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Traffic noise induces oxidative stress and phytohormone imbalance in two urban plant species

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Abstract

Traffic noise pollution is one of the major environmental concerns in crowded cities worldwide. The objective of the present study was to investigate the effects of traffic noise on growth, hormonal balance, oxidative damage, and activity of antioxidant systems in two urban plant species, *Tagetes patula* and *Salvia splendens*. Each of the plant species were equally divided into 2 groups (control and traffic noise treatment) and each group was grown under identical controlled conditions in two separate growth chambers for two months. Traffic noise was recorded during peak traffic hours in a highly congested area of the city. Frequency analysis of traffic noise was conducted on the samples during the recording process. Plants in the traffic noise treatment group were exposed to 16 h of road traffic noise each day for a total of 15 days, while the control group was kept under complete silence. Traffic noise exposure led to significant decrease in growth indices of both plant species. The content of H_2O_2 and malondialdehyde (MDA) significantly increased upon traffic noise treatment in both species, followed by an increase in DPPH (2,2-diphenyl-1-picrylhydrazyl) anti-oxidant activity and the activity of antioxidant enzymes including catalase, peroxidase, and ascorbate peroxidase. Road traffic noise significantly reduced the content of phytohormones including zeatin, salicylic acid, indole-3-acetic acid, and gibberellic acid. On the other hand, the content of stress-related hormones including abscisic acid and jasmonic acid significantly increased in response to road traffic noise in both species. Based on our results, daily traffic noise can negatively affect growth and physiology of plants by inducing of oxidative damage and interfering with hormonal balance.

Introduction

Sound is acoustic energy in the form of oscillatory concussive pressure waves transmitted through mediums such as gases, liquids, or solids (Hassanien, Tian-Zhen, Yu-Feng

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& Bao-ming, 2014). As a fundamental form of energy, many organisms have evolved to respond to the ambient sounds by a wide range of biological processes (Simon, Holderied, Koch & von Helversen, 2011). The ability of plants to sense and respond to different sounds have been revealed in recent years (Jung, Kim, Kim, Jeong & Ryu, 2018; Kim, Lee, Kim, Muthusamy & Jeong, 2021; Schöner, Simon & Schöner, 2016). Emerging studies have demonstrated that sound waves with specific frequencies and intensities can trigger a wide array of cellular, physiological, and molecular responses in plants (Mishra, Ghosh & Bae, 2016; Rodrigo-Moreno et al., 2017). Sound waves have been demonstrated to affect growth, seed germination rate, polyamine production, vitamin C content, and expression of stress-related genes in plants (Cai, He, Zhu & Wang, 2014; Kim et al., 2016; Qin, Lee, Choi & Kim, 2003). Similarly, it has been demonstrated that the signaling and homeostasis of several phytohormones including auxins, abscisic acid, and ethylene are regulated by specific sound waves (Bochu, Jiping, Biao, Jie & Chuanren, 2004; Kim et al., 2015, 2016). While the mechanisms for perception of sound waves remain to be elucidated in plants, there is growing evidence that place the increase in cytosolic Ca^{2+}, proton flux, and reactive oxygen species (ROS) generation at the center of signal transduction pathways (Monshausen, Bibikova, Weisenseel & Gilroy, 2009; Rodrigo-Moreno et al., 2017).

Being sessile organism, plants are exposed to fluctuating and extreme environmental conditions and require a complex signaling network and tolerance mechanisms to respond to stressful conditions, accordingly. Environmental factors pose an adverse effect on the metabolism, growth, and yield of plants, including both agricultural and ornamental species. Recent studies confirmed that sound waves can induce excessive generation of reactive oxygen species (ROS), especially hydrogen peroxide (H_2O_2) in plants (Mishra et al., 2016; Rodrigo-Moreno et al., 2017). The disparity between ROS production and scavenging will lead to oxidative damage to cellular macromolecules including membrane lipids, proteins, nucleic acids, causing severe metabolic impairment (Azizollahi, Ghaderian & Ghotbi-Ravandi, 2019). Plants have evolved a complex network of enzymatic and non-enzymatic antioxidant systems to protect cells from oxidative damage by scavenging various types of ROS (Ghotbi-Ravandi, Shariati, Shahbazi & Shobbar, 2019). Xiujuan et al. (2003) demonstrated that the activities of several antioxidant enzymes including. catalase (CAT), ascorbate peroxidase (APX) and different isozymes of peroxidases (POD) of *Chrysanthemum* significantly increased in response to exposure to sound intensity of 100 db and frequency of 1000 Hz for several days. Similarly, Li et al. (2008) showed that the specific activity of superoxide dismutase (SOD), CAT, POD and APX enhanced in different organs (leaves, stems and roots) of *Dendrobium candidum* in response to exposure to different sound waves.

Recent studies have revealed that during stress condition, an intricate interplay exists between ROS and plant hormones. Elevated content of ROS during stress can modulate the content and function of phytohormones, which play a key role in acclimatory response to adverse environmental conditions (Choudhury, Rivero, Blumwald and Mittler, 2017)

Traffic noise is a major global problem in metropolitan areas which adversely affects various aspects of urban life. Traffic noise can exceed 55 dBA during the day depending on traffic congestion, speed of vehicles, percentage of heavy vehicles, road surface, and gradient as well as number of lanes. The growth of population and increasing number of automobiles in major cities have exacerbated the level of traffic noise in recent decades (Ögren, Molnár & Barregard, 2018). The high levels of traffic noise can impose a devastating effect on living organisms, including plants.

