*., 2005; Long and Naidu, 2002). In the present study, the anatomical studies and physic-chemical parameters of eggplant (*Solanum melongena* L.) grown under different levels of ozone stress with or without combination of ascorbic acid were reported.

MATERIALS AND METHODS

Growing conditions

Seeds of *S. melongena* were obtained from a local market in Riyadh, Saudi Arabia. Healthy seeds were surface sterilized with 1% sodium hypochlorite for 10 min, and then vigorously rinsed with sterilized double distilled water (DDW) before sowing. The seeds were sown in plastic pots on December 10, 2012 at a temperature of 9/20ºC, air humidity 66%, and natural light. Pots of (30 cm diameter and 30 cm height were filled with sterile sandy alluvial soil in a ratio of 1:1 and provided with a fungicide to prevent fungus and supplied with Raukura's nutrient solution (Smith *et al*., 1983). The pots were arranged in a simple randomized design in the different location with a single factor and four replicates. One week after sowing, seedlings were thinned so that each pot contained healthy plants of uniform size. When the plants were at the stage of two to three true leaves and then start treatments.

Experimental Treatments

The plants were divided to six groups; control (Cont.) group was left in King Saud University site without treatment, one group treated with ascorbic acid (AA), two groups were under ozone stress (O_3) one of them transferred to the second industrial city (SIC II), and other one to the second power plant (SPP II), and two groups under ozone stress which treated by AA concentration (300 mg/L) (O_3+AA) one of them transferred to the (SIC II), and other one to the (SPP II). Table 1 presents the measuring of the concentration of ozone gas in the study sites. The effects of ozone in combination with two or more

other environmental factors have been little explored. But, it has been shown in rice that the magnitude of the ozone and elevated carbon dioxide responses and interactions can be influenced by high temperature episodes, nutritional status and intra-plant competition (Reid and Fiscus, 2008).

Irrigate of plants were started at a rate of one time every 15 days until the end of the growing season The plants were sampled at 90 days after sowing to assess their growth parameters [plant height, stem fresh plant^{-1} (stem FW), dry weight plant^{-1} (stem DW), root fresh plant^{-1} (root FW), dry weight plant^{-1} (root DW), leaves fresh plant^{-1} (leaves FW), dry weight plant^{-1} (leaves DW)], leaf number, root length, and area leaf^{-1}, relative water content (RWC) and physio-biochemical attributes [chlorophyll (Chl) a and b, carotene, total chlorophyll, proline (pro)].

Plant growth parameters

Plant height (PH) was measured using a meter scale after removal from the pots. After recording fresh weight (FW) with balance, plants were placed in a 60°C oven for 48 h and then were weighed for dry weight (DW). Leaf area (LA) was measured using a LI-3000 Portable Leaf Area Meter (LI-COR, Lincoln, NE, USA). The relative water content (RWC) was expressed as percentage of the water content at a given time and tissue as related to the water content at full turgor (Slatyer, 1967). The relative water content was calculated using the following formula given by González and González-Vilar (2001): RWC (%) = [(FW - DW) / (TFW- DW)] x 100.

Physiological and biochemical parameters

The Chl was extracted from fresh leaves of experimental plants using the DMSO method based on Barnes *et al*. (1992). Chl absorption in the extract was measured using UV-VIS spectrophotometer. Contents of the Chls were calculated using the following formulas:

$$\text{Chl a} = 14.85\, A_{664.9} - 5.14\, A_{648.2}$$

$$\text{Chl b} = 25.48\, A_{648.2} - 7.36\, A_{664.9}$$

$$\text{Total chlorophyll} = 7.49\, A_{664.9} + 20.34\, A_{648.2}$$

Proline concentration was determined spectrophotometrically by adopting the ninhydrin method of Bates *et al*. (1973). Firstly 300 mg of fresh leaf samples were homogenized in sulphosalicylic acid. To the extract, 2 mL each of acid ninhydrin and glacial acetic acid were added. The samples were heated at 100°C and mixture was extracted with toluene and the free toluene was quantified spectrophotometrically at 528 nm using L-proline as a standard.

