Role Of Ascorbic Acid In Imparting Tolerance To Plants Against Oxidizing Pollutants

Priyanka Sharma, Reena Jain, ChirashreeGhosh

Abstract: Ascorbic acid is an antioxidant in plants which play important role in activation of many physiological and defense mechanisms. The level of ascorbic acid in plants is determinant of its tolerance against the adverse effect of oxidizing pollutants. The present study tries to relate the variation in ascorbic acid content with the tolerance and sensitivity of two selected plant species viz. Azadirachtaindica and Pongamiapinnata by calculating their Air Pollution Tolerance Index (APTI) during winter season from November to March in the urban city, Delhi, of North India. Moreover, ascorbic acid is also an important part of chloroplast; it protects different components of photosynthetic system from oxidative stress. Thus, to understand the role of ascorbic acid in imparting tolerance to plants against oxidizing pollutants the changes in chlorophyll content of the selected plant species with variation in ambient ozone concentration was analysed. It was found that as per APTI values Azadirachta sp. came under tolerant range with highest ascorbic acid content, whereas, Pongamia sp. was under intermediate range with less ascorbic acid content. It was statistically established, that ozone has no significant relation with chlorophyll content of Azadirachta sp. which has the highest ascorbic acid content. Whereas ambient ozone concentrations showed significant negative relation with the chlorophyll content of Pongamia sp. ($p< 0.05$). Thus, it was observed that the plants with high ascorbic acid content are tolerant and have greater ability to remediate pollutants.

Key Words: APTI, Ozone, Ascorbic Acid, Chlorophyll,Azadirachtaindica, Pongamiapinnata.Tolerant.

1 INTRODUCTION

Since industrialization air pollutants that have entered in the environment has changed the chemistry of atmosphere and adversely affected the health of humans and plants. Different oxidizing pollutants like, NOx¸ SOx and O_3 affect plant at biochemical and physiological level by causing oxidative stress and generation of Reactive oxygen species (ROS).Some plant species are sensitive and some are tolerant to such gaseous pollutants. Some of the gases and pollutants present in the environment are phytotoxic and results in different morphological and physiological deformities in plant. The level of such deformities will be high in sensitive plants and low in tolerant ones. Sensitive species are useful, as early warning indicator of pollution, and the tolerant ones help in reducing the overall pollution load leaving the air relatively free of pollutants [1]. Singh and Rao, [2] have suggested a method of determining Air Pollution Tolerance Index (APTI) by synthesizing the values of four different biochemical parameters i.e. leaf extract pH, ascorbic acid, total chlorophyll and relative water contents [3], [4], [5]. Among all the four parameters ascorbic acid acts as first line of defense against the oxidative stress of pollutants. Ascorbate is present in various organelles like-cytosol, chloroplast, vacuoles, mitochondria and also inside the cell wall [6], [7]. Ascorbic acid is a well known antioxidant and cellular reductant with an intimate and complex role in the response of plant to ozone stress [8], [9].

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The effect of oxidative pollutant SO_2 on the ascorbic acid level of three economicaly important crop species Vignaradiata (Mung bean), Lycopersiconesculentum (Tomato) and Zea mays (Maize) was studied and it was recorded that maize crop which maintain high ascorbic acid content under high pollution level exhibited greater resistant to SO_2 stress [10]. It was also studied, in both control and ozone-treated (150 ppb ozone, 5h) leaves that the ascorbic acid pool was approximately two times higher in the ozone resistant Bel B tobacco leaves as compare to the ozone-sensitive Bel W3 leaves [11]. Ascorbic acid impart tolerance to plant by reacting rapidly with superoxide, singlet oxygen, ozone and hydrogen peroxide, thus participating in removal of these ROS which are generated during aerobic metabolism and during exposure to some pollutants and herbicides [12], [13].Thus, the tolerance of plant depends on the amount of Ascorbate present in the plant cellular component which imparts tolerance to plants from the negative effect of oxidative pollutants. With this intend, the present study aims to understand the effect of ascorbic acid level in imparting tolerance or sensitivity to naturalized plants against oxidizing pollution in ambient environment.

2 MATERIAL AND METHOD

Sampling site was selected in urban Delhi in India which has been labeled as most polluted city in the world attributing to its fast paced growth in population and transport sector. Delhi has two major habitat types- 1. Ridge: This is a remnant of dry deciduous forest part of Aravalli hills, one of the oldest mountain range in the world. 2. Riverine: This includes river Yamuna and its fertile flood plain. The study was conducted at Delhi's northern ridge area. The ridge consists of quartzite rocks which covers nearly, 6000 hectares area. Major vegetation of this area includes Azadiractaindica, Cassia fistula, Delonixregia, Pongamiapinnata, Acacia, etc. The northern ridge is surrounded by two major landuse configurations. The vegetation of this area faces heavy pollution load from its nearby high emitting traffic intersection and mixed cluster (commercial cum institutional area).