The acoustic biology has become increasingly popular in recent years and more attention has been paid to the impacts of sound waves on growth and development of plants. However, the majority of studies on responses of plants to sound waves have focused on the effects of specific frequencies on germination rate and growth. Less is known, however, about the effects of noise pollution, and especially traffic noise, on the biochemistry and physiology of plants. The aim of the present study was to examine the impacts of traffic noise pollution, as an environmental stimulus, on ROS formation and phytohormone homeostasis in two common urban plant species (*Tagetes patula* and *Salvia splendens*). These are both common and widely cultivated spring/summer annual plants used in urban green spaces of Tehran.

Materials and methods

Plant materials and growing condition

Two plant species, *Tagetes patula* L. (Lamiaceae) and *Salvia splendens* Sellow ex Schult. (Asteraceae), were used in the experiments. Both species are annual plants that are extensively used in urban green spaces. Seeds of both species were obtained from the parks and recreation office, Tehran municipality. Seeds were surface sterilized in 70% ethanol (v/v) for one minute and 14% commercial bleach (v/v) for 20 min, followed by washing with sterilized distilled water 3 times. Seventy seeds of each species were planted in 5 cm plastic pots containing a mixture of peat moss and perlite (3:2). Plants were grown in a growth chamber under controlled condition (16 h light/ 8 h dark, at 25 °C /18 °C day/night and 40−50% relative humidity under 500 μmol photon m^{-2} s^{-1}) in 70% of soil water holding capacity for two months.

Exposure of plants to daily traffic noise

To determine the most polluted area in terms of traffic noise, environmental noise assessment was performed in
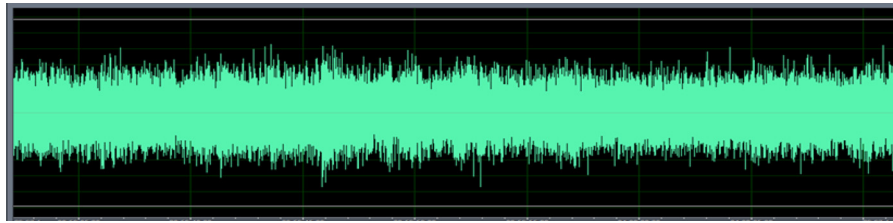
Fig. 1. Image of the recorded daily traffic noise in the cool editor pro 2.1 software. The final waveform of traffic noise was recorded six days a week during high traffic intervals (7 am to 10 pm) in the Cool Edit Pro 2.1 software and applied to treatment groups of plants for 15 days and 16 h a day. The fundamental or peak frequency is approximately 250 Hz.
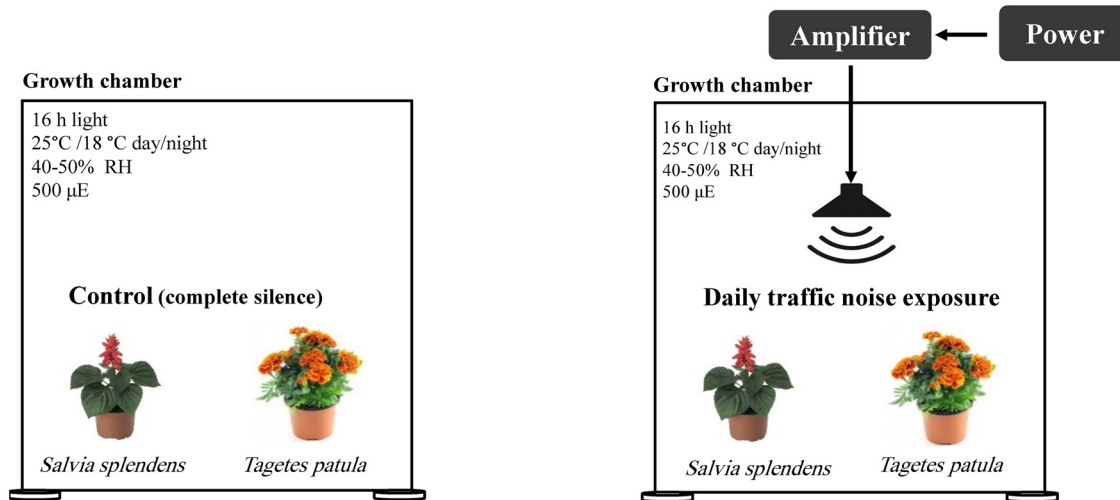

Fig. 2. Schematic diagram of the experiment setup.

several areas with high traffic density. Noise measurements were carried out at five points of the study area in the first six days of the week in high- traffic intervals of 7-9 AM., 9-11 AM, 11-13 AM, 14-16 PM, 4-6 PM, 6-8 PM and 8-10 PM. In this study, the order of selecting stations for sound measurement was random. An image (Cool Edit Pro2.1) of the traffic sound recorded using a Sony ICD-SX2000 is shown in Fig. 1.