Anatomical characters

A leaf surface was cleaned with distilled water, and then silicon rubber imprinting was made for studying epidermal characters according to Lloyd (1908). For studying internal structures, leaf portions was divided into 5 mm pieces, then fixed in FAA (formalin-acetic acid-ethanol 10:5:85), dehydrated in a graded ethanol series and embedded in paraffin wax at 58°C. Sections (25µm thick) were stained with safranin and light green and mounted in Canada balsam. These sections were examined and photographed using Zeiss photomicroscope III. The following parameters were recorded; Stomata Upper epiderm Number (St. U. E. No), Stomata Upper epiderms Length (St. U. E.L), Stomata Lower epiderms No (St. L. E. No), Stomata Lower epiderms Length (St. L. E. L.), Stomata Lower epiderms Width (St. L. E.W.), Palisade cells Length (P. C. L.), Palisade cells Width (P. C. W.), Upper Epidermis Cell Length (U. E. C. L.), Upper Epidermis Cell Width (U. E. C. W.), Lower Epidermis Cell Length (L. E. C. L.), Lower Epidermis Cell Width (L. E. C. X.) Xylem Vessels Number (X. V. No.) and Xylem Vessels Diameter (X.V. D.).

Measurements and photographs

Measurement of concentrations of ozone gas was a day for three months for each of the study sites using a device (AEROQUAL Series Monitor with multihi). Epidermal cells dimensions, stomatal number, stomatal dimensions, palisade cells dimensions, and xylem vessels were taken at Magnification x 40 using leitz light microscope with motic 2000 cam. All measurements and description were taken in the last vegetative growth before flowering.

Statistical analysis

Most ozone studies, however, have been single factor or two-way interaction experiments. However, plant responses to ozone are highly influenced by site conditions, and comprehensive assessment of their relative influences needs attention (Reid and Fiscus, 2008). Each pot was treated as one replicate and all the treatments were repeated five times. All data from morpho-anatomical investigations were expressed as means ± SD and the means were subjected to a one-way

analysis of variance (ANOVA). When the ANOVA indicated a significant difference among treatments, a comparison of the means was done employing Duncan's multiple-range test at a 0.05 level of probability. The data were statistically analyzed using SAS version 8.2 (SAS, 2001) in a completely randomized design (CRD) to test the differences among treatment levels.

RESULTS AND DISCUSSION

Growth responses

The measuring values of growth parameters of *S. melongena* were highly affected by the different stresses of O_3 (Table 2). The plant height was highly significant affected (P<0.0001) by AA (32.66±8.08 cm/plant) followed by the control treatment (29.00±5.19 cm/plant) and the lowest highest was found by SPP II (12.00±2.00 cm/plant). Moreover, the growth parameters (LN, LA, RL, RFW, RDW, SFW, LFW, LDW and RWC were positively highly affected P< 0.001) by treatment of AA. On the other hand, the SDW was highly enhanced by the combination of SPPII+AA stress (2.37±0.73g plant^{-1}). Also, some of the growth parameters were enhanced by the combination AA with SPPII. For example, the plants showed RL in a value of 7.69±0.68 cm plant^{-1} but by introducing the AA with SPPII, the value increased to 120.25±5.19 cm plant^{-1}. On the other hand, the introducing of AA with SIC II which have an O_3 with a concentration of 136.66 pbb, didn't significantly enhanced the growth parameters, for example, the RL showed a value of 25.17±5.22 cm plant^{-1} under the O_3 stress (SICII) but by introducing the AA with SPPII, the value increased to 29.61±6.36 cm plant^{-1}. At low O_3, Pääkkönen *et al*. (1995) reported that the relative area of leaf epidermis significantly decreased of *Betula pendula*. Similarly, decreased yield and quality of O_3-exposed *Paspalum notatum* and *Lespedeza cuneata* (Muntifering *et al*. 2000; Powell *et al*. 1999) were of sufficient magnitude to have nutritional implications in their utilization by mammalian herbivores (Krupa *et al*. 2004). It has been reported that as a highly reactive oxidizing agent, the superoxide production by ozone induces cell death lesions in plant leaves when it enters the symplast (Overmeyer and Kangasjarvi, 2003). On the other hand, the initial acceleration in flowering in blackberry was found with elevated O_3 and more flowers were initiated (Chappelka, 2002). Most of the growth parameters were enhanced be the combination of AA with SPPII. On the hand, significantly, the plants grows under the O_3 stresses were showed the lowest growth parameters.

Several studies (Kobayashi *et al*., 1995) demonstrate that elevated ozone levels reduced growth and yields of rice (*Oryza sativa* L.). Wahid *et al*. (1995) have reported significant reductions in grain yields of wheat cultivars at 6 h daily mean ozone concentrations of 25-45 ppb in Punjab, Pakistan. Some plants can be used as O_3 bioindicators because they show characteristic responses when exposed to this pollutant (De Temmerman *et al*. 2004; Klumpp *et al*. 2001).

