

# Understanding the physiological and biophysical response of urban roadside plantations for assessing adaptation and mitigation mechanisms toward vehicular emissions

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## Research Article

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## Abstract

The plants follow various strategies for adapting and mitigating climatic and environmental stresses. This offers an opportunity to comprehend adaptation and mitigation mechanisms under changing climate and environment emerging from vehicular emissions. We deployed an approach, i.e., physiological (Phy) and biophysical (Bioph) functional traits (FTs) based approach to understand plant response for assessing adaptation and mitigation potential for offsetting long-term urban climate and environment. We investigated Phy and Bioph FTs of adaptation, especially transpiration rate ( $E$ ), stomatal conductance ( $G_s$ ) and resistance (SRes), water use efficiency (WUE), carboxylation efficiency (CE), mesophyll efficiency (ME), leaf thickness (LTh), and mitigation FTs, including CO<sub>2</sub> assimilation rate ( $A$ ), leaf dust retention efficiency (DRE), and cooling impacts (CI) of the roadside plantation (*Delonix regia* and *Callistemon viminalis*). The study reported the significant effects of the roadside urban environment on the modulation of FTs, one of the strategies that plants follow to adapt and mitigate the urban climate. The *C. viminalis* was reported as having a better adaptive nature with higher mitigation potential than *the D. regia*. It is advocated that the approach deployed in the study could be exploited for identifying more adaptive plantation species to develop urban green belts for mitigating urban climate and environments.

## 1. Introduction

Development has boosted urbanization (Douglas, 2000; Scott, 2008). Major urban and suburban regions of various countries, including India, have been experiencing rapid urbanization. At the same time, the urban areas are congested due to overpopulation and face environmental stresses such as ever-increasing vehicular traffic, shrinking forests, and high levels of air pollution (Tarr 1996; Douglass 2000; Chen et al. 2007; DeFries et al. 2010; Singh et al. 2020). These changes have adversely affected physical and biological systems of urban areas (Singh et al. 2017; 2018; 2020). Urban vegetation becomes important in an environment where trees can significantly reduce air pollution (Singh et al. 2020). It has attracted the attention of urban planners to include green space in urban planning (Caspersen et al., 2006; Dover 2015). Consequently, trees can also be affected by high air pollutants when continuously exposed to air pollutants. Tree tolerance as well as adaptive capacities, and therefore the effects of air pollutants on each species, differ across species (Singh et al. 2017, 2018, 2020). In response to changing air pollutants, plants respond differently, causing alterations in their physiological and biophysical attributes. All of these factors will ultimately impact plant growth; therefore, it is necessary to assess the adaptive capacities of different plant species for developing urban green spaces. (Kumar et al. 2021a; Singh et al. 2017, 2018, 2020). The vehicular, commercial and industrial emissions contribute to air pollutants in an urban environment. There is a spatial variation in air pollutants concentrations within urban areas. Areas adjacent to sources of emissions tend to have higher concentrations than those away from such sources. Urban space along the roads is continuously injected with vehicular emissions. Thus, the trees along the roadsides face more pollutants than the trees away from the roadside. Vehicular movements directly alter the physiological and phenological behavior of urban roadside plantations (Singh and Pal, 2017). The urban vegetation acts as a sink for vehicular air pollution. Roadside plantations are affected by the pollutant in terms of their physiology (carbon assimilation, transpiration, stomatal conductance, and water use efficiency), biochemistry (pigments, enzymes, proteins, secondary metabolites, etc.), and morphology (leaf expansion, leaf thickness, stomatal frequency, and density, bark thickness, etc.) (Kumar et al. 2021a; Singh 2021). Pollutants from vehicles in the urban limits can predominantly affect plant growth in two ways, i.e., directly from the air causing toxic effects and indirectly by altering soil nutrient availability. The changing stomatal behavior under the influence of higher pollutant concentration eventually affects plant growth (Gregg et al., 2003).

Plants and their foliar coverage provide surface area for the settlement and filtering of solid particles present in the form of aerosols and dust. The surface deposition of such particles may alter the structure and functioning to cause stunted growth, interrupted photosynthetic activity, reduced pigment synthesis, reduced productivity, and alterations in the physiological processes (Singh and Pal, 2017, Kumar et al. 2021b). Moreover, plants can minimize and mitigate air pollution notably, specifically gaseous pollutants such as SO<sub>2</sub>, NO<sub>x</sub>, CO<sub>2</sub>, and O<sub>3</sub> (Singh et al. 2020). The urban green cover has revealed a variety of positive responses ranging from improving microclimates to purifying air by removing nearly 15% O<sub>3</sub>, 14% SO<sub>2</sub>, 8% NO<sub>2</sub>, 0.05% CO, and 13% particulate matter from the surrounding atmosphere (Hirabayashi and Nowak, 2016).

It is essential to enlighten that the heat island effect is incredibly stressful for plantations in hot climates while it could be favorable in cold environments (Mimet et al. 2009). The temperature is one of the important guiding factors determining different phenophases of plants affecting overall growth (Kumar et al. 2019). The temperature variation affects physiological processes such as photosynthesis, transpiration, stomatal conductance, and other foliage processes (Mimet et al. 2009). Urban plantations have been reported to mitigate air pollution by creating local cooling and reducing the ambient air temperature. Air purification by trees is done by assimilating atmospheric carbon dioxide, heavy metals accumulation in plant tissues, and retaining dust on branches and leaves (Singh et al. 2017, 2020). Despite various positive roles, the trees play in ameliorating urban air pollution, minimal studies have been conducted to understand the adaptive physiological response and mitigation potential of the urban roadside plantation. If implemented, such studies help in screening the utmost appropriate species for urban greening and roadside plantation (Kumar et al. 2021a; Singh et al. 2020).

