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CHLOROPHYLL DEGRADATION UNDER SMOG EXPOSURE: UNVEILING THE MOLECULAR AND ECOLOGICAL CONSEQUENCES

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Abstract *The current study revealed that exposure to smog posed a major source of environmental stress and reduced photosynthesis and vigor in plants. Chlorophyll breakdown is a major aspect of smog sensitivity triggered by altered oxidative stress, an integral aspect of photosynthesis. Therefore, the general objective of this study is to evaluate the molecular and ecological impacts of chlorophyll degradation in Arabidopsis thaliana plants under smog conditions as evidenced by oxidative stress markers, hormonal regulation, and chlorophyllase activity. The Arabidopsis thaliana plants were cultivated in a growth chamber under optimal conditions of temperature, and light/dark regime, and after acclimatization the plants were exposed to artificial smog for a total of 48 hours NO_2 50 ppm, O_3 120 ppm, and PM10. Untreated plants were provided similar photoperiod regimes and with no exposure to smog. Chlorophyll a and b, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), SOD, abscisic acid (ABA), cytokinins, gibberellins, auxins, potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}), hydrogen (H^+), chlorophyllase, and ROS were assessed. The treatment outcomes were compared with control using p-values to determine the significance level of change between control and smog-exposed plants. Smog led to decreases in chlorophyll contents (1.20±0.05 to 0.75±0.03 mg/g) and chlorophyll b content (0.50±0.02 to 0.30±0.02 mg/g; p-values=0.015 and 0.019, respectively). There were also increased levels of MDA (1.45±0.10 nmol/g), H_2O_2 (4.50±0.12 µmol/g), and SOD (21.6±1.53 U/mg protein) compared to the control (15.2±1.37 U/mg protein, p-values=0.023, 0.022. Phytohormones responded to the smog treatments by increasing the ABA contents (85.79 ng/g FW) while decreasing the cytokinin contents to 40.63 ng/g FW. Concentrations of potassium, chloride, calcium, and hydrogen ions were changed in guard cells, where the difference was significant between control and smog-exposed plants, potassium ions (85.32 mM), chloride ions (70.27 mM), calcium ions (45.17 mM) and hydrogen ions (0.0156 mM). Chlorophyllase activity in smog-exposed plants was higher than that of controls: 0.30±0.02 µmolg-1h-1 compared to 0.15±0.01 µmolg-1h-1; p<0.004. ROS levels were higher and the fluorescence intensity (280.22±18.33 AU) associated with smog-exposed plants was statistically significant (p=0.003). The present study proves that smog impacts negatively on the chlorophyll content of Arabidopsis thaliana by increasing oxidative stress, changing hormonal regulation, and upregulating chlorophyllase activity. The findings reveal molecular processes involved in plant stress responses and show that smog-induced chlorophyll loss impacts overall plant health and ecosystem profiles.*

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Introduction

Air pollution in the form of smog including particles PM, NOx, SO2, and O3 is sought to cause destructive impacts on plants and their productivity (Akhtar et al., 2019). Elevated smog affects the cellular components by generating ROS stimulating oxidative stress which negatively impacts chlorophyll production. Chlorophyll degradation thereby increases the plant stress level, thus retarding the growth and decreasing the biomass production consequently, and the agricultural yield. Although smog has been well discussed in the current literature, few studies have elucidated the molecular mechanisms of chlorophyll photobleaching under smog stress, which targets