2.1 Biomonitoring

The sampling site was situated near the Ridge area and characterized by the similar flora, which is a tropical, thorny and xerophytic in character. Two trees species which were abundant in sampling site were selecte- Azadirachtaindica (Family: Meliaceae, Evergreen tree) and Pongamiapinnata (Family: Fabaceae, Decideous tree) Different physiological and biochemical analysis were carried out to decipher their tolerance and sensitivity level in relation to their ascorbic acid content. Foliar samples were collected during winter month (November to March), to study the monthly variation during this period. Sampling was done twice in a week from November to March. Since, ozone concentration increases during post monsoon month these months were appropriately selected to comprehend the oxidizing effect of O3 on plants. The regular Sampling was done with same plant species and three replicas of each species were taken for the authenticity of the data. To understand their tolerance and adaptive features to the pollution dynamics of that area Air Pollution Tolerance Index (APTI) was calculated. APTI was calculated by using the formula developed by Singh and Rao [2].

$$APTI = \frac{A(T+P) + R}{10}$$

Where,
A= Ascorbic acid content of leaf in mg/g fresh wt [14]
T= Total chlorophyll content of leaf mg/g fresh wt [15]
P = Leaf pH [2]
R= Percent Relative water content [2]

2.2 Pollution Monitoring

Ozone data was collected from System for Air Quality Forecasting and Research (SAFAR) which is developed by the Indian Institute of Tropical Meteorology, Pune and weather information and forecasting is done by Indian Meteorological Department (IMD), both are constituents of Ministry of Earth Sciences, Govt of India.

2.3 Data Analysis

The statiatical analysis of the data (n=8 for each month) was done by using Statistical package for social sciences (version SPSS 22.0). Parameters were expressed as mean ± S.D. A correlation analysis was done to infer the effect of ambient ozone on chlorophyll content of selected plant species.

3. Result and Discussion

3.1 Seasonal Variation in APTI Values- Role of Ascorbic acid in imparting Tolerance to plants

During winter season monthly variation in total chlorophyll content, ascorbic acid and APTI for the three plant samples is given in Table 1. Monthly variation of chlorophyll concentration was quiet distinct due to pollution stress. Among two tree species, A.indica possessed higher concentration of Chlorophyll compared to P.pinnata, which can be supported by various reports [16], [17]. The presence of azadirachtin, an insect repellent in the leaves of A.indica may be responsible for its higher chlorophyll content than the P.pinnata, which is a deciduous tree prone to worm mining especially in the month of December. Interestingly we can see that the ascorbic acid content first decreases and then increases from November onwards till February, in all the three plant species. It has been observed that, when total chlorophyll decreases ascorbic acid content which is an antioxident increases to combat the stress. According to Gadallah [18] ascorbic acid protects chloroplast membrane integrity and chlorophyll degradation. It has been also reported that increase amount of dehydroascorbatereductase maintains higher amount of chlorophyll content and photosynthetic functioning [19; 20]. Even APTI value increases with increase in ascorbic acid content, thus, we can infer that it is a basic factor that provides tolerance to plants. The highest ascorbic acid content is seen in A.indica, exhibiting the capacity of ascorbic acid content to improve the tolerance of the plants to the effect of pollutants. This can further be verified with the observation reported by Nivane [16] according to which the ascorbic acid content was maximum in A.indica, followed by a small plant Bougainvilleaespectabilies and least for P.pinnata. APTI values obtained from the Table 1 for different plants including herbs and trees depicted their sensitivity or tolerance level. In A.indica APTI value ranged from 31.84 to 46.59 from months of November to March. Other authors [16, 17, 21] also suggested that a plant species known to be sensitive or tolerant in one geographical area may behave differently in another area. The study highlights that plants sensitivity level also changes with season. As the pollutant chemistry, temperature and other factors changes with season, plant tries to adapt to such variation by modifying different physiochemical and biological processes. Besides A.indica, P.pinnata was found to have APTI value varied between 14.93 to 25.71 respectively. The deciduous and evergreen trees differ in the sensitivity levels to pollutants. In general, deciduous trees are more sensitive to pollutants than the evergreen trees [22] which can be supported with our observation of evergreen tree (A.indica) and deciduous trees (P.pinnata).In view of this consideration the APTI of different plant groups have been categorized into different classes. The APTI value represented in Table 1 showed that amongst the two tree sample, A.indica had a higher APTI value (39.53) and falls under tolerant category and P.pinnata (18.92) comes under intermediate category as per APTI index.