Leaf chlorophylls, carotene and proline concentrations

Leaf Chl a,b, TChl, carotene and proline concentrations were highly affected significantly (P<0.001) by the different treatments (Table 3). The

Table 1. Average monthly reading of ozone gas in the study sites

Study sites	Month	Ozone gas conc. (ppb)	Average Ozone conc. (ppb)
Control	January	42	44.33
	February	44	
	March	47	
the second industrial city (SIC II)	January	132	136.66
	February	137	
	March	141	
The second power plant (SPP II)	January	85	92
	February	98	
	March	93	
Limit air pollution to global gas ozone (ppb)	30-25		

growing plants planted in SPP II (exposed to approximately 92 ppb) and enhanced by introducing of AA (SPP II+AA), exhibited the higher amounts of Chl a (27.45± 1.03mg g^{-1} FW), Chlb (10.43±1.05 mg g^{-1} FW), TChl (47.35±3.41 mg g^{-1} FW) and Caro (9.46±1.35 mg g-1 FW). On the other

Table 2. Statistical analysis of the effects of different treatments of O_3 on *S. melongena* growth responses

Growth responses [a]	Treatments						
	Cont.	AA	SICII	SICII+AA	SPPII	SPPII+AA	P value [b]
PH (cm $plant^{-1}$)	29.00± 5.19ab	32.66± 8.08a	17.00± 5.56cd	22.66± 1.52bc	12.00± 2.00d	25.33± 1.52abc	0.0016***
LN	20.66± 4.16a	21.33± 2.30a	13.00± 2.81abc	8.33± 1.85bc	5.33± 0.57c	15.66± 1.15ab	0.0043***
LA (cm^2)	120.79± 16.51a	123.45± 14.64a	95.38± 3.45b	91.71± 1.42b	45.89± 2.90c	120.25± 5.19a	<0.0001***
RL (cm $plant^{-1}$)	29.36± 4.33a	31.36± 4.02a	25.17± 5.22a	29.61± 6.36a	7.69± 0.68b	25.35± 3.37a	0.0003***
RFW (g $plant^{-1}$)	5.30± 1.42ab	6.97± 2.12a	5.54± 2.90ab	3.19± 0.83bc	0.42± 0.13c	2.61± 0.08bc	0.0038***
RDW (g $plant^{-1}$)	0.41± 0.02b	0.61± 0.09a	0.23± 0.03c	0.14± 0.03c	0.14± 0.03c	0.51± 0.07a	<0.0001***
SFW (g $plant^{-1}$)	11.29± 2.78ab	14.29± 3.24a	8.50± 1.52bc	8.14± 0.61bc	0.54± 0.05d	6.59± 0.27c	<0.0001***
SDW (g $plant^{-1}$)	0.58± 0.04b	0.71± 0.18b	0.50± 0.10b	0.29± 0.05b	0.42± 0.01b	2.37± 0.73a	<0.0001***
LFW (g $plant^{-1}$)	3.46± 0.35ab	3.69± 0.20a	3.20± 0.21b	2.78± 0.17c	1.01± 0.03e	1.78± 0.20d	<0.0001***
LDW (g $plant^{-1}$)	0.39± 0.51ab	0.59± 0.33a	0.10± 0.02ab	0.11± 0.02ab	0.08± 0.01b	0.23± 0.07ab	0.1660[ns]
RWC (%)	309.43± 29.35ab	311.76± 28.85a	276.08± 21.45bc	242.91± 4.07c	86.34± 2.08e	132.15± 3.77d	<0.0001***

a: Mean (± SD) for growth responses. Dissimilar letter designations indicate significant differences at $P < 0.05$ (Duncan's new multiple range test). b; Results of the ANOVA: NS = not significant at $P < 0.05$; *, *** = significant at P <0.05, 0.001, respectively.

