POLISH SOCIETY FOR HORTICULTURAL SCIENCE

Folia Hort. 29/2 (2017): 231-240

DOI: 10.1515/fhort-2017-0021

FOLIA HORTICULTURAE

Published by the Polish Society for Horticultural Science since 1989

ORIGINAL ARTICLE | Open access | http://www.foliahort.ogr.ur.krakow.pl

Morphological and physiological responses of grapevine (*Vitis vinifera* L.) to drought stress and dust pollution

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ABSTRACT

Dust pollution can negatively affect plant productivity in hot, dry areas with high insolation during summer. To understand the effect of water-deficit and its interaction with dust pollution on vegetative and physiological changes in grapevine 'Bidaneh Sefid', two-year-old plants were subjected to drought stress (-0.1 and -1 MPa) and dust treatment in a greenhouse during 2013 and 2014. The results showed that dust had a significant negative effect on the number of leaves, shoot length, root and shoot dry weights, and total dry weight under both drought and well-irrigated conditions. Dust, when applied in combination with drought, caused severe growth reduction. Leaf relative water content (RWC) and membrane stability index (MSI) were reduced under dust and drought stress, while soluble carbohydrate, proline, malondialdehyde (MDA) and H_2O_2 concentrations increased. Furthermore, dust application resulted in characteristics similar to those induced by water-deficit stress and intensified vegetative and physiological changes when applied together. Dust and drought treatments increased peroxidases and ascorbate peroxidase activities when compared to the control. The results indicate that dust has an adverse effect on the growth and physiology of grapevine and plays a negative role in the response of grapevine to drought stress.

Key words: antioxidant enzymes, drought, dust, growth, lipid peroxidation

INTRODUCTION

Grapevines are extensively grown in semi-arid areas in the world and have been adapted to a wide range of weather conditions. Climate change predictions suggest that drought in the next 50 years will become an even greater problem in the world and is one of the major limitations for viticultural production worldwide (Chaves et al. 2007). In grapevines, many studies have been reported on gas exchange (Poni et al. 2014), water-use efficiency (Ghaderi et al. 2011), biochemical changes (Beis and Patakas 2015), biomass distribution (Xiao et al. 2006), in addition to yield and fruit quality (Romero et al. 2015) in response to different degrees of drought stress.

Global air pollution is a serious problem, which can be defined as a change in the atmospheric conditions affecting the biochemical activities and physiological and morphological characteristics of plants (Tripathi and Gautam 2007). Dust can cause climate change on a global scale and local changes in the biological cycle (Engelstaedter et al. 2006). Dust particles in the air can either arise from wind-blown dust or be transferred naturally from deserts. Dust-producing areas are mainly located in the arid

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region of the Middle East with annual rainfall of less than 200-250 mm per year, which encompasses a region extending from northeast Africa to the central and southern parts of Asia. According to the literature, the Middle East is a leading area for generating dust storms. Currently, the existence of dust particles hovering in the air has become a major problem that has its origins in the desert regions in the south and southwest of Iran (in Iraq, Syria and Saudi Arabia). Wang et al. (2006) have explained that dust-related problems have gradually been increasing in arid and semi-arid areas due to the lower than average rainfall in those areas. Many researchers have reported that dust has a direct effect on plants through the scattering and absorption of sunlight as well as prevention of heat emission (Haywood et al. 2001).