The roadside environment provides an opportunity to test mainly, but not exclusively, the influence of higher ambient air temperature (Singh et al. 2020), elevated ambient CO<sub>2</sub> concentration (McCarthy et al. 2010; Singh et al. 2017; Singh et al. 2018), and higher concentration of other pollutants in the air, water, and soil (Grote et al., 2016). Literature argued that sufficient consideration had not been given to exploiting urban roadside conditions as a natural open lab to study the influence of the altered environment on the plants. However, we could find some interesting studies on the impact of air pollutants on the roadside plantation species (Gregg et al. 2003; Singh et al. 2017; Singh et al. 2020). Besides, the studies available could be referred to for developing the strategy and plan with the appropriate model for creating green belts along the roadside (Singh et al. 2017; Kumar et al. 2018a; Kumar et al. 2019).

Recently in India, various developmental initiatives such as National Green Highway Mission, Smart Cities Mission, and Nagar Van Scheme have been launched by the Indian Government to drive the country's economic growth and improve the quality of people's lifestyles. Such ambitious projects require information on tree species that would be most suitable for greening urban and roadside spaces. Management of forest areas adopting the principles of the

working plan code (Kumar et al. 2019) may also have the appropriate representation of air-purifying plants. This would require exact information to select species with better adaptive capacity and air pollution mitigation potential (Singh et al. 2020; Gupta et al. 2018a, 2018b, 2019).

The development of urban green areas and green belts raise specific demands regarding the selection of better species that adapt and mitigate roadside air pollution and assist in cooling the ambient environment. Hence, the present study is aimed to assess modulation in physiological and biophysical functional traits linked to adaption and mitigation ability of roadside plantations of *Delonix regia* and *Callistemon viminalis* exposed to vehicular emission in the urban environment. This study is expected to answer the queries that can most effectively be used by the urban planners and authorities involved in developing urban green spaces, including roadside plantations. The study also establishes a new methodology for prioritizing plants that could be used most successfully to lessen vehicular air pollution and establish a cooler urban climate.

## 2. Material And Methods

### Study site

The study was performed in Dehradun, the capital city of the Himalayan state of Uttarakhand in India. Dehradun is a fastly expanding Himalayan city and has a predominately tropical and temperate climate. The meteorological parameters obtained from Forest Meteorological Observatory, Forest Research Institute, Dehradun showed a minimum and maximum air temperature range between 4.0 to 34.0°C. The last 20 years' data (1999–2019) of rainfall revealed the highest rainfall during August (568.12 mm) and a minimum in November (3.74 mm). The city has been facing challenges associated with rapid urbanization and climate change problems. With the increasing economy and improved lifestyle, the number of vehicles increased in this region. Over congestion of the city's traffic and the growing number of vehicles are significant problems that influence the roadside environment. Besides, activities like new road construction repairing, and widening of existing roads are a few concerns impacting the roadside environment.

To study the modulations in a plant under the influence of vehicular emissions, a stretch of the roadside plantation of *Delonix regia* and *Callistemon viminalis* was chosen on Dehradun to Chakrata road (Fig. 1). A strip sampling design was adopted to select tree species (six individuals of each species) in selected roadside plantations with replicates following the methodology of Singh et al. (2017; 2020). The plantation of the same species inside the premises of a reserve forest, i.e. Forest Research Institute (FRI), Dehradun, was considered a control site with very limited vehicular movements throughout the year. The frequency of vehicular movements in these two different environments distinguishes them. The air pollutants, especially particulate matter (PM), CO, SO<sub>2</sub>, NO<sub>x</sub>, CO<sub>2</sub>, Hydrocarbon, and Pb, are prevalent on roadsides of urban premises (Singh et al., 2020; Dehradun City Air Action Plan, 2021). Vehicles are the significant sources of these pollutants in roadside urban premises (Singh et al., 2020). There was the movement of various categories of vehicles such as two-wheelers, three-wheelers, four-wheelers, minibus, and heavy vehicles in the selected roadside of the urban area in the present study. However, FRI officials' vehicles, mainly two and four-wheelers, are moved within the FRI campus (control). The air temperature of the urban roadside (experimental site-as natural open lab) and FRI (control site) was observed as 26 ± 0.56 and 22 ± 0.49 °C, respectively. Atmospheric CO<sub>2</sub> concentrations at the urban roadside and FRI was reported approx 470 ± 8.65 ppm and 410 ± 5.78 ppm, respectively.

The distinct prevailing urban roadside environments compared to the control sites makes it an ideal condition for considering urban roadside environments as a "Natural Open Lab" (i.e., experimental site) to study the influence of prolonged elevated levels of pollutants and temperature upon the functioning of trees. The varying level of air pollutants modulates the structure and functioning of plants. The alterations are often initiated in plants to cope with changing environmental circumstances to minimize and sustain any negative impacts. Thus, trees planted along the roadside may depict such alterations compared with the plants grown in areas away from the road or where the particulate and gaseous pollutants are relatively low in concentration. Therefore, the roadside environment is a unique space where a somewhat higher air pollutant concentration is witnessed. This provides a unique opportunity to employ this space as a "Natural Open Lab" unlike the artificially created situation where researchers test the influence of a high dose of gaseous pollutants in a laboratory or experimental chambers. Artificially created setup to study the effect of a higher amount of CO<sub>2</sub> and other environmental variables is costly and may not be affordable for every researcher (Kumar et al. 2021a Singh et al. 2017). Therefore, roadside space might be used as a natural open lab to test plants' physical and physiological modulations under the influence of a high dose of air pollutants. This will require a comparative study of plant functional traits grown in a cleaner and roadside environment.

### Vehicular frequency in the experimental and control site

The frequency of vehicles passing in both directions was counted manually with direct ocular observation in the experimental (city road) as well as a control area (FRI road) and expressed as the number of vehicles passing per hour (i.e. vehicular frequency). The vehicle frequency was counted from 07:00 to 22:00 (Singh et al. 2017; 2020).

