Assessment of Metabolic Potential and *In Silico* Analysis of Enzymes Involved in Inducing Air Pollution Tolerance in Some Angiosperm Plants

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A B S T R A C T

To analyze the air pollution tolerance capacity of ten selected plant species, their biochemical analysis was done, while they were growing in ambient polluted air. For this purpose, there ambient air quality monitoring was done for one year with parameters SO2, NO2, PM10 and PM2.5. Plants species selected were *Alstonia scholaris*, *Anthecephalus kadamba*, *Bauhinia variegate*, *Cassia fistula*, *Tectona grandis*, *Ficus rumphii*, *Mangifera indica*, *Polyalthia longifolia*, *Pongamia pinnata* and *Sarca indica*. Biochemical parameters analyzed were Ascorbic acid, chlorophyll, pH, and relative water content. Results indicate that some can be used as indicators and some as a sink of air pollution. Air Pollution Tolerance Index (APTI) was calculated by calculating biochemical parameters. *S. India* (49.36), and *A. scholaris* (33.66) *F. rumphii* (30.17) showed high; and *A. kadamba* (17.71), *B. variegate* (19.69) and *Pongamia pinnata* (17.72) showed intermediate response while *M. indica* (12.39), *P. Longifolia* (11.88), *Cassia fistula* (9.92) and *T. Grandis* (9.68) showed low tolerance as calculated through a change in their biochemical parameters in response to enhanced air pollution. *In silico* study of enzymes involved in the air pollution tolerance in the above plants was also done and in the phylogenetic analysis of all 9 enzymes, it was found that two clusters were formed in both the cases i.e. nucleotide and protein sequences. Cluster A Superoxide dismutase and Expansion two enzymes while in Cluster B, Catalase, Peroxidases, Glycosyl-transferase, Phenylalanine ammonia lyase, Sucrose synthase, Polygalacturonase, Laccase were clustered. The *in silico* and biochemical analysis strongly suggested that these enzymes were highly expressed in the air pollution condition and might play a major role in the air pollution control.

**Keywords**

Air Pollution Tolerance Index (APTI), Biochemical parameters, SO2, NO2, PM10, PM2.5. *In-silico*, Domain, Phylogenetics, Moradabad.

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**Introduction**

Plants can effectively be used as indicators and pollutant scavengers. Cultivation of trees can serve to be an effective and economical device for the abatement of air pollution. Planting of certain fast growing trees which are resistant to and can withstand the
Increasing air pollution is significantly useful for the air pollution control [14]. Most plants experience physiological changes before exhibiting visible damage to leaves when exposed to air pollutants [18-19]. Pollutants can cause leaf injury, stomatal damage, premature senescence, and a decrease in photosynthetic activities, disturb membrane permeability and reduce growth and yield in sensitive plant species [36]. Certain air pollutants have been reported to reduce chlorophyll content [14-15, 36] while others increase it [1].

Vegetation is an effective indicator of the overall impact of air pollution and the effect observed is a time-averaged result that is more reliable than the one obtained from the direct determination of the pollutants in the air over a short period. [28-30]. A large number of trees and shrubs have been identified as dust filters to check the rising urban dust pollution level. Plants provide an enormous leaf area for impingement, absorption, and accumulation of air pollutants to reduce the pollution level in the air environment [6] with various extents for different species [19, 34]. The use of plants as bio indicators of air pollution has long been established because these are the initial acceptors of air pollutants due to having scavenging property for many air pollutants [3]. Plants show varying degree of sensitivity and tolerance to air pollution stress. Chlorophyll content [7]; ascorbic acid content [13]; leaf pH [17-18] and relative water content have been used in the evaluation of the impact of air pollution on plants. Although, it was observed that separate parameters gave conflicting result for a particular plant [11-12]. Air pollution tolerance index based on Air pollution tolerance index [23-25, 29, 32].

Different Studies showed that some enzymes of the roadside plants are directly or partially involved in the development of tolerance against air pollution. The activity of plant enzymes including peroxidase, catalase, and ascorbate peroxidase was investigated using spectrophotometric methods. A higher level of peroxidase and catalase enzymes were measured in both plant samples collected from the polluted area [2].