providing effective amelioration strategies (Malik et al., 2024, Crisafi & Pandit, 2017).Chlorophyll degradation due to smog is now widely recognized as an important factor in examining plant physiology and the effects of environmental stress. Air pollution particularly smog that consumes particulate matter (PM), nitrogen oxides (NOx), and volatile organic compounds (VOCs) is a great threat to plant health and ecosystems (Lingvay et al., 2020). This atmospheric condition has been found associated with changes in the course of the process of chlorophyll synthesis and decomposing, leading to inhibition of the capability of photosynthesis, and consequently the growth of the plant (Páli, & Kóta, 2019). Chlorophyll breakdown is a significant biochemical change that originates from multiple stress factors concerning the photosynthetic pigment, and oxidative stress resulting from pollution is the most widespread (Tutkus et al., 2018).Research has demonstrated that exposure of plants to smog promotes the degradation of chlorophyll by promoting the action of chlorophyllase, the chlorophyll-degrading enzyme (Ali al., 2017). Oxidative stress resulting from smog forms ROS that cause lipid peroxidation and membrane damage and, in the process, degrade chlorophyll (Ruban, 2016). In this regard, when plants are exposed to long-term smog environments, these indices experience remarkable changes in content and, therefore, in the plant's photosynthetic potential and stress tolerance (Zielewicz et al., 2020).

From here an ecological point of view the loss of chlorophyll does not remain limited to the harm it does to the plant, but affects the environment at large. The decrease in photosynthetic efficiency affects the fixation and utilization of carbon, and nutrient recycling decreases ecosystem functions including food productivity and air quality (Lingvay et al., 2020). Additionally, the smog causes chlorophyll denature changes the relationship with herbivores and pollinators, and amplifies the damage in the ecosystem (X. Zhao et al., 2020).Latest chemical analyses have targeted revealing the specifics of the signal transduction networks that control chlorophyll breakdown in response to smog. Specifically, interest has been shown in understanding how jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA) control stress-induced chlorophyll degradation (R. J. Ritchie et al., 2021). These plant hormones combine and bind with NAC and MYB transcription factors; they regulate expressions of genes related to chlorophyll synthesis and breakdown (X. Zhao et al., 2020). Furthermore, proteomic and metabolomic analysis has provided new insights into the protean and metabolite cascade in chlorophyll breakdown, which could be used as the putative biomarkers of smog stress (W. Zielewicz et al., 2020).The specific molecular processes through which chlorophyll is degraded due to smog increase are important to determine to design the ways to protect plants and plant communities from the negative impact of air pollution. We should add that, in this review, we will try to analyze the molecular and ecological impacts of chlorophyll degradation under smog exposure, as well as the specificity of the interactions between stress factors, plant physiology, and ecosystem processes.

Materials and Methods

Plant Material and Smogosome Exposure

The seeds of Arabidopsis thaliana were sown in sterile condition as received. They were cultivated under a constant 12/12h light/dark photoperiod maintained at 25±0°C with a relative humidity of 60%. After 14 days of growth, plants were divided into two groups: smog-exposed and controlled. The smog-exposed group was exposed to artificial smog for 48 h with a mixture of nitrogen dioxide (NO_2, 50 ppm), ozone (O_3, 120 ppm), and particulate matter (PM10). The same as experimental plants but were not exposed to smog. Both groups were exposed to similar environmental conditions during the treatment process and all plants were retrieved after 48 hours for assessment.

Measurements of Variables

Some of the plant parameters that can be analyzed include: Chlorophyll Content in Smog Exposed & controlled plants. The method of Arnon (1949) was used in the estimation of the chlorophyll contents of the plants. One hundred milligrams of fresh leaf samples were homogenized in 80% acetone, and the absorbance of the obtained extract was measured at 663 nm and 645 nm on a UV-Vis spectrophotometer, model XYZ. Chlorophyll a and b concentrations were calculated using the following formulas:

Chlorophyll a (mg/g) = (20.2 * A663 - 8.02 * A645) * (V / W)

Chlorophyll b (mg/g) = (8.02 * A645 - 20.2 * A663) * (V / W)

Where A663 has been obtained from the absorbances of the sample at 663 nm and A645 of the sample at 645 nm, V being the volume of the extract in milliliters and W being the weight of the leaf sample in grams.