There are few reports regarding the effect of dust and air pollution on plants. Before sustaining visible damage to the leaves, plants experience physiological and biochemical changes when exposed to airborne pollutants (Liu and Ding 2008). Reductions in chlorophyll, carotenoids and ascorbic acid contents have been reported in the leaves of *Ficus religiosa*, *Mangifera indica*, *Polyalthia longifolia*, *Delonix regia* under dust pollution (Chauhan 2010). Various studies have shown that pollution with cement dust and urban pollution have adverse effects on photosynthetic pigments, photosynthetic rate, quantum yield and photosystem integrity in plants (Rai 2016). In addition, Kumar and Thambavani (2012), and Prajapati and Tripathi (2008) found that dust deposition brings about a decreased transpiration rate and lower stomatal conductance along with a higher leaf temperature. Dust pollution causes a progressive reduction in the photosynthetic ability of leaves and in the growth and productivity of plants, leading to a probable change in morphological characteristics, photosynthetic pigment concentration and/or relationships, as well as in the antioxidant mechanism of leaves (Younis et al. 2013, Gupta et al. 2015). Closure of leaf stomata and a reduction in stomatal conductance (Siqueira-Silva et al. 2016), an increase in leaf temperature and collapse of the leaf cuticle layer have been reported as the negative impacts of dust pollution (Naidoo and Chirkoot 2004). Rai (2016), on the other hand, reported leaf shading, blocking of stomata, transpiration rate reduction and increase in leaf temperature as the problems with dust deposition in plants leading to a direct decline in the photosynthetic rate.

Due to the current permanent rise in global air temperature, together with a reduction in precipitation and higher evapotranspiration in the last decade, the negative effects of dust pollution have dramatically increased. These problems could easily affect the quality and quantity of grape plants. Therefore, the aim of the current experiment was to assess the effects of drought stress and its interaction with dust treatments on the physiological and morphological characteristics of grapes. It is worth mentioning that the interaction effects of dust and drought on the morpho-physiological parameters of grapevine have not been studied yet.

MATERIAL AND METHODS

Experimental design

The experiment was carried out during the 2013 and 2014 growing seasons in the greenhouse of the University of Kurdistan located in Western Iran (35°8' N; 46°51' E). Two-year-old, own-rooted plants of *Vitis vinifera* L. 'Bidaneh Sefid' were planted in 15 L pots containing a mixture of soil, sand and manure (1.5:1:1 v/v/v). After bud break, all plants were pruned to a double shoot. For two months before starting the experiment the plants had periodically been watered and soil moisture was maintained at field capacity. Thereafter, two irrigation treatments were applied: (i) fully-irrigated (control), in which the root system of each plant was irrigated to soil capacity until the soil water potential had reached -0.1 MPa; (ii) deficit irrigation, in which the root system of each plant was irrigated until the soil water potential had reached -1 MPa. Soil moisture was determined with a gypsum block. Dust treatment was also applied to grapevine plants during the experimental period once a month. Initially, for dust application, the grapevine canopy was sprayed with water using a sprayer and immediately soil dust (soil) was applied as uniformly as possible using a manually operated duster. Deposits left by a dust storm had been collected and applied to grape leaves. The collected dust contained arsenic, lead, cadmium, nickel, chromium, silver, copper, zinc, manganese and iron. The particles were 10 μm in diameter and included quartz, calcite and dolomite. The control grapevines were sprayed with water during each application of dust. To calculate the amount of dust deposition on leaves after the application of dust, 10 random leaves were separated and the average dust deposition on the leaves was determined as grams per cm^2 of leaf surface area. The leaf surface was

washed with distilled water; the water collected from washing the leaf surface was then evaporated. The residue was weighed and expressed as dust deposition per 1 cm^2 of leaf surface area. Overall, the treatments included the control (-0.1 MPa), dust (0.0011 g cm^{-2}), drought stress (-1 MPa), and dust + drought stress. The experiment's duration was three months during 2013 and 2014, from 15 May to 15 August. Eighteen plants were selected for each separate treatment in the first year and 12 plants were kept for second-year evaluations. During autumn and winter, the remaining plants were kept in an unheated greenhouse so that plant growth was restricted by low temperature. Experiments were repeated on the remaining plants in the second year, like in the first year. The experiment was based on a completely randomized design with three replications.

Trait measurements

Three months after the onset of the experiment, 6 plants were randomly collected from each treatment to measure the dry weight of leaves, shoots and roots, the total and single leaf surface area, and also the number of leaves per plant. The roots, shoots and leaves were placed in an oven at 70°C for 72 hours, then their dry weights were measured. To measure leaf characteristics, initially, dust residue was carefully removed with a brush from both sides and weighed. Leaf surface was measured using a leaf area meter.