In the present study, we aim to evaluate air pollution tolerance of 10 plant species growing in the vicinity of Moradabad city, by using the Air Pollution Tolerance Index (APTI) method. For this purpose, we did ambient air quality monitoring in Budh Bazar of Moradabad for one year (Sep 2014 – Aug 2015) with parameters SO₂, NO₂, PM₁₀ and PM₂.₅. After gathering the data of the ambient air pollution we analyzed the biochemical parameters of 10 plant species growing in the same region and control site (Shahpura).

**Materials and Methods**

**Respirable suspended particulate matter (PM₁₀)**

RSPM samples were collected with the help of Respirable Dust Samplers APM-460 NL (Envirotech, New Delhi) at rate of two samples per week on Whatman glass fiber filter paper – GF-A for 24 hrs (three shift i.e. 8 hrs.) with air flow rate of 1-1.5 m³/min. The difference in initial and final weight of the filter paper gave the total quantity of RSPM collected over the 24 hours period. The values of RSPM were reported in µgm⁻³. Calculation of mass concentration of RSPM

\[ RSPM = \frac{W_1 - W_2}{V} \]

Where, \( W_1 \) = Initial weight of filter paper, \( W_2 \) = Final weight of filter paper, \( V \) = Volume of air
Particulate matter 2.5

Gravimetric method is also used for measuring the mass concentration of PM$_{2.5}$. The instrument employed is Fine Particulate Sampler (FPS) (Envirotech, New Delhi, Model: APM-550). The operating flow rate for the machine is 1 m$^3$h$^{-1}$(± 5%), which separates particulates with a larger diameter. The NRPM fraction (>10μm) is separated on the inlet surface of the machine. The particle fraction of 10 - 2.5 μm diameter is separated at surface of glass fiber filter (Whatman GF/A 37 mm dia) wetted with silicon oil. The PM$_{2.5}$ fraction escaping is collected on a teflon membrane filter (Whatman of 47 mm dia). Dividing the difference between initial and final weights of the teflon membrane filter by the total volume of air sampled gives the mass concentration for PM$_{2.5}$.

Calculation of mass concentration of PM$_{2.5}$(μg/m$^3$) = \( \frac{W_2 - W_1}{V \times 10^5} \)

Where, \( W_1 \) = Initial weight of filter paper, \( W_2 \) = Final weight of filter paper, \( V \) = Volume of air

Sampling and analysis of gaseous pollutants

Sulphur dioxide

Colorimetric method is used for gaseous sampling. The instrument employed for gaseous sampling is APM-411 fitted and run simultaneously with APM-460 NL RDS. The impingers of 35 ml capacity were filled with 20 ml absorbing reagents, i.e. Potassium tetra chloromercurate(Na$_2$HgCl$_4$, TCM) exposed SO$_2$ gas in the four hours. Air is sucked through the absorbing reagents in the impingers at a flow rate of 1 L m$^{-1}$. The reagents after reacting with the corresponding gas and converted into the Sulfitomercurate complex. Samples were brought in the laboratory and maintained the loss by additional absorbing reagent and adjusted the volume to 10 ml. Added 1 ml Sulphamic acid, 2 ml Pararosaniline hydrochloride, and 2 ml Formaldehyde and wait for 30 minutes for color development then analyzed colorimetrically. SO$_2$ was analyzed employing the modified West–Gaeke method (1956) on a spectrophotometer at a wavelength of 560 nm. Chemicals (Merck, GR-Grade) along with deionized water were used for the preparation of all the reagents and the blanks. Sulphurdioxide concentration in the sample was determined from calibration curve and calculated by the formula- SO$_2$(µg/l) = \( \frac{OD \times F \times 1000}{V} \)

\( OD \) = Optical density displayed by spectrophotometer
\( F \) = Factor calculated by calibration graph
\( V \) = Volume of Air (\( A = \text{hours} \times \text{minutos} \times \text{avg. of rotameter reading} \))

Nitrogen dioxide

NO$_2$ was analyzed employing the modified Jacob–Hochheiser method. The colourimetric method is also used for NO$_2$ sampling.