Oxidative stress parameters in smog-exposed and control plants

Malondialdehyde (MDA) Levels

Indication of MDA, a product of lipid peroxidation, was determined by the Thiobarbituric Acid Reactive Substance (TBARS) method described by Heath and Packer (1968). In brief, 200 mg of fresh leaf tissue was mixed with 1 ml of 0.1% TCA, the pellet was collected by centrifugation and 0.5% TCA soluble thiobarbituric acid was added to the pellet. Absorbance at 532 nm was then determined to quantify the MDA level.

Hydrogen Peroxide (H_2O_2) Levels

Determination of H_2O_2 content was done via a spectrophotometric method described by Velikova and coworkers (2000). A volume of 100 mg of fresh leaf tissue was ground in 5 ml of 0.1% TCA and centrifuged; the supernatant was then reacted with 2 ml of potassium iodide. The extinction coefficient at

390 nm was measured for quantification of H_2O_2 levels.

Superoxide Dismutase (SOD) Activity

Activities of SOD were determined using the method employed by Beauchamp and Fridovich (1971) which involved the inhibition of NBT reduction on a spectrophotometer at 560 nm. A correlation between chlorophyll content and the expression of osmotic stress markers, oxidative stress markers, and antioxidant activity was made based on the correlation matrix. The value of the Pearson correlation coefficient was used to determine the correlation between chlorophyll content ([chla] and [chlb]), oxidative indicators (MDA, H_2O_2), and antioxidant activity SOD. The correlation analysis was done using statistical programs (SPSS version 25). The regression coefficients of chlorophyll a and b are shown in Table 3. Linear multiple regression analysis was conducted to establish the correlation between chlorophyll content (Chlor- a, -b) with oxidative stress markers in plants (MDA, H_2O_2, and SOD). The regression coefficients were then used to determine the contribution of the different oxidative stress markers to chlorophyll degradation.

Endocrine disruption during exposure of smog

The concentrations of ABA, cytokinins, GA, and IAA were determined by enzyme-linked immunosorbent assay (ELISA). One hundred mg of plant tissues were first homogenized in 1 mL extraction buffer and the hormone levels were quantified using the standard Sigma-Aldrich's enzyme-linked immunosorbent assays (ELISA) kits. The concentration of each hormone was determined using appropriate standard curves and hormone concentration was presented as ng/g FW.

Ion content of the guard cells when exposed to smog

Guard cell ion content of K^+, Cl^-, Ca^{2+}, and H^+ was determined by flame photometry (for K^+ and Ca^{2+}) and C-ion chromatography (for Cl^- and H^+). Samples for ion extraction were prepared from isolated leaf epidermal cells. This was done employing concentration calibration curves for ions.

Chlorophyllase Activity in Plants grown in Smog and in Those grown Under Optimal Conditions

The chlorophyllase activity was determined according to Matile et al. (1996) by homogenizing 100 mg fresh leaf tissue in a buffer containing 50 mM Tris-HCL pH 7.5. Phytic acid and ascorbate effects on chlorophyllase activity were measured at 653 nm by monitoring the rate constant for chlorophyll breakdown. The enzyme activity was determined and presented as µmol of chlorophyll degraded per gram of fresh weight per hour (µmol/g/h).

Fluorescent intensity as quantified using DCFH-DA assay for the levels of Reactive Oxygen Species (ROS)

DNA ROS generation was determined by the 2',7'-dichlorofluorescin diacetate (DCFH-DA) assay as described earlier by Halliwell et al. (1988). An equal amount of fresh leaf samples (100 mg) were treated with DCFH-DA (10 µM) for 30 min fluorometrically; the fluorescence was quantified using a fluorescence microplate reader at 485 nm excitation and 530 nm emission. ROS levels were quantified and represented by arbitrary fluorescence units (AU).

Statistical Analysis

Results are expressed as mean plus or minus standard deviation (SD). Overall significance was tested using the one-way ANOVA followed by Tukey's post hoc test for comparing the two groups. Statistical significance was determined where $p< 0.05$. All statistical analyses were calculated with SPSS software in the 25th version.