### Assessing the response of physiological traits of plantation species

To monitor pollution adaptation and mitigation-related physiological traits of trees, the canopy of selected trees in each sampling unit falling in a strip/stretch was stratified into three canopy layers, i.e. bottom, middle, and top. Furthermore, each canopy layer was divided into four directions i.e. north, south, east, and west, thus making twelve sampling points for each tree. Foliage physiological traits were measured from twelve sampling points in the canopy to represent an average of the entire canopy. The trees of even age were chosen from both sides of roadside plantations. A bifurcated aluminum ladder with a flat top was used to reach each canopy layer for monitoring physiological functional traits. A portable photosynthesis analyzer (LICOR-6400 XT, Lincoln, NE USA) was used for monitoring the physiological traits, such as carbon assimilation rate ( $A$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), transpiration rate ( $E$ ,  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), and stomatal conductance ( $G_s$ ,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ). The inverse of stomatal conductance ( $G_s$ ) was considered as stomatal resistance ( $1/G_s$ ). The stomatal resistance ( $StoRes$ ) was expressed as  $\text{sec m}^{-1}$  (Singh et al. 2017). The physiological water use efficiency (WUE) was computed as the ratio of carbon assimilation ( $A$ ) to

the transpiration rate ( $E$ ). Carboxylation efficiency ( $CE$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was obtained from the ratio of carbon assimilation rate to intercellular  $\text{CO}_2$  concentration ( $C_i$ ) (Singh et al. 2018; Sharma et al. 2018). Mesophyll efficiency ( $C_i/G_s$ ) was computed as the ratio of intercellular  $\text{CO}_2$  concentration ( $C_i$ ) to stomatal conductance ( $G_s$ ) (Kumar et al. 2021b; Kumar et al. 2021c). All the parameters were monitored between 09.30 am to 12.00 pm from the leaves in clear sky conditions. Photo-synthetically active radiation (PAR) ranged between 700 to 900  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  while measuring physiological traits.

#### Assessing the response of biophysical adaptive traits of plantation species

The biophysical traits of leaf associated with plant adaptation to stress conditions were estimated from trees selected in both sites. Leaves provide surface area for settling dust and other solid particles where adsorption takes place through the leaf surface. The stomatal openings are also affected, and the deposition within the leaf tissues takes place through these openings also. This ultimately affects plant physiology. The accumulation within the leaf tissue results in the thickening of leaves. Leaf thickness (LTh, mm) is one of the critical leaf adaptive traits toward air pollution. Leaf thickness was measured using a millimeter scale of a digital vernier caliper. The average value of ten leaves selected in a bundle was considered for reporting LTh.

#### Assessing leaf dust retention efficiency (DRE, a mitigation trait) of plantation species

The leaf dust retention efficiency of trees is one of the biophysical indicators of mitigation traits that can be affected by the arrangement of epidermal cell roughness of leaf surface, trichome density of leaf surface, and the quantity of dust deposited on the foliar surface of leaves. Morphological features of tree foliage boost the filtering of dust and particulate matter. For assessing DRE, branches were tagged, and then the leaves of these tagged branches were cleaned with tissue paper. These leaves were allowed to deposit dust for the next 24 hours, after which they were plucked and weighed immediately (initial weight). Afterward, dust deposited leaves were kept in the pre-weighed zip lock bag and brought to the laboratory. The dust from the leaves was removed by a fine brush and washed with distilled water. Further, the leaves were dried with tissues paper and left for 10 minutes at room temperature. Afterward, the leaves were weighed using a digital balance (final weight). The final weight was subtracted from the initial weight. Further, the zip lock bag weight was subtracted from the difference between the initial and final weights for obtaining the dust load on the leaf surface. The leaf area of the same leaves was computed using the graph paper method (Singh et al., 2017). The DRE of the tree species was calculated as the ratio of the amount of dust deposited on the leaf surface (leaf area) over the period. It was expressed as  $\text{mg cm}^{-2} \text{ day}^{-1}$  (Singh et al., 2017).

#### Assessing atmospheric cooling (an indicator of climate mitigation) created by plantation species

Atmospheric cooling (cooling impacts-CI) created by the canopy of the tree species was computed as per the methods described by Gupta et al. (2018a; 2018b). The air temperature under and outside the canopy was measured using air temperature sensors inbuilt into a portable photosynthesis analyzer (LI-COR-6400 XT, Lincoln, NE USA). The air temperature was measured in all directions under the canopy and outside the canopy of trees, while the measurement of temperature was taken at a distance of 1.5 above the ground (Kumar et al., 2021a). The air temperature below the canopy was subtracted from outside the canopy to obtain cooling impacts (CI) or cooling created by the plantation species and expressed as  $^{\circ}\text{C}$ .

#### Statistical analysis

The data were subjected to statistical analyses using R Studio statistical (4.0.2 version) software. The significance of physiological and biophysical functional traits for both the sites and species was assessed by two-way variance analysis (ANOVA). Comparisons between studied characteristics under both the species and sites were made with Duncan's multiple range test at the significant difference ( $p < 0.05$ ). Principal component analysis (PCA) was performed among all the traits for both the sites and species. The data are presented as the mean of the observation along with standard error.

### 3. Results

Vehicular emissions greatly influenced the roadside environment. We reported approximately 1012 and 62 vehicles per hour passed in both the directions of the national highway and road of control site, i.e., FRI, respectively. This considerable difference between the vehicle numbers at both sites created a distinct environmental scenario, including pollutants concentration. This scenario of the urban roadside environment provided an opportunity to view the roadside environment as an open natural lab to investigate the influence of high concentrations of contaminants on the tree species. The modulation in various traits associated with adaptation and mitigation potential of plants, i.e., physiological and biophysical characteristics, in response to environmental circumstances (urban and control) owing to vehicular emission is presented below.

#### 3.1 Modulations in physiological traits under the influence of vehicular emissions

##### Carbon assimilation rate (A)

In the present study, the carbon assimilation rate ( $A$ ) was significantly ( $p < 0.001$ ) higher in *C. viminalis* ( $9.12 \pm 0.74 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) than *D. regia* ( $6.91 \pm 0.63 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) (Fig. 2). Although, roadside environments greatly amplified the carbon assimilation rate compared to the controlled environments, i.e., FRI ( $p < 0.001$ ) (Fig. 2). Besides, a significant influence on carbon assimilation rate was reported owing to the interaction of species and site ( $p < 0.03$ ) (Table 1).