To record daily traffic noise, the CEL-450/490 analyzer device (Casella-CEL Co., UK) with continuous data recording capability was used. To ensure the accuracy of the measurement by the sound meter, the calibrator of the CEL_110.1 model was used. It should be noted that the sound meter was calibrated before the start of the measurement and the noise measurement stations were chosen as far away as possible from reflector surfaces. Also, the measurement of sound was in dry conditions and the direction of the wind at a slow speed. The windscreen was used to remove the effect of the airflow on the sound meter microphone. The sound meter in each measurement station was adjusted on the weighing network of frequency A and fast response speed. The measurement time per station was 30 min. In each station, sound parameters such as sound pressure level (SPL) and equivalent level of noise exposure (Leq) and frequency analysis were measured in a one-octave band (See

Appendix A). The average sound equivalent level is approximately 73 dB

Two months after sowing, each plant species was equally divided into two groups (control and traffic noise exposure) and each group was transferred into a separate growth chamber with identical conditions. The exposure group was exposed to 16 h of road traffic noise each day for 15 days, while the control group was kept in complete silence (Fig. 2). Samples were taken simultaneously from the youngest fully expanded leaves and were immediately frozen in liquid nitrogen and stored at -80 °C for further analysis.

Measurement of plant weight

At harvest, plants were weighed and the biomass of plants was reported as g plant^{-1}.

Determination of hydrogen peroxide (H$_2$O$_2$) content

The H$_2$O$_2$ content of samples were determined according to Velikova, Yordanov & Edrev (2000). Fresh samples (100 mg) were homogenized in an ice-cold mortar using 1 ml trichloroacetic acid (TCA) (0.1% w/v). The homogenate was centrifuged at 12,000 g for 15 min at 4 °C, and

0.1 ml of the supernatant was added to 0.5 ml potassium phosphate buffer (10 mM, pH 7.0) and 0.5 ml KI (1M). The absorbance of the supernatant was measured at 390 nm by spectrophotometer (CARY 300, Agilent, Santa Clara, California, USA). The content of H_2O_2 in the samples was calculated based on the standard curve obtained by measuring the absorbance of H_2O_2 standard solutions prepared in TCA (1%).

Assessment of lipid peroxidation

The lipid peroxidation level was determined in terms of malondialdehyde (MDA) concentration, as a secondary product of the oxidation of polyunsaturated fatty acids that can react with thiobarbituric acid (TBA). MDA content of samples was measured according to the Heath & Packer (1968) method. Fresh samples (500 mg) were homogenized in 5 ml (0.1%, w:v) of trichloroacetic acid (TCA). The homogenates were centrifuged at 10,000 g for 20 min. Supernatant (0.5 ml) was added to 1 ml of 0.5% TBA in 20% TCA. The mixture was heated at 100 °C for 30 min followed by cooling the mixture in ice bath. Samples were centrifuged at 10,000 g for 5 min and absorbance of supernatant was monitored at 532 and 600 nm using UV−VIS spectrophotometer (CARY 300, Agilent, Santa Clara, California, USA). The MDA concentration was determined by subtracting the absorbance at 600 and 532 nm using extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Measurement of antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

The free radical scavenging activity of the samples was determined based on Shyu & Hwang (2002). In this method, 2 ml of leaf extracts were added to 2 ml of 0.1 mM DPPH in ethanol. The mixture was vortexed and kept in the dark at 30 °C for 30 min. Then the absorbance of the samples was measured at 517 nm. For the control, the extracts were replaced with ethanol. The DPPH radical scavenging activity of the samples was calculated via the following formula:

$$I(\%) = [1 - (A_1/A_0)].100$$

where A_1 is the absorbance of the sample and A_0 is the absorbance of the control (Li, Gao, Zhao & Wang, 2007). The results are presented as IC_{50} which is the amount of anti-oxidant needed for the DPPH concentration to reach 50% of its initial amount.

Determination of antioxidant enzyme activity

Extraction of enzymes was performed according to Larkindale & Huang, 2004. Fresh samples (250 mg) were homogenized in ice-cold mortar using 100 mM phosphate buffer (pH = 7) containing PVP (1%) and EDTA (1 mM). Homogenates were centrifuged at 10,000 g (15 min, 4 °C). The resulting supernatants were used for enzyme analysis. The Protein content of samples was measured based on Bradford (1976).

The specific activity of catalase (CAT, EC: 1.11.1.6) was determined according to Aebi (1983). The Reaction was started by adding 100 μl H_2O_2 (100 mM) to a mixture of 100 μl enzyme extract and 1 ml potassium phosphate buffer (50 mM, pH = 7.0). Changes in absorbance due to H_2O_2 decomposition were recorded at 240 nm for 3 min. CAT activity was expressed units mg^{-1} protein.

The specific activity of the peroxidase enzyme (POX, EC: 1.11.1.7) was determined based on the method of Ruley et al. (2004). Reaction mixture contained the enzyme extract (100 μl), H_2O_2 (100 μl,17 mM), guaiacol (250 μl, 2%) and phosphate buffer (780 μl, 10 mM). Peroxidase activity was determined by measuring the increase in absorbance at 470 nm for 3 min and expressed as units mg^{-1} protein.

The specific activity of Ascorbate peroxidase (APX, EC 11 .1 .11 .1) was determined according to Nakano & Asada (1987). Reaction mixture (1 ml) contained potassium phosphate buffer (50 mM, pH = 7), ascorbic acid (0.5 mM), EDTA (0.2 mM), H2O2 (0.25 mM) and 50 μl enzyme extract. The reaction was initiated by addition of H_2O_2 to the mixture. The absorbance was read at 290 nm for 3 min. Ascorbate peroxidase activity was expressed as units mg^{-1} protein.