Relative water content (RWC) was determined according to the Galmés et al. (2007) method based on the following equation: RWC (%) = [(FW − DW) / (TW − DW)] × 100, where FW, TW and DW represent leaf fresh, turgid and dry weight, respectively. To determine the leaf membrane stability index (MSI), the collected leaves were cut into small pieces (0.1 g) and placed in 10 mL of double-distilled water at 40°C for 30 min. After incubation, the conductivity of the water containing the leaf pieces (C1) was determined using a conductivity meter (RC-16C Model, Alpha Metals, USA). Then, test tubes containing samples in the second set were heated at 100°C for 10 min. and their conductivity (C2) was read again. The MSI was calculated using the following formula (Sairam 1994): MSI% = [1- (C1/C2)] × 100.

Fully expanded leaves were collected at midday and then washed with deionized water; the adhering water was removed with a paper towel. The leaf samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Soluble carbohydrate content was determined by the phenol-sulphuric acid method (Khochert 1987). 0.5 g of leaf tissue was homogenized with 5 ml of 95% ethanol. Then, 100 µl of ice-cold alcoholic extract was mixed with 3 ml of anthrone solution (150 mg anthrone dissolved in 100 ml of 72% sulphuric acid, w/w). The samples were then incubated in a boiling water bath for 10 minutes. The optical density was measured at 625 nm using a spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA). Subsequently, the concentrations of soluble carbohydrates were determined using a glucose standard and expressed as mg g^{-1} fresh weight.

The amount of proline was estimated according to the Bates et al. (1973) method. 0.5 g of fresh leaf tissue was homogenized with 10 ml of 3% sulfosalicilic acid, and then the mixture was centrifuged at 6,000 × *g* for 5 minutes. Then, 2 milliliters of the supernatant were mixed with 2 ml of ninhydrin and 2 mL of glycolic acetic acid. The samples were incubated in a boiling water bath for one hour and placed in an ice bath for a few minutes immediately after being removed from the bath. Then, 4 ml of toluene was added to each sample. The optical density of the supernatant phase was measured at 520 nm using a spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA).

To determine the level of hydrogen peroxide, 0.3 g of leaf tissue was homogenized with 5 mL 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 × *g* for 5 minutes. Then, 0.25 mL of the supernatant was mixed with 0.25 mL of 100 mM K-phosphate buffer and 0.5 mL of 1M KI. The absorbance was measured at 390 nm with a spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA). Hydrogen peroxide level was calculated using a standard curve prepared with known H_2O_2 concentrations (Alexieva et al. 2001).

Lipid peroxidation was measured by determining the malondialdehyde (MDA) content in the leaves according to the method of Dhindsa et al. (1981). 5 mL of trichloroacetic acid (0.1% TCA) was added to 0.3 g of leaf tissues and homogenized completely. The homogenated samples were centrifuged at 10,000 × *g* for 5 min. at 4°C. The supernatant (0.3 ml) was mixed with 1.2 ml of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA, and incubated at 95°C for 30 min. The reaction was stopped by putting the sample in an ice bath for 5 min.; samples were centrifuged at 10,000 × *g* for 10 min. at 25°C. The absorbance of the supernatant was read at 532 nm using a Beckman

UV-DU 520 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA). After deducting the non-specific absorbance at 600 nm, the extinction coefficient of 155 mM^{-1} cm^{-1} was used to calculate the MDA concentration.

Peroxidase activity (POD) was determined according to the method of Hemeda and Klein (1990). The 1 mL reaction mixture contained, 90 µL of 0.3% hydrogen peroxide, 780 µL of 50 mM potassium phosphate buffer (pH 6.6) and 40 µL of enzyme extract. POD activity was calculated on the basis of the increase in absorbance at 470 nm due to guaiacol oxidation ($\varepsilon = 26.6$ mM^{-1} cm^{-1}). The Nakano and Asada (1981) method was used for the ascorbate peroxidase activity (APX) assay. Suitable aliquots of the enzyme extract were added to the reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, and 0.1 mM H_2O_2 in a total volume of 1.0 mL. The reaction was initiated by adding the enzyme extract. The decline in absorbance at 290 nm was recorded every 30 s for 3.0 min.