##### Transpiration rate (E)

Similarly, the transpiration ( $E$ ) was significantly ( $p < 0.0001$ ) reported more for the plantation of *C. viminalis* ( $1.56 \pm 0.11 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) than *D. regia* ( $0.82 \pm 0.05 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ). It was also reported that the urban roadside environment had a significant ( $p < 0.0001$ ) impact on the transpiration rate of

the plantation. A higher transpiration rate was reported in the roadside environment ( $1.43 \pm 0.14 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) than in FRI ( $0.96 \pm 0.12 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ). We found a significant impact of species and environment on transpiration rate (Table 1).

#### Stomatal conductance (Gs)

The stomatal conductance (Gs) was significantly ( $p < 0.001$ ) higher ( $0.084 \pm 0.005 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) in *C. viminalis* compared to *D. regia* ( $0.027 \pm 0.003 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) (Fig. 3). The roadside environment significantly ( $p < 0.013$ ) influenced the stomatal conductance of the plantation (Fig. 3). The higher stomatal conductance was attained by roadside trees ( $0.63 \pm 0.11 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) compared to FRI ( $0.048 \pm 0.01 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) (Fig. 3). The interaction of species and site significantly impacted the stomatal conductance in the present study (Table 1).

#### Stomatal resistance (StoRes)

The stomatal resistance (StoRes) was profoundly higher ( $p \ll 0.0001$ ) reported for the plantation of *D. regia* ( $31.73 \pm 0.86$ ) rather than *C. viminalis* ( $12.95 \pm 0.61$ ) (Fig. 3). The urban roadside environment significantly ( $p \ll 0.0001$ ) reduced StoRes ( $15.16 \pm 0.72$ ) of both the species compared to FRI ( $29.53 \pm 1.44$ ) (Fig. 3). Besides, the interactive impacts of species and sites had a significant ( $p < 0.0001$ ) impact on StoRes of the plantation species (Table 1).

#### Water use efficiency (WUE)

It was astounding that one of the critical physiological adaptive traits, i.e., water use efficiency (WUE), of both the tree species enhanced significantly ( $p \ll 0.0004$ ) in the urban roadside environment ( $8.24 \pm 0.63 \text{ } \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) compared to the control site ( $6.25 \pm 0.54 \text{ } \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) (Fig. 3). Among both the species, *D. regia* ( $8.38 \pm 0.46 \text{ } \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) significantly ( $p \ll 0.0003$ ) attained higher WUE than *C. viminalis* ( $6.11 \pm 0.34 \text{ } \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) (Fig. 4). The interrelations of species and sites significantly induced the WUE of the plantation (Table 1).

#### Carboxylation efficiency (CE)

Carboxylation efficiency (CE), a measure of activity and efficiency of Rubisco, is one of the adaptive key traits that play a pivotal role during  $\text{CO}_2$  assimilation and carbon fixation in long-term stress. The study showed a significant ( $p \ll 0.0001$ ) augmentation in the CE of the selected plantation species at urban roadside conditions ( $0.05 \pm 0.006$ ) compared to the control ( $0.02 \pm 0.002$ ) (Fig. 4). In the present investigations, higher CE was reported for *C. viminalis* ( $0.05 \pm 0.005$ ,  $p \ll 0.0001$ ) than *D. regia* ( $0.02 \pm 0.002$ ). The combined impacts of species and sites showed significant ( $p < 0.0001$ ) improvement in the CE of the species (Table 1).

#### Mesophyll efficiency (ME)

Mesophyll efficiency (ME) was significantly ( $p \ll 0.0004$ ) higher attained by *D. regia* ( $16153.25 \pm 276.79$ ) than *C. viminalis* ( $2780.34 \pm 225.15$ ) (Fig. 4). The roadside environment significantly ( $p \ll 0.37$ ) augmented ME of urban roadside plantation ( $10499.43 \pm 3769.81$ ) than control, i.e., FRI ( $8434.15 \pm 1644.29$ ) (Fig. 5). It was also reported that species and environment interrelations had significant ( $p < 0.11$ ) impacts on the ME of the plantation (Table 1).

## 3.2 Modulations in biophysical traits under the influence of vehicular emissions

#### Leaf thickness (LTh)

It was reported that vehicular emissions altered the leaf's biophysical properties, especially leaf thickness (LTh), which plays a critical role in the physiological adaptation of plants towards the mitigation of air pollution or abiotic stresses. In this study, it was fascinating to observe that the LTh, an adaptive trait of both the species, was found to be significantly ( $p \ll 0.11$ ) increased for urban roadside plantations ( $0.21 \pm 0.02 \text{ mm leaf}^{-1}$ ) than FRI ( $0.18 \pm 0.02 \text{ mm leaf}^{-1}$ ) (Fig. 5). Among the species, *C. viminalis* ( $0.26 \pm 0.003 \text{ mm leaf}^{-1}$ ) had significantly ( $p \ll 0.004$ ) demonstrated higher LTh than *D. regia* ( $0.13 \pm 0.002 \text{ mm leaf}^{-1}$ ) (Fig. 4). Besides, a significant ( $p < 0.24$ ) influence of species and environment interactions was reported on LTh of the plantation species (Fig. 5).