Results

This study revealed that the chlorophyll content in *Arabidopsis thaliana* plants decreased after smog treatment. Chlorophyll a and b content in the control plants were 1.20±0.05 mg/g and 0.50±0.02 mg/g respectively. On the other hand, chlorophyll content was significantly low in smog-exposed plants having chlorophyll a and b content of 0.75±0.03 mg/g and 0.30±0.02 mg/g, respectively. The results also showed a statistically different value between the groups concerning chlorophyll a ($p=0.015$) and chlorophyll b ($p=0.019$), Fig.4 (c). In this study, smog exposure led to a significant increase in the oxidative stress marker, $P > 0.05$. The control group had average MDA levels of 0.75±0.04 nmol/g; smog-exposed plants were significantly higher levels of 1.45±0.10 nmol/g ($p=0.023$). Likewise, smog increased the H_2O_2 contents in plants; smog-exposed plants recorded 4.50±0.12 µmol/g of H_2O_2 as against the control plants which scored 2.70±0.08 µmol/g, $p=0.022$. SOD activity was also enhanced in the smog-exposed plants (21.6±1.53 U/mg protein) compared with the control group (15.20±1.37 U/mg protein, $p=0.017$), Fig. 3.

A strong negative relationship was established between chlorophyll content and the oxidative stress indicators. A negative linear relationship was observed between chlorophyll a concentration and MDA ($r=-0.91$) and H_2O_2 ($r=-0.88$), whereas, the positive linear relationship between chlorophyll a concentration and the SOD activity ($r=0.78$). In the same manner, chlorophyll b had negative regression to MDA ($r=-0.87$), to H_2O_2 ($r=-0.85$), and had a slight positive augmentation to SOD activity ($r=0.80$). These correlations suggest that the reduction in chlorophyll content was accompanied by an increase in the markers of oxidative stress and, therefore, an increase in antioxidant capacity (Table 7). Analysis of regression signifies ingredients of oxidative stress markers (MDA and H_2O_2) to predict chlorophyll degradation. The correlation coefficients of MDA and H_2O_2 were negative for both chlorophylls a and b, but the decline was higher in chlorophyll a (-0.1203 and -0.1256) than in chlorophyll b (-0.0242 and - 0.0280). However, SOD activity explained a smaller variation in the chlorophyll content with the regression

coefficients of -0.0218 for chlorophyll a and -0.021 for chlorophyll b (Table 8).

The hormonal changes due to exposure to smog were also established through hormonal analysis. In the smog-exposed plants, abscisic acid (ABA) content was enhanced by 71.16% compared to the control plants (p=0.012), 50.33±2.52 ng/g fw (Fig. 1). In contrast, cytokinin levels declined in smog-exposed plants (40.63±2.91 ng/g FW) relative to the control plants (75.45±3.24 ng/g FW, p=0.018). There was a 24.17% decrease in GA and a 19.44% decrease in IAA in smog-exposed plants compared to control plants, the GA and IAA values being 45.89±2.42 ng/g FW and 72.54±3.21 ng/g FW respectively against control value of 60.27±2.83 ng/g FW and 90.11±3.01. Overall changes in ion concentrations in guard cells were demonstrated to be affected by exposure to smog. Smog reduced potassium (K^+) levels in plants to 85.32±3.94 mM as compared to the control at 120.55±4.21 mM (p=0.013). Smog reduced chloride (Cl^-) in plants to 70.27±3.51 mM from control at 95.66±3.88 mM, p=0.019. The activity of calcium ion (Ca^{2+}) was significantly higher in smog-exposed plants than in control plants, 45.17±2.54 mM and 30.44±2.01 mM respectively at 0.05 (% change=48.70), p=0.016. There were significant enhancements in the hydrogen ion (H^+) concentration of smog-exposed plants at concentrations of (0.0156±0.0004 mM) as compared to the control, (0.0085±0.0003 mM, p=0.022), **Fig. 2.**