Measurement of phytohormone content

Extraction of phytohormones was conducted based on Michalak et al. (2016) method. In brief, tissue samples (1 g) were frozen and grounded in liquid nitrogen and extracted in methanol (80%) overnight. After that, the samples were centrifuged at 13000 g for 10 min. The supernatants were evaporated and the pellets were dissolved in acetic acid (1%). Hormones were purified on Oasis cartridges (Milford, USA) based on manufacturer's protocol. The extracted hormones were dissolved in 10% methanol and were analyzed by HPLC (Agilent 1100, USA). Phytohormone content of samples was determined according to Górka & Wieczorek (2017).

Statistical analysis

The data were obtained from five independent replications and expressed as mean ± SD. The statistical significance was tested using t-test. The value of $P \leq 0.05$ was used for criterion of significance. Analyses were performed using SPSS v22 and Microsoft Excel. The value of $p \leq 0.05$ was used as the criterion of significance.
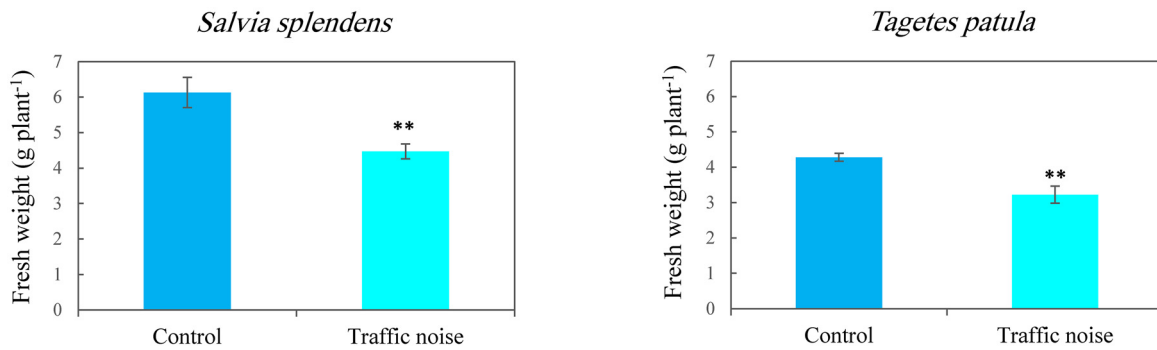
Fig. 3. Effects of daily traffic noise exposure on fresh weight of *Tagetes patula* and *Salvia splendens*. Values are the average of five independent replications ± standard deviation. **, different from control group at $p \leq 0.01$.

Results

Effect of traffic noise on plant biomass

The imposed traffic noise significantly decreased the biomass of both plant species (Fig. 3). Traffic noise led to 27% and 17% decrease in fresh weight of *S. splendens* and *T. patula*, respectively.

Changes in H_2O_2 and MDA content in response to traffic noise exposure

Effects of daily traffic noise on the accumulation of H_2O_2 and MDA are depicted in Fig. 4. The generation of H_2O_2 was significantly ($P < 0.01$) increased under the traffic noise treatment in both species. H_2O_2 content of *S. splendens* and *T. patula* increased 111% and 225%, respectively, compared to the control group. Consistently, traffic noise exposure resulted in significantly higher formation of MDA in both species. In response to traffic noise, the MDA content increased by 144% and 29% in *S. splendens* and *T. patula*, respectively.

Antioxidant activity in response to daily traffic noise

The DPPH radical scavenging activity significantly increased in both plant species in response to daily exposure
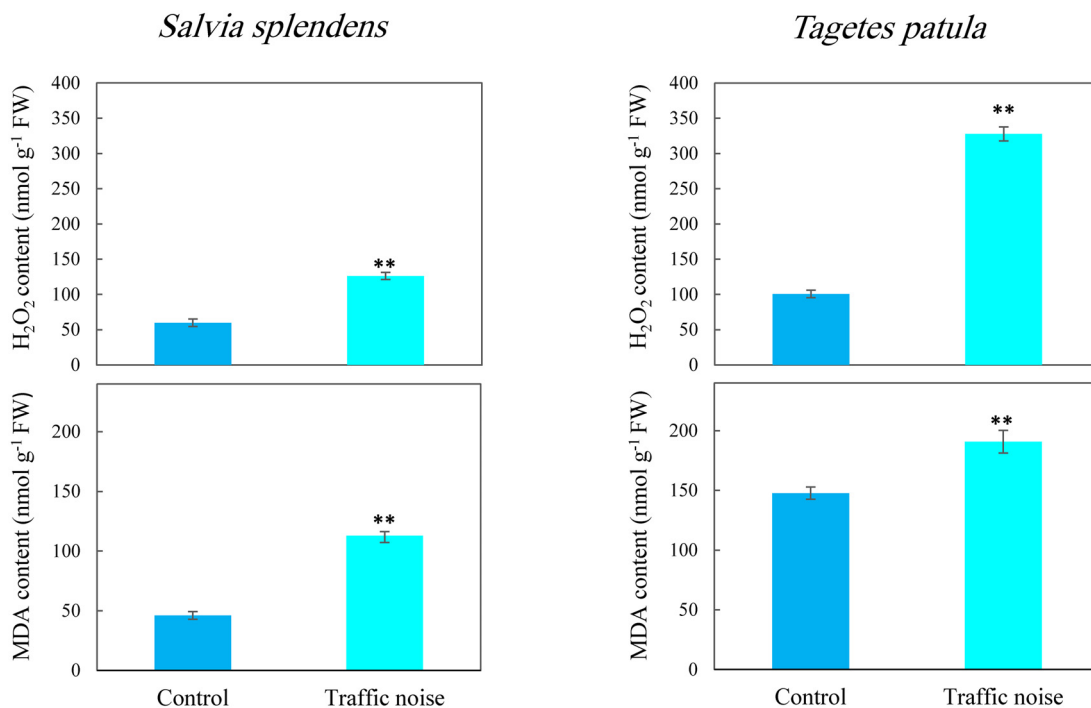