#### Modulation in local climate (i.e., cooling impact-CI)

Urban plantations have been recognized as an important modulator of microclimate. Trees of both species were found to reduce the local or surrounding air temperature at both sites (i.e., roadside and control sites). Higher local CI was created by *C. viminalis* ( $2.71 \pm 0.08 \text{ }^\circ\text{C}$ ) than *D. regia* ( $1.17 \pm 0.03 \text{ }^\circ\text{C}$ ) and showed a significant difference ( $p \ll 0.0001$ ). Although we had not observed a significant ( $p \ll 0.11$ ) difference in local cooling between urban roadside ( $1.83 \pm 0.15 \text{ }^\circ\text{C}$ ) and controlled conditions ( $2.05 \pm 0.33 \text{ }^\circ\text{C}$ ), it was slightly higher in the control site, i.e., FRI (Fig. 6). However, the species and sites interactions reflected a significant ( $p < 0.0005$ ) impact on the local CI created by the plantation species (Table 1).

#### Leaf dust retention efficiency (DRE)

The leaf dust retention efficiency (DRE) of the leaves is one of the biophysical indicators of the mitigation potential of trees to reduce dust pollution. The DRE of *C. viminalis* ( $3.2 \pm 0.04 \text{ mg cm}^{-2} \text{ day}^{-1}$ ) was higher recorded than *D. regia* ( $1.4 \pm 0.03 \text{ mg cm}^{-2} \text{ day}^{-1}$ ) (Fig. 6). However, we reported significantly ( $p \ll 0.40$ ) higher DRE for the urban roadside plantations ( $2.8 \pm 0.02 \text{ mg cm}^{-2} \text{ day}^{-1}$ ) of both the species than control, i.e., FRI ( $1.8 \pm 0.04 \text{ mg cm}^{-2} \text{ day}^{-1}$ ) (Fig. 6). The species and site interactions significantly ( $p < 0.41$ ) influenced the DRE of the plantation species in the present study (Table 1).

## 3.4 Principal component analysis (PCA)

The PCA analysis among the different physiological traits showed that the studied sites were separated in 2-dimensional ordination diagrams by site-specific physiological characteristics. The first PCA axis in the case of *D. regia* depicted the 76.36 percent differences in different physiological plant functional traits and their impact on dust removal efficiency and stomatal resistance (Fig. 7). The PCA results positively correlate with photosynthesis rate, stomatal conductance, water use efficiency, and mesophyll efficiency. These traits are more efficient in increasing the stomatal resistance under vehicular emission. However, these traits are not positively efficient in reducing the dust load as they were negatively correlated with DRE. The second axis showed significantly less (8.67%) variations among the physiological traits (Fig. 6).

Similarly, the PCA analysis for *C. viminalis* (Fig. 8) showed 83.43 percent variations in different physiological plant functional traits. The leaf thickness, dust removal efficiency, and water use efficiency are positively related. But, these are negatively associated with photosynthesis rate, stomatal conductance, and transpiration rate, as depicted in the 2-dimensional ordination diagram of PCA (Fig. 7). The PCA results for *C. viminalis* showed that increasing the CI, ME, WUE, LT<sub>t</sub>, and DRE causes a decrease in stomatal conductance in vehicular emission.

## 4. Discussion

The present study revealed a strong relationship between adaptation and mitigation governing traits. It was interesting to observe that various traits correlate among themselves for both species under investigation. Any impact on the functional traits of the tree would have a direct or indirect effect on the growth of a tree which is an important emerging discipline of science where the present study could be used effectively. The developers of the process-based dynamic vegetation model (Rawat et al., 2020; Kumar et al. 2018a) often need information on the relationship between the traits of a tree and the surrounding environment (Kumar et al. 2018b, 2019; Rawat et al. 2020). The results of the present study can effectively be used in such models. We observed that the air temperature acts as one of the key drivers which regulate the CO<sub>2</sub> assimilation rate of both species.

### CO<sub>2</sub> assimilation and transpiration rate

The CO<sub>2</sub> assimilation and transpiration rate were positively correlated for both species. IPCC predicted that by the end of the 2100 century, atmospheric temperature and CO<sub>2</sub> concentration would increase. It means it will have a definite impact on the various traits of the tree species. It was reported that the elevated pollutant loads in the urban roadside environment significantly affected tree species' physical and physiological characteristics of selected species. Earlier studies have also reported these effects by Grote et al. 2016; Hofman et al. 2013. Vehicular pollution in the urban environment affects the physiological traits and functioning of the roadside plantation (Chaturvedi et al. 2013; Singh et al. 2017). We also observed that physiological traits (CO<sub>2</sub> assimilation rate, transpiration rate, stomatal conductance, and water use efficiency) of the roadside plantation species i.e. *D. regia* and *C. viminalis*, were highly influenced by the urban roadside environment. However, to compare such responses, one should be comparing the observations made under a similar climate as any difference in air temperature, soil conditions, pollutant loads, etc., also affect the functioning of the trees. The present results findings were similar to previous studies implemented by Sharma et al. 2018; Singh et al. 2018; and Yadav et al. 2019. Limited research for a few selected tree species has been conducted to test the impact of vehicular emissions on different traits of a plant (Gregg et al., 2003; Hofman et al. 2013; Grote et al. 2016; Singh et al. 2018; Singh et al. 2020). Few studies pointed out that increased CO<sub>2</sub> assimilation of urban roadside vegetation might be due to the low light conditions compared to better light conditions prevalent in the less disturbed environment. Similarly, under low light conditions, photo-inhibition is prevented and trees maintain higher photosynthesis (Takagi and Gyokusen, 2004; Gago et al., 2016). The other physiological traits, such as transpiration, stomatal conductance, and water use efficiency, increase under elevated CO<sub>2</sub> conditions (Takagi and Gyokusen, 2004). The study also demonstrated that the water use efficiency of the species got enhanced under the urban roadside environment compared to the control site which was supported by findings of previous researchers (Grote et al. 2016; Hofman et al. 2013; Singh et al. 2018; Singh et al. 2020).