The instrument employed for gaseous sampling is APM-411 fitted and run simultaneously with APM-460 NL RDS. The impingers of 35 ml capacity were filled 20 ml with appropriate absorbing reagents, sodium hydroxide with sodium arsenite for NO$_2$. Air is sucked through the absorbing reagents in the impingers at a flow rate of 1 L m$^{-1}$. The reagents after reacting with the corresponding gases were analyzed colorimetrically in the laboratory. The nitrite
ion produced during sampling is reacted with 1ml Phosphoric acid, 10 ml Sulphanilamide and 1 ml n⁻¹ Naphthyl ethylene diaminedihydrochloride (NEDA) and left for color development. Finally readings were taken on the spectrophotometer at a wavelength of 540 nm. Chemicals (Merck, GR-Grade) along with deionized water were used for preparation of all the reagents and the blanks. Nitrogen dioxide concentration in the sample was determined from calibration curve and calculated by the formula-

\[
\text{NO}_2 (\mu g/m^3) = \frac{\text{OD} - F}{V} \times 1000
\]

\(\text{OD} = \) Optical density displayed by spectrophotometer

\(F = \) Factor calculated by calibration graph

\(V = \) Volume of Air

(\(A = \) hours \times \text{minuts} \times \text{avg. of rotameter reading})

**Ascorbic acid content**

For ascorbic acid determination, 500mg of the fresh sample was homogenized with 20 ml of ice-cold extracting solution (500 mg Oxalic acid and 0.75 mg of EDTA, dissolved in 100ml distilled water). The homogenate was centrifuged at 6000 \times g for 15 minutes. The supernatant was used for determination of ascorbic acid by using the Keller method [16]. To 1 ml of supernatant, 5 ml of 20 µg/ml of 2,6dichlorophenol (DCPIP) solution was added with constant shaking and O.D. of the pink colour solution was taken at 520 nm wavelength (Es) on a UV-VIS spectrophotometer (Systronics Model 119, India). The pink color of the solution was bleached by adding one drop of 1% aqueous ascorbic acid and O.D. of the turbid solution was taken of the same wavelength (Es). The blank solution was prepared by adding 1 ml distilled water, 5 48 ml of DCPIP solution, and O.D. was taken at the same wavelength (Es). A calibration curve was prepared by using varying concentrations of ascorbic acid solution. Ascorbic acid content was calculated by using the following formula:

\[
\text{Ascorbic acid (mg/g)} = (\text{Eo} - \text{Es} - \text{Et}) \times F
\]

Where Eo, Es and Et are the optical densities of blank, plant samples and samples with one drop of 1% ascorbic acid which was added to it. Calibration curve for ascorbic acid was prepared using chemically pure ascorbic acid.

**Relative leaf water content (RWC)**

Fresh weight was obtained by weighing the fresh leaf samples collected from different sites. The leaves were then immersed in water overnight, blotted dry and then weighted to get turgid weight. Then leaves were dried overnight in an oven at 70°C and reweighed to obtain the dry weight. According to the method described by Liu and Ding (2008), relative leaf water content was determined and calculated with the formula:

\[
\text{Leaf RWC (%)} = \left(\frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}}\right) \times 100
\]

Where: FW = fresh weight; TW = turgid weight; DW = dry weight

**Leaf extract pH**

Five grams of the fresh leaves were homogenized in 10ml deionized water. This was then filtered and the pH of leaf extracted determined after calibrating pH meter with a buffer solution of pH4 and 9 [1].

**APTI determination**

The air pollution tolerance index (APTI) was computed by using the equation and formula [32].

\[
\text{APTI} = \left[\text{AA} \times \left(\text{TCh} + \text{pH}\right) + \text{RWC}\right] \div 10
\]
Where: AA = Ascorbic acid content (mg/g), TCh = total chlorophyll (mg/g), pH = pH of leaf extract, and RWC = relative water content of leaf (%).

**In silico analysis of enzymes**

*In silico* analysis of 9 different enzymes involved in air pollution tolerance was performed through different bioinformatics approaches.

**Sequence retrieval**

NCBI database was used to retrieve the nucleotide sequence of all enzymes of different plants.

**ORF prediction and conserved domain prediction**

ORF finder tool of NCBI was used to predict open reading frame in the nucleotide sequence. The domain is the functional part of sequence and prediction of the domain in sequence provide functional information about the protein. The conserved domain was predicted by the CD-Search tool of NCBI.