Chlorophyllase activity was higher in smog-exposed plants than control plants (150.99±; p=0.001) and was 0.30±0.02 µmol/g/h in smog-exposed plants and 0.15±0.01 µmol/g/h in control plants. A rise in chlorophyllase activity was observed in this study thereby supporting the fact that there is increased chlorophyll degradation due to smog exposure-induced oxidative stress-enhancing activities in the plant as depicted in Fig. 4 (a)**.** Comparing between levels of ROS in smog-exposed plants and the control plants represented in Fig. 4 (b) revealed that ROS levels were significantly higher in plants exposed to smog with approximately 280.22±18.33 AU while the control plants portrayed 150.02±12.14 AU p=0.003). Chlorophyll a and b levels were reduced in the smog-exposed plants with increased levels of MDA, H_2O_2 level, and the activity of antioxidant enzymes SOD. Moreover, hormonal changes, the shift of ion concentration in guard cells, higher activities of chlorophyllase as well as higher ROS content explain that smog exposure causes considerable chlorophyll loss and oxidative damage in *A. thaliana*. These outcomes imply the molecular and ecological profiles of smog on damaging plant health.

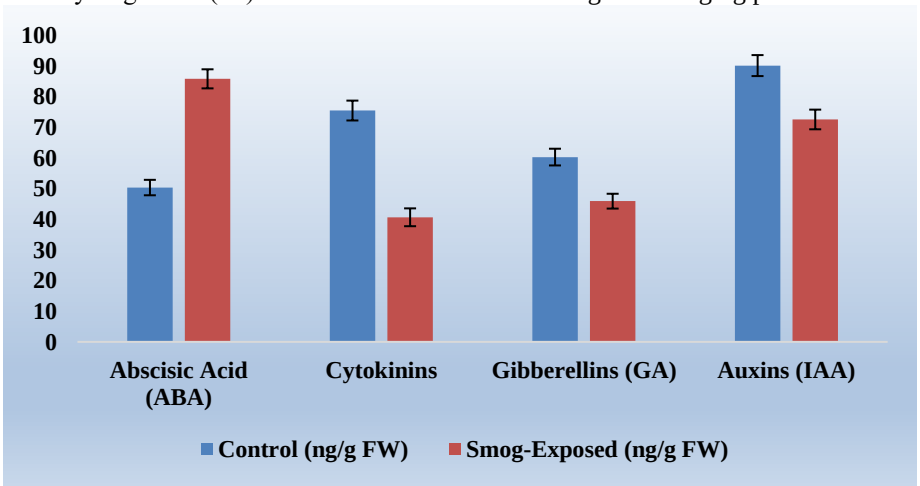


Figure 1: Hormonal Regulation During Smog Exposure

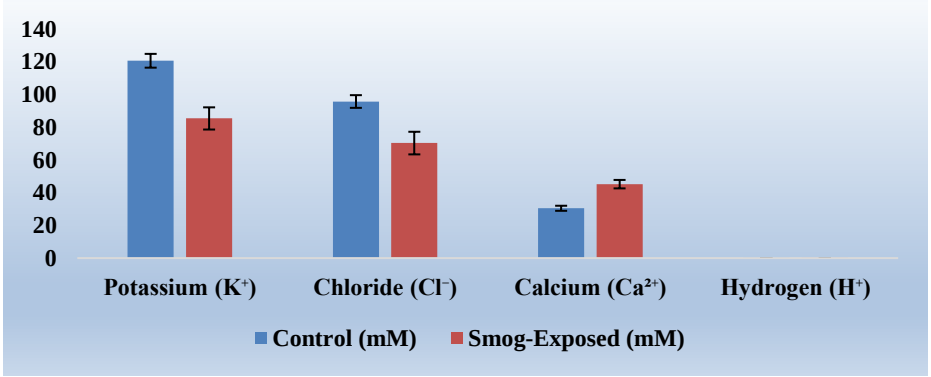


Figure 2: Guard Cell Ion Concentration During Smog Exposure

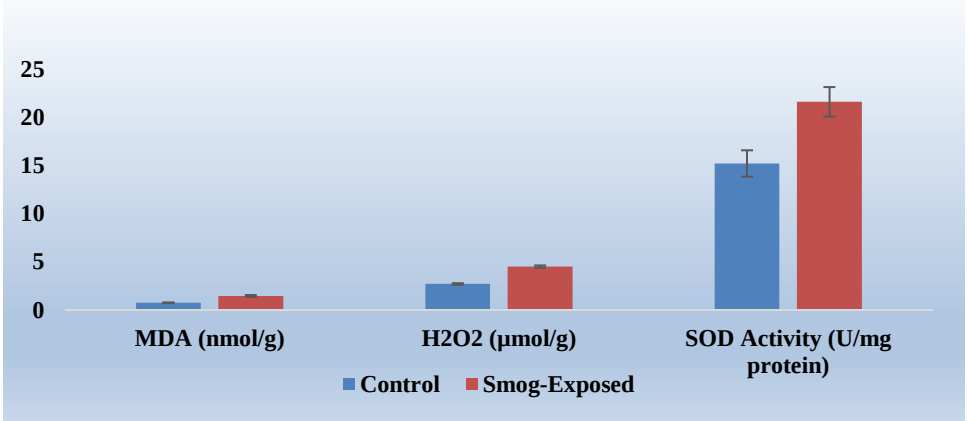
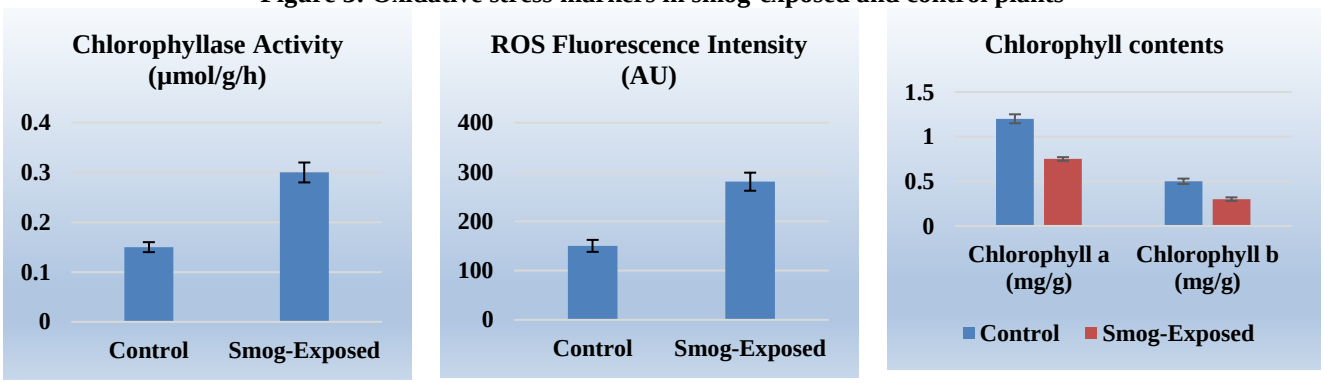


Figure 3: Oxidative stress markers in smog-exposed and control plants