Fig. 4. Effects of daily traffic noise exposure on hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content of *Tagetes patula* and *Salvia splendens*. Values are the average of five independent replications ± standard deviation. **, different from control group at $p \leq 0.01$.
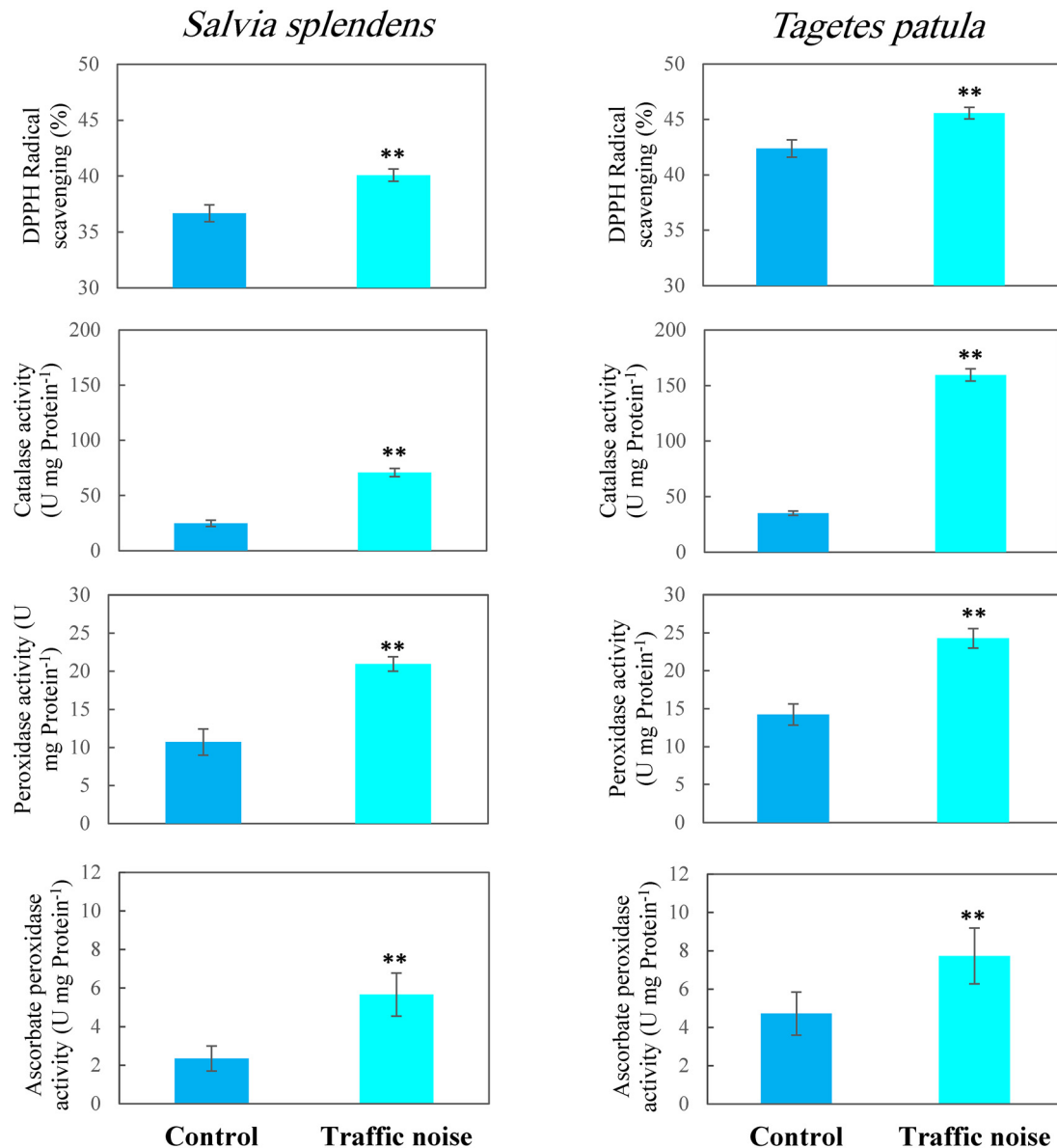
Fig. 5. Effects of daily traffic noise exposure on DPPH (2,2-diphenyl-1-picrylhydrazyl) radicle scavenging capacity, activity of catalase, peroxidase and ascorbate peroxidase antioxidant enzymes of *Tagetes patula* and *Salvia splendens*. Values are the average of five independent replications ± standard deviation. **, different from control group at $p \leq 0.01$.

to traffic noise (Fig. 5). Daily traffic noise treatment led to 9% and 7% increase in radical scavenging activity of *S. splendens* and *T. patula,* respectively.

Daily traffic noise significantly increased the activity of antioxidant enzymes in both plant species, as compared to the control group (Fig. 5). The specific activity of CAT, POX and APX increased by 185%, 95%, and 141%, respectively, in *S. splendens* in response to traffic noise exposure. Similarly, in *T. patula,* 355%, 70%, and 63% increase in activity of CAT, POX, and APX was observed under traffic noise treatment, respectively.