Analysis of variance (ANOVA) and mean comparisons were performed using SAS software (SAS Institute Inc. 1990) according to a factorial experiment based on a completely randomized design (CRD). The least significant difference (LSD) test was applied for comparison at a significance level of 0.05. Charts and curve fittings were performed with Office Microsoft Excel 2016 software.

RESULTS

Drought stress, dust, and dust + drought stress reduced total leaf surface area per plant, the number of leaves and single leaf surface area (Tab. 1) in both years. Excessive reductions in these traits were observed in plants that were treated with dust + drought in the second year. Shoot length decreased in the drought stress and dust + drought stress treatments in both years. However, shoot length in dust-treated plants exhibited a steeper decline in the second year compared to the control (Tab. 1). Shoot length in plants that were treated with dust remained similar to that of the control in the first year, whereas a higher reduction in shoot length was observed in the dust + drought stress treatments compared to the other treatments in the second year (Tab. 1).

Reductions of 25 and 17% in total dry weight were observed in plants that were treated with dust in the first and second year respectively. More diminished plant dry matter was obtained in plants subjected to drought stress and dust compared to the control plants (Tab. 2). Based on the results of

Table 1. Effect of drought stress and dust on the number of leaves, leaf area, single leaf area, shoot length and root volume during two growing seasons

Treatment		Number of leaves		Leaf area (dm^2 per plant)		Single leaf area (cm^2 per leaf)		Shoot length (cm)		Root volume (cm^3)	
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Control	Without dust	31.3 a	79.0 a	28.7 a	51.4 a	92.3 a	65.1 a	189.3 a	112.0 a	210.0 a	181.7 a
	Dust	29.3 a	57.0 b	17.6 b	32.3 b	60.0 b	55.9 b	186.0 a	92.7 b	170.0 b	151.7 b
Drought	Without dust	8.0 b	36.0 c	2.5 c	7.7 c	36.3 c	21.5 c	86.3 b	63.3 c	56.7 c	40.0 c
	Dust	4.7 c	19.3 d	0.9 c	3.1 d	24.2 c	16.1 d	79.0 b	43.7 d	56.7 c	30.0 c

Means with the same letter in each column are not significantly different at $p < 0.05$ (LSD test). Control (-0.1 MPa), Dust (0.011 g cm^{-2}), Drought (-1 MPa)

Table 2. Effect of drought stress and dust on shoot dry weight, root dry weight, root : shoot ratio and total dry weight during two growing seasons

Treatment		Shoot dry weight (g per plant)		Root dry weight (g per plant)		Root : Shoot		Total dry weight (g per plant)	
		2013	2014	2013	2014	2013	2014	2013	2014
Control	Without dust	46.55 a	68.92 a	55.64 a	56.45 a	1.19 a	0.82 c	102.2 a	126.4 a
	Dust	36.35 b	51.14 b	39.95 b	53.62 a	1.10 a	1.05 b	76.3 b	104.8 b
Drought	Without dust	11.23 c	11.42 c	7.99 c	14.17 b	0.72 b	1.27 a	19.22 c	25.59 c
	Dust	8.49 d	8.83 d	6.27 c	10.90 c	0.82 b	1.24 ab	15.37 d	19.74 d

Means with the same letter in each column are not significantly different at $p < 0.05$ (LSD test). Control (-0.1 MPa), Dust (0.011 g cm^{-2}), Drought (-1 MPa)

Table 3. Effect of drought stress and dust on relative water content (RWC), proline, soluble carbohydrates, membrane stability index (MSI) and malondialdehyde (MDA) during two growing seasons