**Sub cellular localization prediction**

Subcellular localization of different enzymes was predicted using a neural network-based tool (http://www.cbs.dtu.dk/services/Target P/). The location with the highest score is the most likely according to Target P 1.1 tool. The localization of peptide may be chloroplast transit peptide, mitochondrial targeting peptide or a signal peptide.

**Phylogenetic analysis**

Multiple sequence alignment and Phylogenetic analysis of all enzymes was done by MEGA V6.0 on both the nucleotide as well as Protein level with the bootstrap value 1000 [33].

**Physicochemical analysis**

Prot-Param (http://www.expasy.org/tools/prot param.html) computes various physicochemical properties that can be deduced from a protein sequence. The proteins can either be specified as a Swiss-Prot/TrEMBL accession number or ID, or in the form of a raw sequence. Physicochemical analysis of each was computed using Prot-Param tool [9].

**Results and Discussion**

The ambient air quality monitoring of one year revealed the results depicted in figures 1, 2, 3, 4, 5. Gaseous pollutants, as well as particulate matter, was found to be higher than the permissible limits of WHO (40 µg/m$^3$ for PM); USEPA (100 µg/m$^3$ for PM) and NAAQMS (140 µg/m$^3$). Temperature variation shows that for many months it is far more than the normal limits.

Monthwise variation of gaseous pollutants shows that it is exceeding the permissible limits of WHO, USEPA, NAAQS. Relative humidity and particulate matter also exceeded the permissible limits.

Humidity showed many spells of variation in a year than any other gaseous pollutant and particulate matter. Ten Plant species growing in such a state of climatic conditions and pollutants were selected and were analyzed for biochemical parameters.

The analysis of biochemical parameters showed a marked variation among species. Chlorophyll content ranged between 2.87mg/g to 16.84 mg/g among all species. In *F. Rumphii* and *S. indica* the chlorophyll content was found highest. The ascorbic acid was found in the range of 1.43 mg/g to 16.56 mg/g among the plant species taken for study. The pH ranged 4.87 to 11.2 among all
High pH may increase the efficacy of conversion from hexose sugar to ascorbic acid, while the low leaf extract pH showed the shows good correlation with sensitivity to air pollution.

Relative water content varied from 62% to 85% indifferent plants species. High RWC helps the plants to maintain its physiological balance under stress condition such as exposure to air pollution when the transpiration rates are usually high.

Therefore the plants with high RWC may be tolerant to pollutants under a stressed condition.

Air Pollution Tolerance Index (APTI) has been calculated of 10 plant species growing near BudhBazar Moradabad city of western Uttar Pradesh and the data is presented in figure 6. For this purpose, it is essential to evaluate the tolerance level of different plant species to air pollution using changes in four biochemical parameters namely ascorbic acid content, total chlorophyll, relative water content and pH value (Table 1).

The above biochemical parameters that are analyzed for calculation of APTI values are extremely important to understand the resistivity and susceptibility of different plant species [26-27, 31].

The ascorbic acid content was maximum 16.56 mg/g in Sarcacinindica at study site. The results indicate that the low ascorbic acid concentration range from 2.64 to 16.6 mg/g at the residential site and high at traffic and industrial sites that range from 4.65 to 15 mg/g (Figs. 5 and 6). The present study reveals that total chlorophyll content in all the urban plants varies with the pollution status of the area. The higher pollution level in the form of industrial and urban pollution lowers the chlorophyll content. Total chlorophyll was found maximum 16.9 mg/g and minimum 4.3 mg/g in Ficus rumphii at an industrial site and residential site respectively.

The present study shows that in the case of Anthocepalus kadamba, F. rumphii, and Polyalthia longifolia, the total chlorophyll content in the experimental sample with respect to the control was found high (Fig. 6).

The results of the present investigation indicate that there exist a strong and positive correlation \( r = 0.91 \) between pH of leaf extract and APTI values in experimental and control samples (Fig. 7). Correlation between pH and Ascorbic Acid was found positively significant \( r= 0.85 \) and hence the leaf extract pH on the higher side give tolerance to plants against pollution (Table 2) [2]. Chl and APTI also showed strong positive correlation \( 0.81 \).