(a) **(b)** **(c)**

Figure 4: Role of smog on chlorophyllase activity, chlorophyll contents and ROS fluorescence intensity in control and smog exposed plants

Discussion

It is widely accepted that chlorophyll degradation is an efficient marker of plant stress, and in this research, we investigated the molecular and ecological impacts of chlorophyll-degrading plants under smog conditions. Our experimental data showed that the content of chlorophyll a and b declines in the plants of Arabidopsis thaliana exposed to smog, which confirmed that oxidative stress reduced photosynthesis in plants (Malik et al., 2024, Alahabadi et al., 2017). This reduced value of chlorophyll is similar to the previous studies that explain the negative impact of smog on the photosynthesis process (El-Khatib et al., 2020). The higher concentrations of the oxidative stress indicators MDA and H_2O_2, in plants exposed to smog also support the fact that ROS are involved in chlorophyll breakdown. MDA and H_2O_2 have been regarded as fora of lipid peroxidation and oxidative stress respectively, and the elevation of these biomarkers under smog exposure shows the disruption of the basic cell membrane and metabolic process (Talebzadeh et al., 2021). Our results also indicated that chlorophyll content has negative correlations with MDA and H_2O_2, which is consistent with other authors (Levei et al., 2021) who stated that the degradation of chlorophyll is generally associated with ROS synthesizing and oxidative injury in plants treated with environmental stressors.