Effects of traffic noise exposure on phytohormones content

The phytohormone content of both *S. splendens* and *T. patula* significantly altered in response to 15 days of traffic noise exposure (Fig. 6). Content of indole 3-acetic acid (IAA), gibberellic acid (GA3), zeatin, and salicylic acid significantly decreased in response to traffic noise. Whereas, the content of jasmonic acid (JA) and abscisic acid (ABA) significantly increased in both species, as compared with the control group. Under traffic noise in *S. splendens*, a
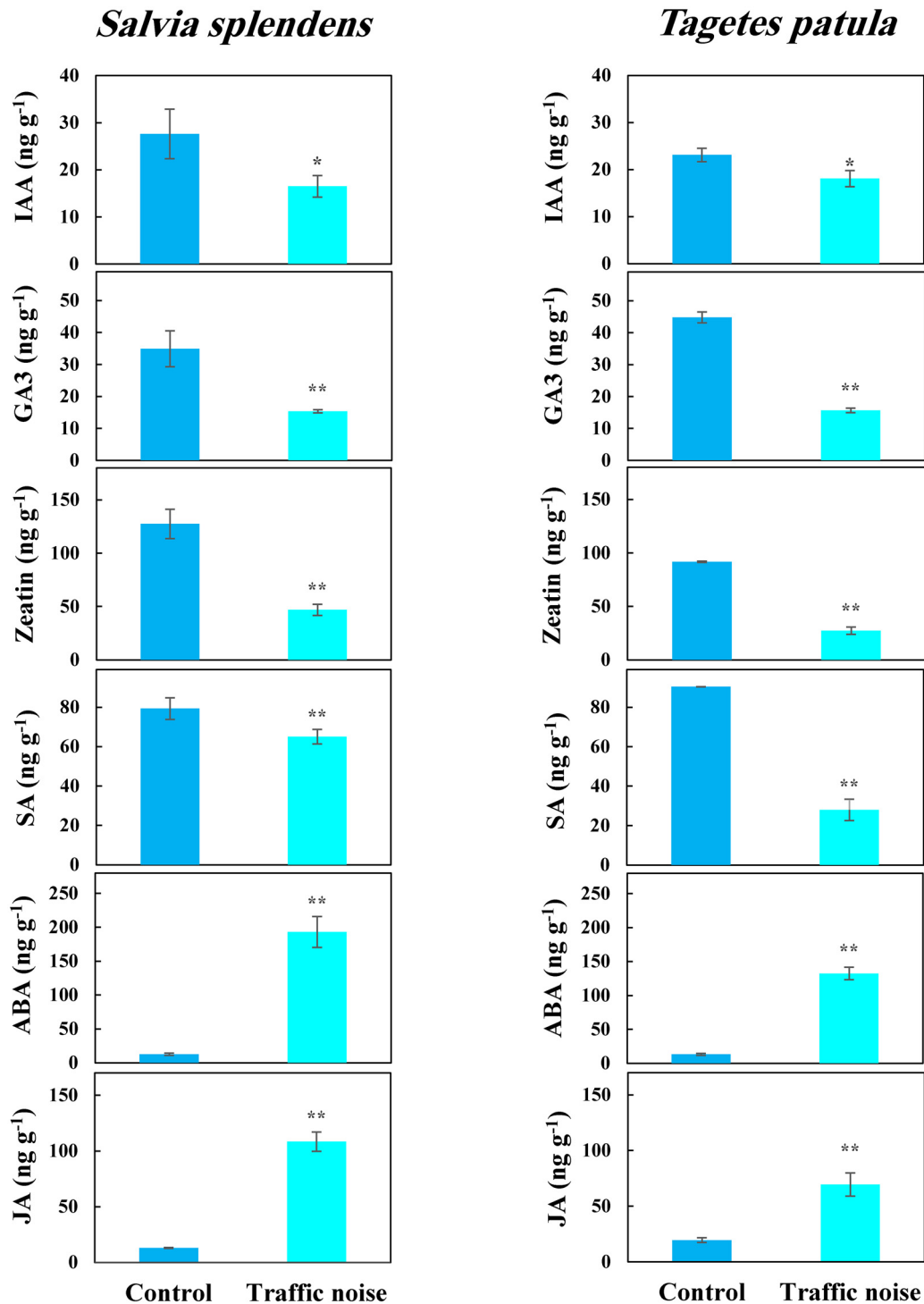
Salvia splendens   *Tagetes patula*

Fig. 6. Effects of daily traffic noise exposure on content of indole-3-acetic acid (IAA), gibberellic acid (GA3), Zeatin, salicylic acid (SA), abscisic acid (ABA) and jasmonic acid (JA) in *Tagetes patula* and *Salvia splendens*. Values are the average of five independent replications ± standard deviation. * and **, different from control group at $p \leq 0.05$ and $p \leq 0.01$, respectively.

reduction of 63.22% in zeatin, 40.26% in IAA, 56.07% in GA3, and 18.01% in SA was observed along with an increase of 1408.09% in ABA and 734.92% in JA. As for *T. patula,* a reduction of 70.09% in zeatin, 21.75% in IAA, 65.04% in GA3, and 69% in SA was observed along with an increase of 911.83% in ABA and 256.03% in JA (Fig. 6)