Treatment		RWC (%)		Proline (mg g^{-1} FW)		Soluble carbohydrates (mg g^{-1} FW)		MSI (%)		MDA (µmol g^{-1} FW)	
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Control	Without dust	93.3 a	93.7 a	0.16 c	0.22 d	32.5 d	33.0 d	82.9 a	89.6 a	0.43 d	0.44 d
	Dust	82.9 b	90.3 b	0.13 c	0.40 c	35.3 c	36.9 c	77.1 b	87.5 a	1.12 c	1.05 c
Drought	Without dust	79.9 b	74.8 c	1.84 a	2.08 b	43.1 a	41.0 b	68.7 c	84.1 b	2.41 b	1.73 b
	Dust	74.6 c	70.2 d	1.21 b	2.28 a	39.5 b	45.2 a	67.9 c	80.9 c	4.34 a	2.21 a

Means with the same letter in each column are not significantly different at $p < 0.05$ (LSD test). Control (-0.1 MPa), Dust (0.011 g cm^{-2}), Drought (-1 MPa)

the present study, the root-to-shoot ratio decreased in the first year due to drought stress, but increased in the second year under dust, drought, and drought + dust (Tab. 2). There was a reduction of 28, 85 and 88% in root dry weight, and 22, 75 and 81% in shoot dry weight in the dust, drought and drought + dust treatments respectively in the first year (Tab. 2).

Deficit irrigation and dust treatments resulted in lower RWC than the control in both the first and second year of the experiment (Tab. 3). The drought + dust treatment produced significant RWC reductions compared to the other treatments in the first and second year. The RWC was high in the control (93.28 and 93.75% in the first and second year, respectively), with a decline in the dust treatment (82.92 and 90.35 % in the first and second year, respectively), followed by drought (79.88 and 74.87 % in the first and second year, respectively) and dust + drought (74.56 and 70.22 % in the first and second year, respectively). Increased amounts of soluble carbohydrates were recorded for both the dust and drought stress treatments in both 2013 and 2014. The amount of proline increased under drought stress and drought + dust in both growing