Table 3. Statistical analysis of different applications of treatments of O_3 on *S. melongena* chlorophyll a, b and Total), Carotenoid and Proline contents

Biochemical parameters	Treatments						
	Cont.	AA	SIC II	SIC II+AA	SPP II	SPP II+AA	P value
Chla (mg g^{-1} FW)	13.79± 1.90d	16.45± 0.40bc	7.82± 1.58e	17.83± 1.25b	14.19± 0.89cd	27.45± 1.03a	<0.0001***
Chlb (mg g^{-1} FW)	6.62± 2.75bc	8.29± 0.69ab	2.25± 0.40d	6.28± 0.92bc	3.90± 1.82cd	10.43± 1.05a	0.0003***
Caro (mg g^{-1} FW)	6.90± 1.49cb	8.57± 0.98ab	2.03± 0.46d	5.83± 0.68c	2.70± 1.04d	9.46± 1.35a	<0.0001***
T Chl (mg g^{-1} FW)	27.32± 6.13cb	29.32± 5.48b	12.11± 2.44d	29.95± 2.68b	20.79± 2.05c	47.35± 3.41a	<0.0001***
Pro (g/100 g^{-1} Fw)	3.85± 0.51b	5.51± 0.75b	8.69± 0.61a	8.07± 0.27a	8.92± 2.08a	4.07± 1.27b	<0.0001***

a: Mean (± SD) for biochemical parameters. Dissimilar letter designations indicate significant differences at $P < 0.05$ (Duncan's new multiple range test). b Results of the ANOVA: NS = not significant at $P < 0.05$; *, *** = significant at P <0.05, 0.001, respectively.

hand, the Pro content was affected by SIC II (8.69±0.61 g/100 g Fw), SIC II+AA (8.07±0.27 g/100 g Fw) and SPP II (8.92±2.08 g/100 g Fw).

Smith *et al*. (2000) reported that *S. melongena* did not show any significant biomass reduction in response to UV-B radiation and no significant alteration in chlorophyll levels by the end of the 14 d experimental period of Ultraviolet-B. Plants with a thick waxy cuticle (*S. melongena*) were UV-B tolerant. Thus, it seems unlikely that the presence of epicuticular wax is in itself a protective feature. Moreover, in both *Quercus* species, exposure to a high O_3 decreased photosynthesis and stomatal conductance. Exposure to an intermediate O_3 had a negligible effect on the measured parameters (Manes *et al*. 1998). Decreases in photosynthesis and stomatal conductance of leaves exposed to high O_3 were transient, with full recovery observed in *Q. ilex* leaves 72 h after exposure and in *Q. pubescens* 288 h after exposure. This suggests that the decrease in photosynthesis during fumigation with a high O_3 in these oak species did not involve damage to biochemical processes, but was a result of alterations in processes, such as increasing resistance to CO_2 diffusion, caused by the transient decrease in stomatal conductance. Ozone may temporarily impair K+ channels involved in stomatal opening (Torsethaugen *et al*. 1999), resulting in down-regulation of photosynthesis rather than permanent inhibition.

Anatomical parameters

The different parameters of anatomical features or stomatal parameters and vessels

Table 4. The anatomical characters of leaf surface of *S. melongena* as affected by different O_3 exposure treatments

Anatomical feature (µ) [a]	Treatments						P value [b]
	Cont.	AA	SIC II	SIC II+AA	SPP II	SPP II+AA	
St. U.E. No.	1.24± 0.45e	2.80± 0.12b	2.48± 0.17bc	2.04± 0.08cd	1.76± 0.16de	3.80± 1.03a	<0.0001
St. U.E.L.	18.74± 0.60d	21.34± 0.82c	23.09± 1.59c	28.66± 0.31b	33.85± 2.74a	30.28± 0.46b	<0.0001
St. U.E.W.	11.63± 1.70d	14.63± 0.24bc	14.91± 0.87bc	19.92± 0.88a	15.38± 0.87b	13.72± 1.64c	<0.0001
St. L.E. No.	4.88± 0.65c	7.68± 0.52a	2.28± 0.10e	4.40± 0.31c	3.68± 0.46d	5.44± 0.16b	<0.0001
St. L.E.L.	17.61± 1.22 d	21.2± 1.13 c	26.04± 1.05 b	36.66± 0.76a	26.13± 1.29 b	26.49± 1.98 b	<0.0001
St. L.E.W.	12.84± 1.23d	14.84± 1.009c	18.44± 1.04b	20.70± 1.24a	14.92± 1.55c	17.09± 0.72b	<0.0001
P. C. L.	26.95± 0.85e	31.15± 1.50d	51.35± 5.22c	65.90± 4.10b	65.30± 1.75b	87.97± 2.02a	<0.0001
P. C. W.	10.98± 1.24d	14.15± 1.11c	15.83± 2.86c	27.77± 0.93a	15.51± 1.23c	21.72± 1.55b	<0.0001
U. E. C. L.	26.31± 1.66f	29.91± 1.32e	35.08± 3.81d	43.73± 3.02b	64.52± 0.91a	40.39± 0.31c	<0.0001
U. E. C. W.	19.84± 1.89c	22.44± 2.15c	21.87± 1.83c	28.81± 0.89b	37.46± 2.33a	36.16± 2.37a	<0.0001
L. E. C. L.	26.59± 1.26e	31.99± 3.14d	38.17± 4.93c	41.15± 2.85c	45.72± 3.75b	59.21± 2.24a	<0.0001
L. E. C. W.	22.40± 5.53b	22.00± 2.38b	22.86± 3.48b	23.51± 0.2b	34.73± 2.02a	37.58± 1.22a	<0.0001
X. V. No.	30.68± 1.07e	35.88± 1.00d	51.48± 1.02c	75.44± 1.75b	31.68± 1.90e	111.16± 4.08a	<0.0001
X. V. D.	5.93± 2.36d	10.53± 0.74c	9.35± 2.08c	20.50± 0.29a	10.00± 1.44c	16.92± 1.26b	<0.0001