Additionally, our data are shown that superoxide dismutase (SOD) activity is enhanced in plants under the influence of smog, which indicates that plants begin antioxidant protection under the conditions of oxidative stress provoked by smog. This result strengthened the hypothesis of plants using antioxidant enzyme synthesis to protect against oxidative damage under stress (El-Amier et al., 2018). SOD one of the schemes crucially participates in the detoxification of superoxide radicals, and the activation of this mechanism is typical for plants subjected to oxidative stress through exposure to environmental toxins, such as ozone and nitrogen oxides (Zielewicz et al., 2020). Plant hormone regulation is another important factor in stress responses and our results indicate hormonal alterations where exposed plants have higher ABA content and lower cytokinins, gibberellins, and auxins levels. ABA is well-documented as being involved in plant stress response, especially in the regulation of stomatal closure and plant water relations during stress (Li et al., 2018). The increase in ABA levels as observed here is in concordance with outcomes from prior research that note the highest ABA level in plants exposed to air pollution On the other hand, the downregulation of cytokinin and gibberellin content

indicates growth restrains where reduced cellular division and elongation might have been retarded under stress conditions (X. Zhao et al., 2020).

Besides, changes in hormone concentrations, ion homeostasis in guard cells was also marked in response to smog exposure. The findings of this study indicated that potassium (K^+), and chloride (Cl^-) contents in smog-exposed plants were reduced while the calcium (Ca^{2+}) and hydrogen (H^+) ion contents were escalated (Malik et al., 2024). These findings bear out the inkling of the prior studies that reveal oxidative stress and air pollution can affect ion transport and homeostasis in plant cells especially guard cells there affecting the stomatal functionality and less efficiency of photosynthesis (Selzer & Busso, 2016). These changes may also amplify the declining rates of chlorophyll content by reducing water and nutrient assimilation and causing enhanced cellular disruption. There was an observed enhanced level of chlorophyllase activity –an enzyme that catalyzes chlorophyll degradation –a sure pointer to the fact that smog hastens chlorophyll breakdown. This result supports the findings of earlier research that revealed increased chlorophyllase activity in plants treated with environmental stress factors such as ozone and nitrogen oxides (Malik et al., 2024). Chlorophyllase is involved in the first stage of chlorophyll breakdown and therefore under the effects of smog, the plants might be trying to mitigate the oxidants' effect by degrading the abnormal chlorophyll molecules (Asad et al., 2016, Levei et al., 2021).

The increased ROS levels analyzed by the DCFH-DA assay in smog-exposed plants conclusively corroborate the hypothesis of oxidative stress. ROS are decisively reactive molecules that lead to cell damage since they oxidize lipids in the cell membrane, alter proteins' structure, and damage DNA which in turn affects chlorophyll degradation (Lingvay et al., 2020). The higher ROS levels were reported by the authors of this work, thus the effects of smog and other forms of air pollution on ROS accumulation in plant tissues discussed in this study conform to the data given in (Akhtar et al., 2016, Páli, & Kóta, 2019). In summary, the findings of the present research indicate that the effects of smog on chlorophyll breakdown are mediated by several molecular factors and physiological processes. These effects include increased oxidative stress due to smog; changes in hormonal content, the disturbance of the ion balance, and the activation of chlorophyllase all of which lead to a decrease in chlorophyll requirement and an inability to photosynthesize effectively. The current study offers useful information about the impact of smog on seed germination and plant growth and development to broaden the research on the effects of air pollution on plants and to develop techniques on how plants can be protected from the destructive effects of smog in environments that are polluted.