Relative water content is associated with protoplasmic permeability in cells causes loss of water and dissolved nutrients, resulting in early senescence of leaves [22]. The results of the present investigation suggest a strong and positive correlation \( r=0.57 \) between % relative water content and APTI values of control and experimental samples of different plant species (Table 2).

The results of the present study reveal that the ten different plant species at polluted site and control site shows considerable variation in their susceptibility to air pollution and they responded differently at each site to air pollutants (Table 1).

The plants with high and low APTI can serve as tolerant, moderately tolerant and sensitive species respectively. Tree species falling under Tolerant species (APTI > 30), moderately tolerant (17 < APTI > 30) and sensitive Species (APTI < 17) are presented in table 1.
Table 1: Biochemical parameters of selected species studied at polluted site (BB), and control site (SP)

<table>
<thead>
<tr>
<th>Name of plant species</th>
<th>SITE</th>
<th>Chl (mg/g)</th>
<th>Ascorbic acid (mg/g)</th>
<th>pH</th>
<th>RWC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alstonia scholaris</em></td>
<td>BB</td>
<td>13.31</td>
<td>9.15</td>
<td>8.10</td>
<td>79.45</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>12.19</td>
<td>8.5</td>
<td>7.23</td>
<td>71.23</td>
</tr>
<tr>
<td><em>Anthocephalus cadamba</em></td>
<td>BB</td>
<td>6.13</td>
<td>5.73</td>
<td>5.93</td>
<td>86.</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>10.53</td>
<td>5.28</td>
<td>8.24</td>
<td>78.</td>
</tr>
<tr>
<td><em>Bauhinia variegata</em></td>
<td>BB</td>
<td>12.77</td>
<td>6.9</td>
<td>6.2</td>
<td>64.</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>11.87</td>
<td>4.3</td>
<td>5.8</td>
<td>62.</td>
</tr>
<tr>
<td><em>Cassia fistula</em></td>
<td>BB</td>
<td>6.87</td>
<td>2.6</td>
<td>5.7</td>
<td>67.</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>5.62</td>
<td>2.2</td>
<td>6.9</td>
<td>64.</td>
</tr>
<tr>
<td><em>Tectona Grandis</em></td>
<td>BB</td>
<td>5.7</td>
<td>2.3</td>
<td>6.9</td>
<td>81.</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>4.9</td>
<td>1.4</td>
<td>9</td>
<td>84.</td>
</tr>
<tr>
<td><em>Ficus Rumphii</em></td>
<td>BB</td>
<td>14.2</td>
<td>9.3</td>
<td>11.5</td>
<td>85.</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>16.8</td>
<td>8.5</td>
<td>5.7</td>
<td>69.</td>
</tr>
<tr>
<td><em>Mangifera Indica</em></td>
<td>BB</td>
<td>4.8</td>
<td>3.7</td>
<td>4.8</td>
<td>77.</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>3.3</td>
<td>1.9</td>
<td>5.7</td>
<td>68.</td>
</tr>
<tr>
<td><em>Polyalthia longofolia</em></td>
<td>BB</td>
<td>3.1</td>
<td>4.9</td>
<td>4.7</td>
<td>68.</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>2.80</td>
<td>4.2</td>
<td>7.1</td>
<td>58.</td>
</tr>
<tr>
<td><em>Pongamia pinnata</em></td>
<td>BB</td>
<td>9.3</td>
<td>6.6</td>
<td>6.9</td>
<td>84.</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>7</td>
<td>3.9</td>
<td>9.3</td>
<td>77.</td>
</tr>
<tr>
<td><em>Sarca indica</em></td>
<td>BB</td>
<td>13.5</td>
<td>16.6</td>
<td>8</td>
<td>81.</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>15.4</td>
<td>14.5</td>
<td>7.5</td>
<td>82.</td>
</tr>
</tbody>
</table>

Table 2: Functional domain analysis of enzymes involved in inducing Tolerance against air pollution