a: Mean (± SD) for Anatomical feature (µ). Dissimilar letter designations indicate significant differences at $P < 0.05$ (Duncan's new multiple range test). b Results of the ANOVA: *** = significant at $P < 0.001$.

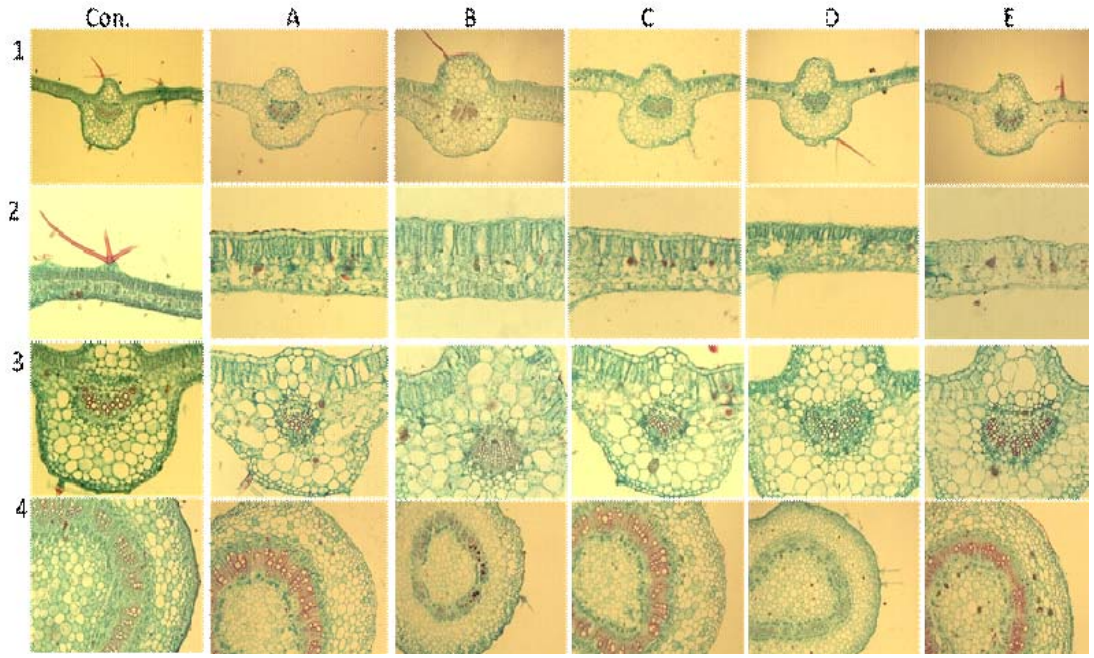

Fig. 1. the anatomical changes of S. melongena as affected by ozone with or without combination of ascorbic acid. Cross-sections in leaf and stem of (Con.): Control. (A): ascorbic acid (AA), (B): Ozone stress (SIC II). (C): Ozone stress and ascorbic acid (SIC II+ AA). (D): Ozone stress (SPP II). (E): Ozone stress and ascorbic acid (SPP II+ AA). Cross-sections in (1) leaf 4X. (2) Intercostals Region 10X. (3) Midrib Region 10X. (4) Stem 4X