Conclusion

Therefore, this study confirms that smog affects the race capability of chlorophyll to degrade and in so doing decreases the photosynthesis rate and growth in the plants. The proposed mechanisms of chlorophyll breakdown are higher oxidative damage that was indicated by enhanced ROS, MDA, and H_2O_2. These changes occur together with shifts in hormones controlling plant development meaning there is increased ABA and decreased cytokinin and gibberellin. Moreover, the disturbance of ion homeostasis, especially in guard cells, aggravates the smog effect on stomatal function and water relations. These findings indicate that plants are actively degrading chlorophyll as a result of oxidative damage and while this is partially remedial, it may not be enough to counter the worst effects of smog experienced over a given period. This study therefore emphasizes the impacts of smog on the ecological viability of vegetation and the ecosystems. Further studies have to be conducted to determine how plants can be protected from the effects of air pollution: antioxidant supplementation and genetic engineering to increase the oxidative stress resistance of plants. The knowledge drawn from molecular and ecological impacts only provides a base to help develop measures to protect plant life in polluted environments to maintain the sustainability of ecosystems that depend on plants for carbon sequestration and oxygen production.

Key contents

Chlorophyll Degradation: *Arabidopsis thaliana* chlorophyll a and b content are reduced due to smog exposure, and the photosynthetic rate reduction supports this conclusion.

Oxidative Stress: Higher concentrations of MDA, H_2O_2 and increased SOD activity showed an increase in smog-induced cellular oxidative stress.

Hormonal Disruptions: The exhibition of smog causes hormonal changes which include an increase in ABA and a decline in cytokinins, gibberellins, and auxins, which products affect development.

Guard Cell Ion Imbalance: Smog exposure affects ion content in guard cells (potassium, chloride, calcium, hydrogen) that affects stomata and water relations.

Chlorophyllase Activity: Chlorophyllase activity increases with smog indicating that smog stress may induce chlorophyll degradation in plants due to oxidative stress.

Reactive Oxygen Species (ROS): Higher ROS values obtained from DCFH-DA assay suggest higher oxidative damage under smog conditions necessary in chlorophyll breakdown.

Learning objectives

Understand the Impact of Smog on Chlorophyll Content: The impact of smog on chlorophyll a and b was studied to determine the loss of this pigment efficiency on photosynthesis in plants.

Explore Oxidative Stress Mechanisms: Analyse the relationship between oxidative stress markers (MDA,

H_2O_2, and SOD activity) and smog-induced injury on plants and the smog's effect on chlorophyll brightness.

Assess Hormonal Changes Under Smog Exposure: Assessment of changes in ABA, cytokinins, gibberellins, and auxin, as well as their regulation of plant development and stress responsiveness.

Evaluate Guard Cell Ion Imbalances: Discover how changes in smog affect concentrations of ion in guard cells, and how such changes can affect stomatal control of water in plants.

Examine Chlorophyllase Activity: The enzyme responsible for the degradation of chlorophyll under smog exposure has been explained, especially its relation to oxidative stress.

Measure Reactive Oxygen Species (ROS) Production: Determine the chlorophyll-reducing effect of smog by using DCFH-DA assay to evaluate ROS levels and their relation with oxidative damage.

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Authors' Contribution

AZ, JI argued about the study idea and shaped the experiment. **JI, MM, HS** conducted the plant exposure to smog, data collection and the experiment involving oxidative stress markers and the chlorophyll data analysis. Accurate and statistical information was analysed by **FA, SA** and the results interpreted. Arif Malik also participated in hormonal measurements of guard cell ion concentration. **QA** was involved in the writing and editing of the manuscript. All authors have read and approved the final manuscript.

Data availability statement

The ideas contributed as part of the present study are within the scope of the article/Supplementary Material; more information is preferable to the corresponding author.

Conflict of Interest

The authors are also in no conflict of interest about the publication of this research. No financial or personal reason has a bearing on the work presented in this study.

Consent for Publication

Not applicable

Ethics statement

Not Applicable

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