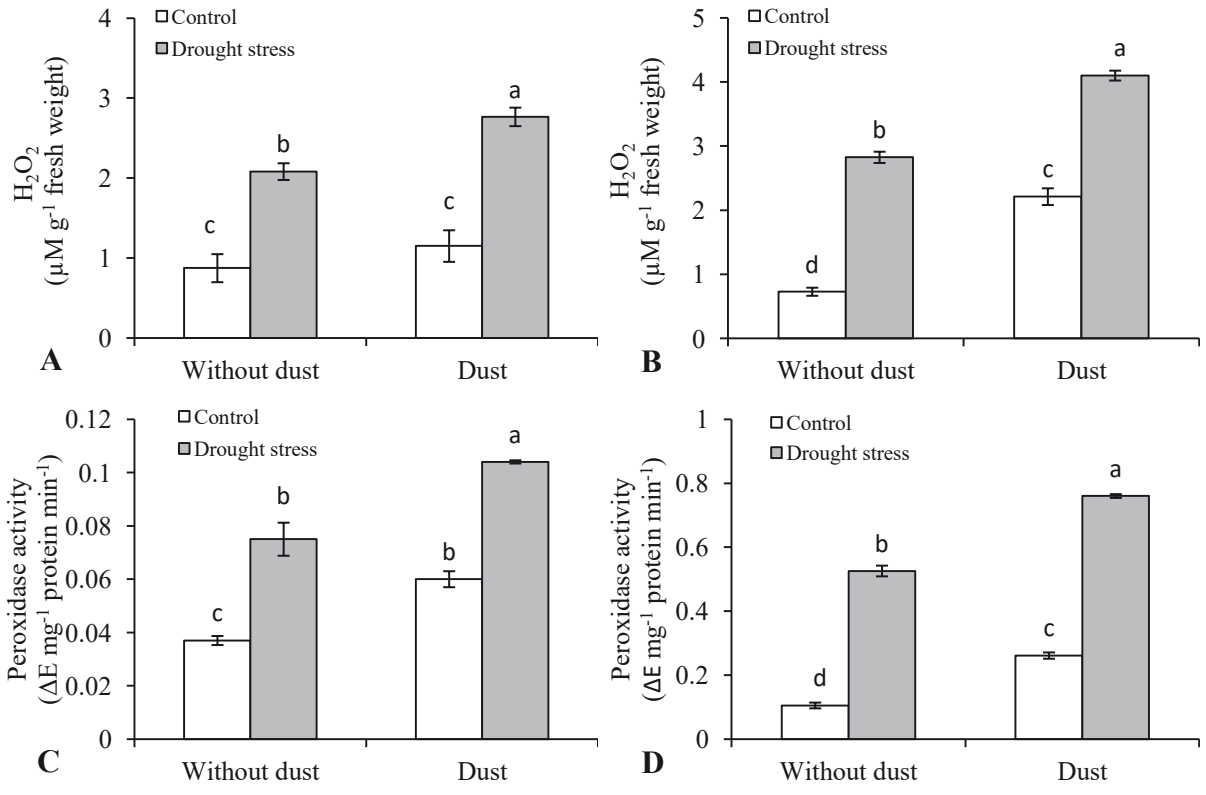

Figure 1. Effect of drought stress and dust on H_2O_2 (A – 2013 and B – 2014) and peroxidase activity (C – 2013 and D – 2014). Means for columns with the same letter are not significantly different at $p < 0.05$ (LSD test). Vertical bars indicate standard error. Control (-0.1 MPa), Dust (0.011 g cm^{-2}), Drought stress (-1 MPa)

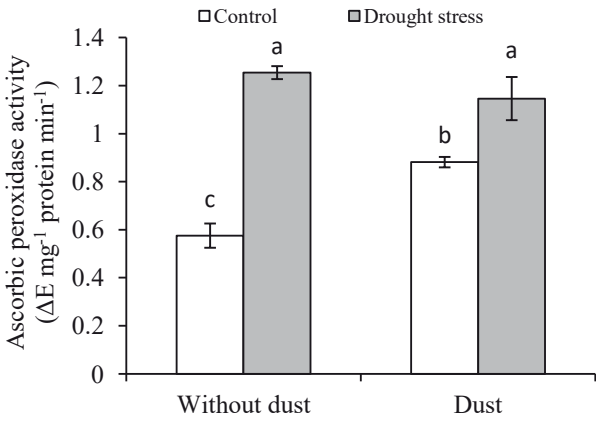

Figure 2. Effect of drought stress and dust on ascorbic peroxidase activity (2014). Means for columns with the same letter are not significantly different at $p < 0.05$ (LSD test). Vertical bars indicate standard error. Control (-0.1 MPa), Dust (0.011 g cm^{-2}), Drought stress (-1 MPa)

seasons. Dust deposition on leaves increased the proline content in the second year. Maximum amounts of soluble carbohydrates and proline accumulated under dust + drought stress, which was recorded in the second year (Tab. 3).

The membrane stability index was reduced in both years in response to dust and drought in comparison with the control (Tab. 3). Drought stress and dust increased the amount of MDA and H_2O_2 during the first and second year. The dust + drought treatment resulted in significantly higher MDA and H_2O_2 concentrations and a lower MSI than in the control. The present results showed that the dust + drought treatment intensified stress, which was shown by the measurements of MSI, MDA (Tab. 3) and H_2O_2 (Fig. 1A, B). Dust and drought resulted in a distinct increase in the POD (Fig. 1C, D) and APX (Fig. 2) activities in grape leaves. Maximum leaf POD activity in both years was observed due to the stress caused by dust + drought.