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Enzyme</th>
<th>Domain</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Catalase</td>
<td>Catalase -like heme-binding proteins and protein domain</td>
<td>1-48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Catalase -like heme-binding proteins and protein domain</td>
<td>335-655</td>
</tr>
<tr>
<td>2</td>
<td>Peroxidase</td>
<td>Protein Disulfide Oxidoreductases and Other Proteins with a Thioredoxin fold</td>
<td>1-363</td>
</tr>
<tr>
<td>3</td>
<td>Super oxide dismutase</td>
<td>Iron/manganese superoxide dismutases, C-terminal domain; superoxide dismutases (SODs) catalyze</td>
<td>52-123</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Iron/manganese superoxide dismutases, C-terminal domain; superoxide dismutases (SODs) catalyze</td>
<td>14-49</td>
</tr>
<tr>
<td>4</td>
<td>Expansine</td>
<td>Rare lipoprotein A (RlpA)-like double psi beta-barrel</td>
<td>294-557</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pollen allergen; This family contains allergens loll pl, pil and pilii from Lolium perenne.</td>
<td>594-809</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Expansine A Provisional</td>
<td>180-963</td>
</tr>
<tr>
<td>5</td>
<td>Glycosyltransferase</td>
<td>Glycosyltransferases catalyze</td>
<td>25-1383</td>
</tr>
<tr>
<td>6</td>
<td>Phenylalanine ammonia layse</td>
<td>Class I like superfamily: contains the lyase class I family, histidine ammonia-lyase and phenylalanine ammonia-lyase</td>
<td>2-451</td>
</tr>
<tr>
<td>7</td>
<td>Sucrose synthase</td>
<td>Glycosyltransferases catalyze</td>
<td>1-345</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glycosyltransferases catalyze</td>
<td>394-795</td>
</tr>
<tr>
<td>8</td>
<td>Polygalacturonase</td>
<td>Polysaccharide Lyase Family 6; Polysaccharide Lyase Family 6 is a family of beta-helical polysaccharide lyases</td>
<td></td>
</tr>
</tbody>
</table>
Table 3 Physiochemical properties of enzymes involved in inducing tolerance against air pollution

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Number of amino acids</th>
<th>Molecular weight</th>
<th>Theoretical pI</th>
<th>Negatively charged residues</th>
<th>Positively charged residues</th>
<th>Chemical Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>108</td>
<td>12595.52</td>
<td>7.87</td>
<td>10</td>
<td>11</td>
<td>C_{580}H_{855}N_{153}O_{150}S_{7}</td>
</tr>
<tr>
<td>Peroxidases</td>
<td>110</td>
<td>12313.06</td>
<td>7.63</td>
<td>12</td>
<td>13</td>
<td>C_{558}H_{861}N_{141}O_{168}S_{4}</td>
</tr>
<tr>
<td>Super oxide dismutase</td>
<td>103</td>
<td>12145.32</td>
<td>9.96</td>
<td>6</td>
<td>15</td>
<td>C_{677}H_{871}N_{159}O_{163}S_{6}</td>
</tr>
<tr>
<td>Expansin</td>
<td>260</td>
<td>28333.46</td>
<td>9.48</td>
<td>11</td>
<td>23</td>
<td>C_{1262}H_{1915}N_{355}O_{352}S_{20}</td>
</tr>
<tr>
<td>Glycosyl-transferase</td>
<td>470</td>
<td>52295.21</td>
<td>6.05</td>
<td>52</td>
<td>47</td>
<td>C_{2360}H_{3725}N_{625}O_{685}S_{15}</td>
</tr>
<tr>
<td>Phenyl alanine ammonia layase</td>
<td>146</td>
<td>15379.47</td>
<td>8.79</td>
<td>10</td>
<td>12</td>
<td>C_{2926}H_{4436}N_{808}O_{878}S_{17}</td>
</tr>
</tbody>
</table>

Table 4 Localization of all enzymes involved in air pollution tolerance

<table>
<thead>
<tr>
<th>Name</th>
<th>Len</th>
<th>cTP</th>
<th>mTP</th>
<th>SP</th>
<th>Other</th>
<th>Loc</th>
<th>RC</th>
</tr>
</thead>
<tbody>
<tr>
<td>gi_257144752_emb_FN4</td>
<td>108</td>
<td>0.151</td>
<td>0.111</td>
<td>0.113</td>
<td>0.752</td>
<td>_</td>
<td>2</td>
</tr>
<tr>
<td>gi_283827716_gb_GU26</td>
<td>110</td>
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Fig.1 Average annual value of temperature in Budd bazaar for the year 2015 (Months)
**Fig. 2** Average annual value of gaseous pollutants in Budd bazaar for the year 2015 (Months)