TABLE 1: *MONTHLY VARIATION IN TOTAL CHLOROPHYLL CONTENT, ASCORBIC ACID AND APTI*

Plant Samples	**November**	**December**	**January**	**February**	**March**
AzadirachtaIndica					
Total chlorophyll content (mg/g)	2.77 ± 0.39	3.48 ± 0.79	2.85 ± 0.78	2.23 ± .06	2.71 ±0.39
Ascorbic Acid (mg/g)	27.58±8.47	22.95±9.58	31.92±7.77	44.47±9.91	40.77±8.24
APTI Value	34.71	31.84	38.79	45.7	46.59
PongamiaPinnata					
Total chlorophyll content (mg/g)	1.97 ± 0.60	3.29 ± 0.13	2.79 ± .43	2.01 ± .38	2.2 ± 0.77
Ascorbic Acid (mg/g)	7.29 ±1.69	5.94 ± 2.48	9.2 ± 0.95	21.54±5.32	16.43±1.66
APTI Value	15.02	14.93	17.79	25.71	21.16

3.2 VARIATION IN CHLOROPHYLL CONTENT WITH THE AMBIENT O3 CONCENTRATION

Variation of ambient O_3 concentration with the total chlorophyll content of the two tree species during winter months have been depicted in Figure 1 respectively. In Figure 1, the average concentration of O_3 ranged between 5.58µg/m^3 – 10.42µg/m^3. It can be inferred from the graph, that ozone showed an inverse relation with chlorophyll content of all the two plant species. The major sources of ozone at the sampling site were, the nearby ring road area which is a major traffic intersection zone in sampling site and since the site was near thickly vegetated area, the biogenic VOC's released from plants like- Mangiferaindica, Dalbergiasisoo, Populus, Morus alba etc. [23, 24] may also play a crucial role in ozone formation of that area. To understand the effect of ozone on the chlorophyll content of selected plant species we establish a statistical relationship between Chlorophyll content and ozone by using correlation analysis and it was clear from the analysis that chlorophyll of Pongamiapinnata significantly ($p<0.05$; r value = -.882) decreased with increase in ozone concentration in the ambient environment. Whereas, chlorophyll content of Azadirachtaindica did not show any significant relation with ozone dynamics.

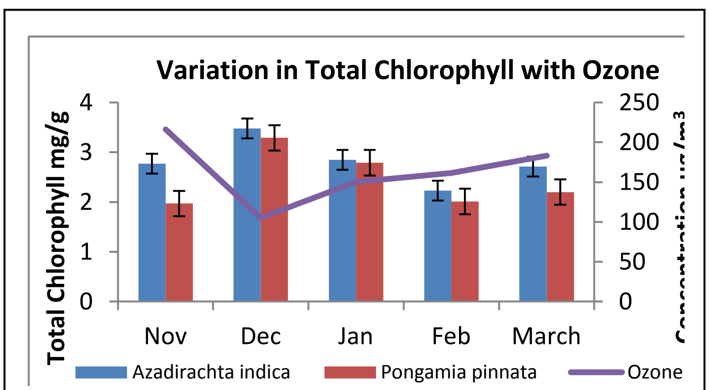

Figure 1: *Variation in Total chlorophyll with* O_3 *Concentration and correlation analysis.*

Ground level O_3 has been shown capable of damaging or inhibiting almost every step of the photosynthetic process from light capture to starch accumulation [25] and also enter leaves via stomata through normal process of gas exchange between leaf and its normal environment. Growing plants are exposed to many interacting biotic and abiotic factors that affect their performance and success and responses to O_3 [26]. One of the common features of the effect of Ozone on plants is the visible leaf injury. Symptom induced by Ozone can be non-specific, appear as chlorosis, bronzing, diffuse pigmentation or senescence [27, 28, 29, 30]. Rinnan and Holopainen [31] reported the decrease in chloroplast area due to prolong ozone exposure.The result depicts that the plant with high ascorbic acid content are more tolerant and can protects its physiological and molecular functioning even under stress. Gallie and Zhong [19] reported that Ascorbic acid is the most abundant antioxidant in the plants and serves as a major contributor to the cell redox state. However, ozone exposure includes number of biochemical and physiological response. One such response includes the ozone induced antioxidant response. As per Sharma and Davis [32] apoplasticascorbate has been proposed to be the first line of defence against O_3 pollution. The study also reported that when chlorophyll content decreased ascorbic acid content increased. Moreover, when ozone concentration increased in the ambient environment ascorbic acid content also increased in response to rescue vegetation from oxidative stress. Similar results were also reported by Urzica et al [33] that exposure of oxidative stress showed increased ascorbic acid level and gene encoding enzymatic components of ascorbate-glutathione system are also up regulated in response to oxidative stress in Chlamydomonas. Ainsworth [34] reported that with increase in chronic exposure to O_3, ascorbate content also increased in Soyabeans. Interestingly in our study Azadirachtaindica had highest ascorbic acid content and its chlorophyll didn't show significant effect with ozone whereas, chlorophyll content of Pongamiapinnata showed significant effect with ozone. So, the study clearly indicates that plants with higher ascorbic acid content can with stand higher ambient pollution stress.

4. CONCLUSION

The study clearly highlights the role of ascorbic acid in imparting tolerance to tree species. Seasonal variation in pollutants affects morphological and physiological structure of plants, specially the components of photosynthetic system. Ascorbic acid in tree species acts as first line defense and helps them to withstand the ill effects of pollutants. APTI study clearly explains that plants with high ascorbic acid content have ability to treat pollutants and are tolerant. Thus, further study should be done to explore the role of such tolerant plants in phytoremediation.

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