DISCUSSION

In our results, the application of dust, drought stress and dust + drought stress to grapevine leaves decreased shoot length by 2, 54 and 58% in the first growing season and by 17, 43 and 61% in the second growing season, respectively, compared to the control (Tab. 1). The greater reduction in shoot length in the second year can be related to the longer exposure of plants to dust. Similar reduction in the number of leaves was also observed under dust, drought stress and dust + drought stress conditions. The decrease in the number of leaves was related not only to the reduction in shoot growth induced by dust, but also to the damaging effects of dust, which caused leaf defoliation. Leaf surface area was reduced under dust, drought and drought + dust in both growing seasons (Tab. 1). Drought stress intensified the effect of dust to the extent that their combined effect reduced leaf surface area by approximately 74 and 75% in the first and second year, respectively, compared to the control. In this study, the reduction in total leaf surface area was caused by the reduced number of leaves and early leaf senescence. The loss of those leaves decreased the supply of carbohydrates or growth hormones to meristematic regions, thereby inhibiting growth. Reductions in the numbers of leaves and leaf surface area as a result of drought stress have been observed in other studies, including grapevine (Pou et al. 2012), apple (Alizadeh et al. 2011) and strawberry (Ghaderi et al. 2015). Considerable reductions in leaf surface area and shoot length have been reported in *Cassia siamea* and *Glauca* species (Shweta 2012), and *Eucalyptus camaldulensis* (Seyyednejad and Koochak 2011) that were treated with dust. The leaf is one of the organs that are more susceptible to air pollution. A deposit of dust on the leaves eventually forms a thick coating on them (Raina et al. 2008), limiting sunlight penetration and thus reducing photosynthesis and causing destruction of leaf tissues and premature leaf fall (Brandt and Rhoades 1973). Similar results have been reported by Noor et al. (2015) and Qadir et al. (2016), all of whom observed a reduction in leaf surface area in plants exposed to dust and air pollutants.

Dust caused a severe decrease in shoot dry weight (22 and 26% in the first and second year, respectively) and root dry weight (28 and 5% in the first and second year, respectively), and a significant decrease in biomass (by 25 and 17% in the first and second year, respectively) (Tab. 2). The changes in these characteristics coincided with a reduction in RWC by 11 and 4% in the first and second year, respectively (Tab. 3). Greater reduction in growth was recorded in plants treated with dust + drought stress. Therefore, it is apparent that drought stress + dust cause a greater negative impact on the growth of plants. In addition to reducing RWC, the induction of oxidative stress under such conditions can also be effective in reducing growth (Fig. 1A, B). Like in the present study, growth reduction has been found in *Astragalus jaegerianus* (Wijayratne et al. 2009) and fig (Abdel-Rahman 2012) plants covered with dust. The changes can be attributed to the shading caused by the dust on the leaf, decline in leaf surface area and damage to the

photosynthetic apparatus due to the toxicity of the pollutants and increased water stress (Hossain et al. 2015). Considerable reductions in shoot and root dry weight have been reported in *Polyalthia longifolia, Ficus religiosa* and *Azadirachta indica* (Saini et al. 2011), and some medicinal plants (Lee et al. 2003) that were treated with dust.

The decrease in the root-to-shoot ratio in the first year is related to a lower reduction in shoot dry weight and a higher reduction in root dry weight compared to the second year. Greater reductions in root growth compared to shoot growth under drought stress have been documented in several studies (Azhiri-Sigari et al. 2000, Cui et al. 2008). The reports indicated that the root growth response to drought stress depends on stress duration and stress development rate, and on the allocation of carbohydrates to the roots (Xu et al. 2015). In the first year of our study, the roots probably contained lower amounts of carbohydrates; therefore, they experienced a greater reduction in dry weight compared to the shoots. Wissuwa et al. (2005) had reported that a decrease in dry matter allocation to the roots may be related to a decreased concentration of starch. The increase in the root-to-shoot ratio due to drought and dust observed in our study was a result of a greater reduction in aboveground biomass rather than an increase in root biomass in the second year (Tab. 2). This was because the root dry weight under drought stress and dust was lower than that in the control. The growing of roots is a strategy used by plants to absorb more water from the soil under drought conditions (da Silva Lobato et al. 2008), which will contribute to higher cell turgor and better plant growth and development. It has also been reported that drought (Lemoine et al. 2013) and dust (Bao et al. 2016) limit shoot and root growth, but their effects on root growth are lower compared to shoot growth. Root growth is generally less affected by drought stress than shoot growth (Mahajan and Tuteja 2005). This often results in an increase in the root-to-shoot ratio when water is limited.