**Fig. 3** Average annual RH value in Budd bazaar for the year 2015 (Months, Data obtained from meteorological department)

**Fig. 4** Average annual particulate matter value (µg/m³) in Budd bazaar for the year 2015 (Months-Sep to Aug)
**Fig. 5** Variation in APTI of different species growing in Budh bazar and control site; Series 1=BB (Budh Bazar). Series 2=Control Site Shapura (SP)

**Fig. 6** Biochemical parameters of various plants species exposed to enhanced level of air Pollution around Budh bazaar area (Chl and Ascorbic acid mg/g, R.W.C in %)
Fig. 7 Correlation between biochemical parameters of plants studied in the polluted area (BB). The red color shows the maximum correlation, yellow shows the minimum correlation and other color shows moderate correlation.

Fig. 8A Phylogenetic analysis on the basis of nucleotide sequence of enzymes of different plants involved in air pollution tolerance.
**In silico analysis**

Nucleotide Sequence retrieval of all enzymes involved in air pollution tolerance like Catalase, Peroxidase, Superoxide dismutase, expansion, UDP-glucose pyrophosphorylase, glycosyl transferase family, phenylalanine ammonia-lyase (PAL), caffeoyl-CoA 3-0-methyl-transferase (CCoAOMT), Sucrose synthase, polygalacturonase, and Laccase was done by NCBI database [20].

The open reading frame of above enzymes was done by ORF Finder and a different number of the open reading frame was found for each enzyme of different plant species. A conserved domain in the nucleotide sequence was analyzed by CD-Search tool of NCBI and different functional domains was found in the nucleotide sequence of enzymes. Some of them have only one domain and some have more than one. Domain and the position in the nucleotide sequence have been described in detail in the (Table 2) [20, 35, 37]. To find the physicochemical property of each peptide sequence of different enzymes was done by Protparam tool. The nature of protein and number of positive and negative amino acid, isoelectric point, the number of atoms (C, N, H, O) was predicted and the detail of all physiochemical enzyme is listed in table 3 [9].

The location with the highest score is the most likely according to Target P, and the relationship between the scores may be an indication of certainty of the prediction. According to the prediction results, three proteins act as the signal peptide and two as mitochondrial targeting peptide and rest of all are located at other places. The detailed description of the localization site prediction was shown in (Table 4) [21].

The phylogenetic analysis described that two clusters were formed on both the level i.e. nucleotide and protein sequence of enzymes. In Cluster A; Catalase, C-glycosyl transferase, Peroxidase, Sucrose Synthase, Polygalacturonase, Phenylalanine ammonia lyase and Laccase enzymes are clustered while in cluster B Superoxide dismutase and expansineis clustered. In the case of the peptide sequence, multiple sequence
alignment and phylogenetic analysis were done and it was found that two clusters A and B were formed. In cluster A, peroxidase, superoxide dismutase, expansin and laccase were clustered while in cluster B rest of all enzymes were clustered (Figs. 8A and 8B).

In conclusion, the highest value of APTI was recorded for Sarca indica (49.36) and least value of APTI in T. grandis (9.68). T. Grandis, C. fistula, P. longifolia fall in sensitive category. Bauhinia variegata was found moderately tolerant to air pollution. Out of ten different species, four were found insensitive category thus they can be considered as sensitive plant species. They can serve as an indicator of air pollution. The most obvious damage was reported in the leaves i.e. chlorophyll content. The major damages caused by air pollutants to plants include chlorosis, necrosis, and epinasty. In present study S. indica, F. Rumphii and A. Scholaris showed remarkable tolerance to ambient air pollution. Since there biochemical and physiological response augments their tolerance. Therefore these species can be planted in large numbers in air pollution hotspots.

The in silico characterization of enzymes involves in inducing air pollution tolerance to lead us to conclude that there are certain conserved domains which have a different binding site for proteins at different positions and that protein important role in enhancing air pollution tolerance. On the basis of their metabolic potential, enzymes were clustered in two groups. The in silico analysis is an attempt here to understand the functioning of ‘enzymes of interest’ at the molecular level.

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