diameters of *S. melongena* grown in the locations with high level of O_3 in comparisons with the control plants were significantly affected ($P<0.0001$) (Table 4). Figure 1 is showing the anatomical changes of *S. melongena* as affected by ozone with or without combination of ascorbic acid. The Stomata U.E. No and Stomata U.E.L were highly affected by the treatment of SPP II+AA (3.80±1.03µ) and SPP II (20.44±1.71µ), respectively. Evans *et al*. (1996) reported that the high stomatal densities and high percentage of intercellular space among palisade mesophyll cells are associated with ozone sensitivity was observed. It is well established that, usually before other cell organelles the chloroplast structure is altered by ozone (Holopainen *et al*., 1996). The *S. melongena* treated with SIC II+AA showed a high Stomata U.E.W (19.92±0.88µ), Stomata L.E.L (36.66± 0.76µ), Stomata L.E.W (20.70±1.24µ), Palisade cells W. (27.77±0.93µ) and X.V.D. (20.50±0.29µ). The highest values of E.C.U. E.L, E.C.U.E.W and E.C.L.E.W were observed by 64.52±0.91µ, 37.46±2.33µ and 34.73±2.02µ as affected by the stress O_3 (SPP II). The percentage of intercellular space, especially in the palisade layer, has been shown to be related to the sensitivity of O_3 (Evans and Ting, 1974). The total leaf thickness and the palisade mesophyll thickness were smaller and the percentage of spongy mesophyll layer was higher for O_3 sensitive trees (*Fraxinus pennsylvanica* Marsh. and *Prunus serofina* Ehrh.) compared to tolerant individuals (Bennett *et al*. 1992; Al Sahli *et al*., 2013).

Tiwari and Agrawal (2011) reported that O_3 was the most significant pollutant affecting the brinjal (Solanum melongena L. var. Pusa hybrid-6) performance. Photosynthetic rate and stomatal conductance declined the test plants. Lipid peroxidation was moderately increased. The constitutive levels of the antioxidants as well as their increments upon O_3 exposure were of higher magnitude. The data indicate that O_3 triggered the protective mechanisms in plants which resulted in increments in enzymatic and non-enzymatic antioxidants of O_3-exposed plants. These data

support the hypothesis that ozone sensitivity is associated with leaf parameters that relate to the ability of ozone to diffuse into leaves (high stomatal densities) and the ability of ozone to diffuse among the target cells (high percentage of intercellular spaces among palisade parenchyma cells).

On the studied species, the increase of stomatal density can be related to a higher efficiency on the stomata opening closure mechanism. Relations between O_3 sensitivity, and different parameters of stomata were discussed by some authors. The increase of stomatal density in *Eugenia uniflora* exposed to the same local of our study, in comparison to control plants kept in a place little affected by air pollutants (Pedroso and Alves *et al*. 2008). The physiological and structural parameters in symptomatic leaves of *Fraxinus ornus*, observed a stomatal conductance reduction due to the changes in stomata or in other epidermal cells (Paoletti *et al*. 2009). Furthermore, the most sensitive cultivar of *Nicotiana tabacum* 'Bel-W3' with the most tolerant (K63) did not observe significant differences in stomatal density (Saitanis and Karandinos 2002). While Pedroso and Alves (2008), also comparing tobacco cultivars, 'Bel-W3' (O_3 sensitive) and 'Bel-B' (O_3 tolerant), registered a higher stomatal density in the tolerant cultivar. O_3 pollutant enters the leaf surface firstly via the open stomata and rapidly dissociates in apoplast, resulting in an excess of reactive oxygen species (Schraudner *et al.* 1998) that may result in a biphasic oxidative burst. The second oxidative peak is absent or reduced in ozone-resistant plants and can be considered a biochemical marker for ozone plant sensitivity (Wohlgemuth *et al.* 2002). The density of stomata, the stomatal index in *Solanum* and the size of stomatal pore and epidermal cells all decreased on long-term exposure to coal-smoke pollutants, while the frequencies of epidermal cells and trichomes increase. Trichome length decreases on the upper surface, but increases on the lower surface of leaves (Gupta and Ghouse, 1986; Al Sahli *et al*., 2013).

CONCLUSIONS

The anatomical and physic-chemical parameters of *S. melongena* L. grown under different levels of ozone stress (two different polluted locations) with or without combination of ascorbic acid were investigated. The results indicate that the AA treatment as well as the combination of the second power plant (SPP II) +AA had the great effect on the growth responses of *S. melongena* L. Moreover, the growth parameters (LN, LA, RL, RFW, RDW, SFW, LFW, LDW and RWC were positively highly affected $P< 0.001$) by treatment of AA. The introducing of AA with SIC II which has an O_3 with a concentration of 136.66 pbb, didn't significantly enhanced the growth parameters. On the other hand, significantly, the plants grown under the O_3 stresses were showed the lowest growth parameters.

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