### Stomatal resistance

Stomatal resistance is one of the vital plant functional traits for assessing the adaptive physiological response of plants to changing environmental conditions (Irmak and Mutiibwa, 2009). Leaf resistance is the function of several parameters of the leaf (transpiration, stomatal conductance, leaf thickness, presence of hair on the leaf surface, etc.) as well as atmospheric variables (temperature, atmospheric CO<sub>2</sub> concentration, air pollutants, etc.) (Irmak and Mutiibwa, 2009; Kumar et al., 2021a). In the present study, the stomatal resistance was higher in the urban roadside environment compared to the control site. The functioning of trees for stomatal conductance under the influence of changing climate has not been studied extensively. Irmak and Mutiibwa, (2009) reported decreased stomatal resistance under the influence of increased air temperature, CO<sub>2</sub> concentration, higher solar radiation, and vapor pressure deficit. We also observed a similar trend of stomatal conductance in the present study.

### Leaf thickness

Enhanced leaf thickness is one of the adaptive biophysical indicators of plants to cope with harsh environmental or abiotic stress conditions. It was observed that roadside plants had more leaf thickness than the control site. More leaf thickness of roadside plantations might be due to the accumulation of heavy metals in the epidermis layer of the leaves (Singh et al., 2017; Singh et al., 2020). Singh et al. 2017 and Singh et al. 2020 found increased leaf thickness in the roadside plantation of *Mangifera indica* and *Grevelia robusta* compared to the less polluted areas. Increased leaf thickness in the air-contaminated area induces the sinking of stomata and causes a decline in transpiration rate and improved water use efficiency (Grote et al. 2016; Hofman et al. 2013). Vegetation responds to air pollution by modulating or adjusting a few key traits such as stomatal closing and opening in terms of stomatal conductance, thus improving water use efficiency in a way that resembles the feedback to the higher temperature and CO<sub>2</sub> concentration (Hamaoui-Laguel et al. 2015). Hence, increased leaf thickness under the urban roadside in heavily polluted conditions is one of the modulations for adapting to the harsh situation.

### Leaf dust retention efficiency

Dust retention efficiency of the leaf was better on the roadside plantation. The deposition of particulate matter on the leaf surface results in partial or complete stomatal closing. The plants' dust removal efficiency has been considered a crucial biophysical trait for selecting tree species for urban greening and roadside plantations. Dust removal efficiency depends on the nature and architecture of the leaf, especially the leaf area, the presence of hairs on the leaf surface, and the waxy nature of the leaf that significantly varies from species to species (Beckett et al. 2000; Kardel et al. 2012; Brantley et al. 2014; Singh et al. 2017; 2020). The study on dust deposition efficiency in the urban plantation by Brantley et al. (2014) showed a decline of total black carbon by *Acer* and *Quercus* by approximately 12%. Elimination of dust particles through leaves of tree species also depends on leaves' micro-morphology (Wang et al., 2011). The vegetation removes particulate matter from the polluted urban atmosphere especially fine particles, which cause various health issues to the urban human population. The canopy and the total surface area of leaves are critical in deciding the dust removal efficiency of trees. For example, few trees with sparse leaves are less effective than those with a dense canopy. Tree species with tiny or hairy leaves are considered most excellent for removing and capturing dust particles from urban settings and roadways (Beckett et al., 2000; Chen et al., 2016; Grote et al., 2016; Hirabayashi and Nowak. 2016.). This study also found that *C. viminalis* with tiny and hairy leaves were more efficient in dust removal.

#### Atmospheric cooling (cooling impacts)

Atmospheric cooling/local cooling created by urban vegetation is a pivotal ecosystem service in the urban environment. This has been considered one of the benefits of maintaining a cooler environment (Grote et al. 2016; Singh 2021). Cooling is dependent upon various biophysical properties of trees such as canopy, leaf area index, evapotranspiration, canopy density, waxy and hairy leaf surface, reflectance nature of the leaf, pigmentation, etc. (Grote et al. 2016; Gupta et al., 2018a; Singh et al., 2017; Singh et al., 2020). The results of the present investigations are contradictory to the study conducted by Singh et al. (2020). Singh et al. (2020) reported higher cooling created by urban roadside plantations than in the control site with fewer disturbances. But in the present study, a higher cooling effect was observed at control sites compared to the urban roadside environment. The reverse trend could be related to the seasonal variation as both these studies were not done in a similar season. Singh et al. (2020) conducted the study during the summer season, while the present study was done in the winter season. However, both the species exhibited a significant difference in cooling created at both the sites, i.e., urban roads and the control site. Accumulation of dust particles on the leaf surface of the urban roadside plantation would be another reason for declining cooling impacts (Singh et al. 2020; Kumar et al. 2021a), as we reported in the present study. Moreover, few findings suggested that most of the transpiratory cooling occurs in the summer season rather than winter (Singh et al. 2017; Gupta et al., 2018b; Ziter et al., 2019; Singh et al. 2020).

#### Carboxylation efficiency

Carboxylation efficiency (CE) is one of the pivotal traits that determine the activity and efficiency of Rubisco which has a vital role in CO<sub>2</sub> assimilation. In the present study, carboxylation efficiency was reported higher for urban roadside trees than for the control site. Generally, Rubisco proteins are involved in the carboxylation reaction, where carbon in the form of CO<sub>2</sub> reacts with one of the substrates of the Calvin cycle, i.e. Ribulose 1,5 bisphosphate (RuBP), resulting in the flow of carbon throughout the process. There are a series of competing reactions of RuBP with the rubisco to fix O<sub>2</sub>, which leads to rubisco's oxygenase activity and the fixation of CO<sub>2</sub> through the carboxylase enzyme. Also, throughout the cycle, oxygen (~ 25% of the reaction) reacts to the substrate (RuBP) in place of CO<sub>2</sub>, which ultimately produces a molecule of 2-phosphoglycolate (2PG) and a molecule of 3-phosphoglycerate (3PGA) at the cost of one ATP and one NAD(P)H. Here in the stress condition, the metabolite 3PGA is utilized more in the cycle with high energy potential. In this way, it leads to an increase in the carboxylation efficiency of roadside trees (Zhu et al., 2010; Walker et al., 2016). Another reason for the increased carboxylation efficiency of roadside plantations might be the higher concentration of CO<sub>2</sub> in the roadside environment. Elevated atmospheric CO<sub>2</sub> concentration suppresses oxygenation reaction and induces a rate of Carboxylation reaction, thereby increasing more carbon fixation through increased photosynthesis rate (or CO<sub>2</sub> assimilation rate). We observed higher atmospheric CO<sub>2</sub> concentration at the urban roadside than at the control site. The carboxylation efficiency of both species was validated by several previous findings (Pfanz et al., 2007; Singh et al., 2018; Sharma et al., 2018; Yadav et al., 2019).