Discussion

Sound waves, as an environmental factor, can significantly impact a variety of biological functions in plants (Jung et al., 2018). In our study, exposure of two urban plant species (*S. splendens* and *T. patula*) to 16 h of traffic noise

for 15 days led to significant increase in H_2O_2 formation in both cultivars, as compared to the control group. Consistent with our results, significant accumulation of H_2O_2 was reported in *Dendrobium officinale* cultures treated by ultrasound at 300 W for 5 min (Wei, Yang & Wei, 2012). Increase in ROS content, including H_2O_2, was also reported in *Panax ginseng* cell cultures as a result of low-energy ultrasound (Wu & Lin, 2002). Oxidative stress is a central component of environmental stress damage. Different unfavorable environmental conditions promote the accumulation of H_2O_2 in plant cells (Ahangir, Ghotbi-Ravandi, Rezadoost & Bernard, 2020; Ghotbi-Ravandi, Sedighi, Aghaei & Mohtadi, 2021; Behdad, Mohsenzadeh, Azizi & Moshtaghi, 2020). H_2O_2 is one of the most harmful oxygen species due to its longer half-life. Being relatively stable, H_2O_2 can spread within biological membranes causing the peroxidation of membrane lipids (Ghotbi-Ravandi et al., 2021). Lipid peroxidation is one of the most harmful processes that occur in the cells which alters the membrane fluidity and integrity, increases solute leakage from cell, and inactivates membrane protein complexes (Garg & Manchanda 2009). In the present study, MDA content of both *S. splendens* and *T. patula* plants significantly increased; indicating a rise in lipid peroxidation level in these plant species due to traffic noise treatment. This increase in the content of MDA was consistent with the elevation in H_2O_2 formation in response to traffic noise in both species. Consistent with our finding, Chen, Huang, Wang & Huang (2008) reported an 48% increase in the MDA content of *Porphyridium cruentum* in response to ultrasound treatment. Similarly, Li et al. (2008) reported that MDA content of *Dendrobium candidum* exhibited a rising trend in response to sound waves with the intensity of 100dB and frequency of 1000Hz in 15 days of treatment.

The magnitude of tolerance to oxidative damage is highly correlated with ROS scavenging capacity of plant cells. Enhanced antioxidant system is a protective mechanism adopted by plants to eliminate excess ROS. In the present study, exposure to traffic noise significantly induced the activity of antioxidant enzymes and radical scavenging capacity in both *S. splendens* and *T. patula.* The activity of CAT, APX, and POX enzymes which primarily decompose H_2O_2 significantly increased along with a higher rate of noise-induced H_2O_2 formation. Increased activity of CAT, APX, and POD indicates the protective roles of these enzymes against traffic noise-induced oxidative stress. Consistent with our findings, Safari et al. (2013) reported that in *Corylus avellana*, exposure to low-intensity ultrasound (29 KHz), led to increase in the activity of CAT and APX enzymes by 4 and 1.7 times compared to the control group. Li et al. (2008) also reported an increase in the activity of antioxidant enzymes (CAT, APX, and POD) in *Dendrobium candidum* when exposed to 100 dB intensity sound waves at 1000 Hz. Corroboratively, an increase in the activity of SOD, CAT, and POD was reported in *Dendrobium officinale* exposed to ultrasound (Wei et al., 2012).

Plant hormones play a key role in the growth, development, and acclimatory responses of plants to unfavorable environmental conditions. In addition to playing a key role in numerus growth and developmental processes, Auxins (principally IAA) are an essential component of stress-induced morphogenic response in plants to limit the detrimental effects of environmental factors (Wolters & Jürgens, 2009). Many auxin responsive genes have been shown to repress by abiotic stimuli such as oxidative stress. Suppression of auxin is considered a mechanism to increase tolerance to different environmental stresses (Bartoli, Casalongué, Simontacchi, Marquez-Garcia & Foyer, 2013). In the present study, the IAA content of both species significantly decreased in response to daily traffic noise exposure. The reduction in IAA content was more prominent in *S. splendens* compared to *T. patula.* Consistent with our findings, Yiyao, Wang, Xuefeng, Chuanren & Sakanishi (2002) observed the increase in the activity of IAA oxidase followed by a reduction in IAA content in *Chrysanthemum* callus exposed to different sound waves, especially at 100 dB and 800 Hz. Similarly, Wei et al. (2012) reported a decrease in endogenous IAA content as a result of ultrasound treatment in the differentiation of protocorm-like bodies of *Dendrobium officinale* to shoot.

Gibberellins have also been implicated in response to environmental cues, including light, temperature, and stresses (Bartoli et al, 2013). Gibberellins (GAs) responses are mediated by relieving the constraints imposed on gene expression by DELLA proteins. DELLA proteins are negative regulators of gibberellin (GA) signaling that act downstream of the GA receptor. Their name was coined on the basis of a short stretch of amino acids (D-E-L-L-A) in their N-terminal region, which is tightly conserved among all higher plant species. In addition to restraining growth, DELLA proteins accumulate in plants exposed to adverse environmental conditions and play a key role in tolerance to biotic and abiotic stresses. It has been reported that Arabidopsis mutants with reduced GA content exhibited more salt tolerance than wild type plants (Achard et al., 2006). Plants exposed to salt stress, contained lower content of GA, higher content of DELLA proteins, and exhibited lower ROS accumulation and increased expression of antioxidant enzyme genes (Achard, Renou, Berthomé, Harberd & Genschik, 2008). It has been proposed that stress conditions that increase the ABA/GA ratio, lead to accumulation of DELLA proteins (Finkelstein, Reeves, Ariizumi & Steber, 2008). In the present study, exposure to traffic noise stress resulted in a significant reduction in gibberellic acid (GA3) content in both *S. splendens* and *T. patula* plant species. It can be argued that the reduction in GA content accompanied by the increase in ABA content may favor the enhanced tolerance of these species to traffic noise stress. Consistent with these findings, Ghosh et al. (2017) reported a significant decrease in GA content after 48 h exposure to 500 Hz and 80 dB sound waves.