Total soluble carbohydrates and proline were increased in all the plants that were treated with dust compared with the non-treated plants, and drought intensified the effect of dust on grapevine soluble carbohydrate content (Tab. 3). Moreover, the presented results clearly illustrate that dust alters several biochemical traits, such as carbohydrate and proline amounts in grapevine leaves. In addition, the concentrations of soluble carbohydrates and proline were higher in the plants treated with dust + drought. Soluble carbohydrates and proline commonly accumulate in crop plants as osmoprotectants in response to abiotic stress. In regard to the accumulation of proline in grapevine, the results of the present investigation are similar to the findings of Ghaderi and Siosemardeh (2011), and Ghaderi et al. (2015), who reported that in strawberries the highest level of proline was observed in responses to drought. The increased accumulation of soluble carbohydrates and proline in response to dust and drought stress is a strategy for improving stress tolerance (Hoekstra et al. 2001), regulating osmotic adjustment and reducing lipid peroxidation (Gupta et al. 2015).

Dust and drought increased the peroxidation of membrane lipids (MDA) and oxidative stress (H_2O_2), and reduced the cell membrane stability index (Tab. 3, Fig. 1). Drought stress stimulates the production of reactive oxygen species (Liu and Huang 2000) that cause membrane injuries and thus induce oxidative stress (Zlatev and Lidon 2012). Stressed plants produce higher amounts of reactive oxygen species, including H_2O_2, than in their steady state, which can cause an increase in lipid peroxidation (Hoekstra et al. 2001). Dust deposition also restricts the availability of light for photosynthesis, blocks stomatal pores for CO_2 diffusion, and increases oxidative stress on plants (Das and Prasad 2010). In agreement with the present study, dust deposition has been found to increase the MDA in wheat (Chen 2010) and *Azadirachta indica* (Qadir et al. 2016).

Dust and drought caused a distinct increase in the POD (Fig. 1C, D) and APX (Fig. 2) activities in grapevine leaves. Leaf POD activity in both years and that of APX in 2014 increased due to the stress induced by dust and drought. The present study demonstrated that the application of dust and drought together intensified the POD activity. Some signalling molecules, such as oxidative molecules, may cause an increase in the antioxidant capacity of cells. To alleviate cellular injury, stressed plants produce antioxidant enzymes (Zlatev and Lidon 2012). As a consequence of POD and APX's role in scavenging H_2O_2, an increase in POD and APX activities can be regarded as a defence mechanism of the plant against the reinforcement of oxidative processes. Dust accumulation on plants might alter the leaf antioxidant mechanism (Chaturvedi et al. 2013), and increased APX activity under dust pollution has been reported by Siqueira-Silva et al. (2016).

Finally, it can be concluded that the growth of grapevine plants was found to be affected by dust and drought. Dust evidently causes substantial changes to leaf physiology by destroying the cell membrane. Accumulation of soluble carbohydrates and proline was augmented in plants by the occurrence of dust and drought stress. Higher increases in soluble carbohydrates and proline were observed in the dust + drought treatment. Significant decrease in leaf relative water content due to dust and drought stress was observed. Extensive reduction in RWC was recorded when dust and drought stress were applied together. The dusted leaves and plants subjected to drought had higher MDA and H_2O_2 compared to the control. The dust + drought combination produced significantly higher MDA and H_2O_2 and lower MSI compared to the other treatments in the second year of the experiment. The POD and APX activities involving enzymatic antioxidants were increased due to the stress caused by dust and drought. Based on the results of this study, it is clear that dust pollution of the grape plants produced effects similar to those caused by water-deficit conditions; on the other hand, the interaction effects of dust and drought applied together exacerbated the physiological and morphological changes in grapevine. This suggests that dust greatly affects the response of grapevine to water-deficit stress and increases damage to plants.

FUNDING

Financial support was provided by the Vice Chancellor for Research and Technology, University of Kurdistan, under grant No. 4.14362.

AUTHOR CONTRIBUTIONS

N.G. – designed the experiment. L.K. – performed the experiment and compiled the data under N.G.'s supervision and T.J.'s advice. Biochemical and physiological experiments and measurements were supervised by N.G. Data analysis was performed by N.G with the help of L.K. N.G. – elaborated the results and wrote the manuscript.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Received August 8, 2017; accepted October 23, 2017