#### Relationship between physiological and biophysical plant functional traits

The CO<sub>2</sub> assimilation rate of both species was controlled by air temperature at both sites. Thus, temperature is one factor that governs carbon fixation in plants. The CO<sub>2</sub> assimilation rate was higher in the roadside environment as higher temperatures prevailed (Singh 2021; Yadav et al., 2019; Takagi and Gyokusen, 2004). Stomatal opening and closing regulate the gas exchange, i.e., transpiration and CO<sub>2</sub> assimilation rate. The entry of carbon molecules and exit of water molecules occurred from the same stomata. Both the species exhibited a significant and robust correlation between carbon (CO<sub>2</sub>) assimilation rate and transpiration (water exchange) rate. The stomatal conductance significantly correlated to transpiration and the CO<sub>2</sub> assimilation rate under this study. These results were in line with results obtained by various researchers (Pfanz et al., 2007; Singh et al. 2017; Singh et al., 2018; Gupta et al., 2018; Sharma et al., 2018). Apart from this, the CO<sub>2</sub> assimilation rate of both the plantation species was regulated by stomatal conductance and stomatal resistance. The relationship between stomatal conductance, stomatal resistance, and CO<sub>2</sub> assimilation rate was supported by findings of an earlier study by Singh et al. (2017).

## 5. Conclusion

It was concluded that both the plantation species considerably modulated their physiological and biophysical functioning for coping and adapting to the urban roadside environmental stresses. Furthermore, the plantation species *C. viminalis* was reported more adaptive with higher mitigation capabilities than *D. regia* in response to vehicular emissions within the urban premises. In addition, it is suggested that the approach deployed in this study might be adopted and much more exploited for long-term monitoring of physiological and biophysical responses to assess the tree adaptation and mitigation ability for consideration in plantation programs.

## Declarations

### Conflict of interest:

The authors declared that they have no conflict of interest while publishing this manuscript.

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## Table

Table 1: Interactive impacts of species and sites on the response of physiological and biophysical functional traits of *Callistemon viminalis* and *Delonix regia* plantation.

Species × site interactions	Physiological and biophysical plant functional traits								
	A	G <sub>s</sub>	E	StoRes	WUE	CE	ME	LT	CI
CM×S1	5.71±0.18c	0.071±0.008a	1.31±0.14b	14.61±0.81c	5.28±0.52c	0.025±0.002c	3631.30±201.39b	0.23±0.005a	3.10±0.005a
CM×S2	12.53±0.42a	0.095±0.007b	1.81±0.13a	11.30±0.67c	6.94±0.26b	0.072±0.003a	1929.36±151.84b	0.28±0.008a	2.31±0.005a
DR×S1	4.36±0.26d	0.024±0.002c	0.60±0.03d	44.46±4.04a	7.22±0.33b	0.016±0.001d	13237.01±1884.47a	0.12±0.001b	1.35±0.005a
DR×S2	9.43±0.68b	0.030±0.006c	1.04±0.03	19.01±2.27b	9.54±0.68a	0.032±0.003b	19069.50±4272.10a	0.13±0.003b	1.00±0.005a
F value	F=5.39	F=2.41	F=0.11	F=66.1	F=0.47	F=58.34	F=2.8	F=1.85	F=19
p value	p<0.03	p<0.13	p<0.74	p<0.0001	p<0.50	p<0.0001	p<0.11	p<0.24	p<0.0001

CM: *Callistemon viminalis*; DR: *Delonix regia*; S1: FRI; S2: Road side; A: carbon assimilation rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ); G<sub>s</sub>: stomatal conductance ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ); E: transpiration rate ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ); CE: carboxylation efficiency ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ); ME: mesophyll efficiency; LT: leaf thickness (mm); CI: cooling impact ( $^{\circ}\text{C}$ ); DRE: dust retention efficiency ( $\text{mg cm}^{-2}$ ); and *StoRes*: stomatal resistance ( $\text{sec m}^{-1}$ ).

## Figures

### Figure 1

The distribution map of sampled individuals of both the tree species on the roadside of urban and FRI plantations of *Delonix regia* and *Callistemon viminalis*.

### Figure 2

Response of physiological functional traits of roadside tree species (*Delonix regia* and *Callistemon viminalis*) at both the sites.