Many studies reported a decrease in the level of endogenous cytokinin under different stress conditions. It has been suggested that reduced levels of endogenous cytokinin is necessary for the improvement of plant tolerance to environmental stresses (Kohli, Sreenivasulu, Lakshmanan & Kumar, 2013). The reduction in cytokinin level under stress is often associated with a higher rate of ABA accumulation. ABA can promote the reduction in cytokinin content by inhibiting the expression of genes involved in cytokinin biosynthetic pathway as well as activating the cytokinin breakdown enzymes (Vysotskaya et al., 2010; Wang et al., 2011). Consistently, in the present study, the remarkable increase in ABA content in response to traffic noise is concomitant with a significant decrease in zeatin content in both species.

ABA is a key hormone responsible for stress responses in plants. Plants exposed to different environmental stresses such as water stress, salt stress, and UV-B elicit the overproduction of ABA. At basal level, ABA modulates several physiological and developmental processes of plants like embryo morphogenesis and dormancy, and synthesis of storage compounds. However, during various abiotic stresses, a prominent elevation in ABA content triggers the adaptive responses such as changes in the physiological process and re-programming the gene expression in plants (Sreenivasulu, Harshavardhan, Govind, Seiler & Kohli, 2012). In the present study, exposure to daily traffic noise led to remarkable increase in ABA content of both *S. splendens* and *T. patula*. Consistent with these results, Hassanien and Li, 2020 reported an increase in ABA content of strawberries after 65 days of exposure (5 h per day) to sound frequencies of 0.1-1 kHz and sound pressure level (SPL) of 70-100 dB at different growth stages.

Salicylic acid (SA) is another plant hormone whose content is negatively affected by high ABA concentration. ABA induces the down-regulation of SA biosynthesis and inhibits SA-mediated responses (De Torres Zabala, Bennett, Truman, Grant, 2009). SA is one of the key phytohormones involved in plant response to environmental stresses as well as pathogen infection (Thevenet et al., 2017). In the present study, the SA content of both *S. splendens* and *T. patula* significantly decrease in response to traffic noise. The reduction in SA content was consistent with the increase in ABA content in response to traffic noise treatment.

Jasmonic acid (JA) plays a key role in plant defense mechanisms to wounding by pathogens as well as resistance to different abiotic stresses (Dar, Uddin, Khan, Hakeem & Jaleel, 2015). Increase in JA content and expression of JA-associated defensive genes occurs in the response to a wide range of abiotic stresses such as drought, salinity, heavy metals, and thermal stresses (Dar et al., 2015; Ahmad et al., 2016; Sharma & Laxmi, 2016; Farhang-Abriz & Ghassemi-Golezani, 2018). JA can protect plants from adverse effects of abiotic stresses by up regulating of the expression of stress related genes as well as regulating several physiological responses. JA can enhance the antioxidant (enzymatic and non-enzymatic) capacity of the plant cell, and therefore, low-off the ROS content and maintain the integrity of plant cells (Farhang-Abriz & Ghassemi-Golezani, 2019). JA accumulation in response to drought stress increased the transcripts and activity of several antioxidant enzymes including ascorbate peroxidase as well as enhancing the capacity of the non-enzymatic antioxidant system (Shan & Liang, 2010). In the present study, exposure of *S. splendens* and *T. patula* plant species to traffic noise led to significant increase in JA content, which is in consistent with increase in antioxidant capacity of these plant species under traffic noise. Similar to ABA, JA also interacts antagonistically with SA and suppresses the SA biosynthesis and signaling (Overmyer, Brosche and Kangasjärvi, 2003). Prominent increase in JA in response to traffic noise stress may have led to reduction in SA content observed in both *S. splendens* and *T. patula.*

Plant growth promoting hormones, including auxins, gibberellins and, cytokinins, play the ultimate role in plant growth by increasing the rate of cell division as well as cell expansion. In the present study, the biomass of plants treated with traffic noise decreased significantly compared to the control group. Reduction in the content of growth hormones IAA, GA3, and zeatin is the main reason for the stunted growth of both *S. splendens* and *T. patula* plants in response to daily traffic noise treatment.

Conclusions

Our results revealed that daily traffic noise can be considered an environmental stress for urban plant species, since it imposes the characteristic adverse effects of stress as well as inducing stress response mechanisms in plants. Similar to other environmental stresses, exposure of two different urban plants to traffic noise led to stunted growth and higher rate of H_2O_2 formation, followed by oxidative damage to bio-membranes, which is marked by an increase in MDA content. To overcome the oxidative damage, the activity of enzymatic and non-enzymatic antioxidant systems was increased in both *S. splendens* and *T. patula* species. The phytohormone content of both species was significantly affected by traffic noise exposure. Changes in phytohormones content are an essential component of the stress response in plants. Our result demonstrated a high degree of plasticity in response to traffic noise stress in both *S. splendens* and *T. patula*, as manifested in significant increase in enzymatic and non-enzymatic activity as well as alteration in hormonal content. The antioxidant metabolism is a key factor determining the ability of plants to withstand the noise-induced oxidative stress and alteration in the content of phytohormones and contributes to the proper regulation of responses to traffic noise stress.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.baae.2022.01.010.

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