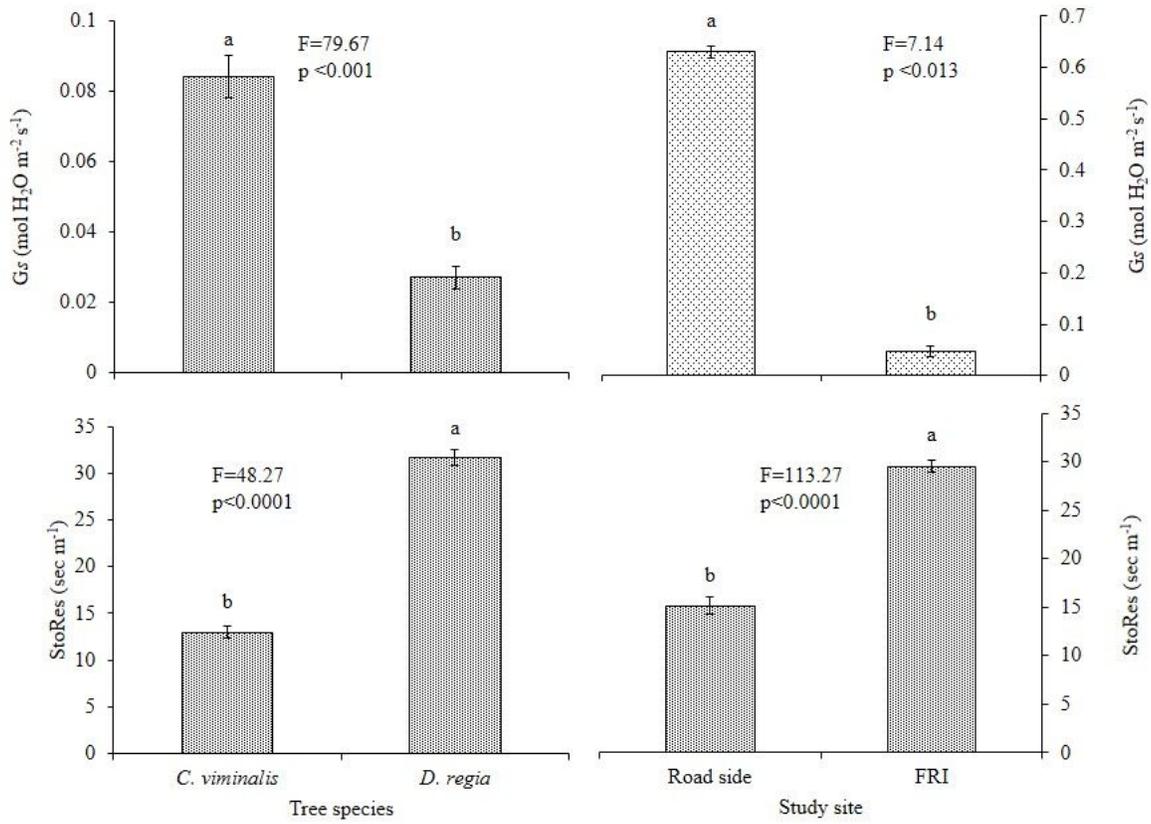


Fig. 3 Response of physiological functional traits of roadside tree species (*Delonix regia* and *Callistemon viminalis*) at both the sites.

Figure 3

Response of physiological functional traits of roadside tree species (*Delonix regia* and *Callistemon viminalis*) at both the sites.

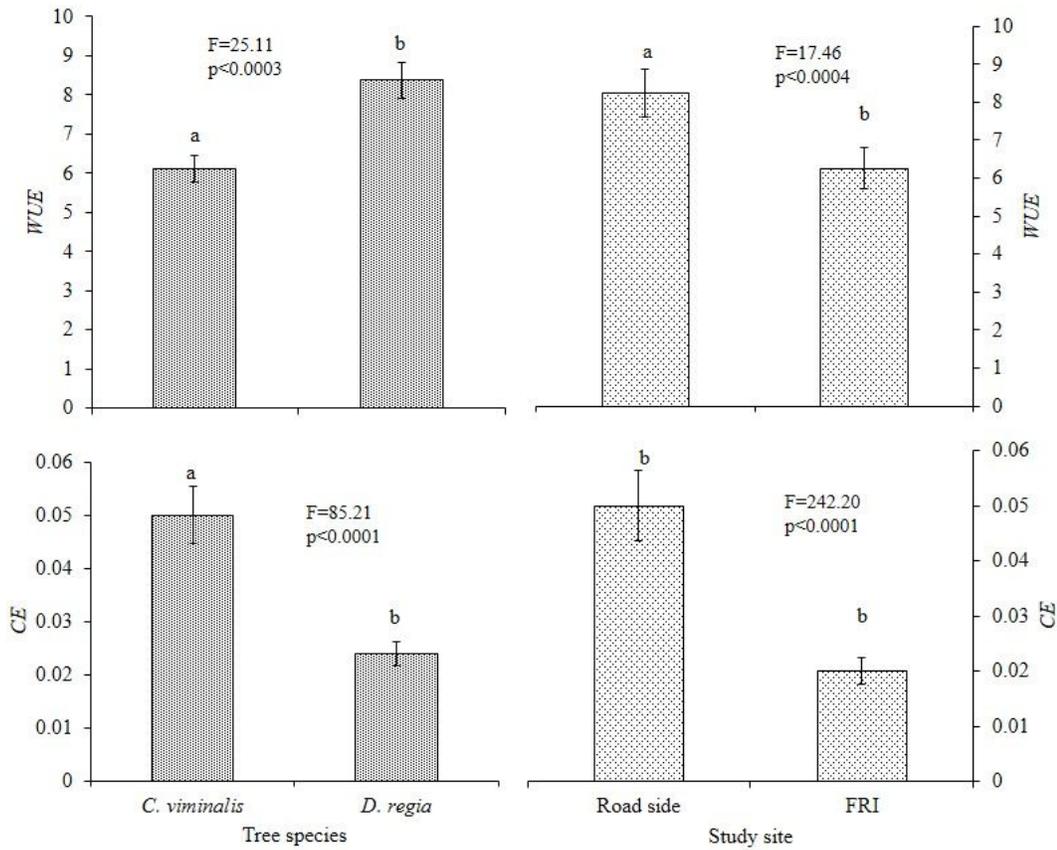


Fig.4. Response of physiological functional traits of roadside tree species (*Delonix regia* and *Callistemon viminalis*) at both the sites.

**Figure 4**

Response of physiological functional traits of roadside tree species (*Delonix regia* and *Callistemon viminalis*) at both the sites.

**Figure 5**

Response of physiological and biophysical functional traits of roadside tree species (*Delonix regia* and *Callistemon viminalis*) at both the sites.

**Figure 6**

Response of biophysical functional traits of roadside tree species (*Delonix regia* and *Callistemon viminalis*) at both the sites.

**Figure 7**

Principal component analysis of physiological and biophysical functional traits of *Delonix regia*.

**Figure 8**

Principal component analysis of physiological and biophysical functional traits of *Callistemon viminalis*.