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Research article

DETOXIFYING HYDROGEN PEROXIDE ENZYMES ACTIVITY IN TWO PLANT SPECIES EXPOSED TO AIR POLLUTION IN ABIDJAN CITY (CÔTE D'IVOIRE).

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ABSTRACT: The activity of antioxidant enzymes were investigated in *Jatropha integerrima* and *Cassia surattensis* exposed to air pollution in Abidjan city. The main objective of this study is to assess the activity of detoxifying peroxide enzymes to be used as indicators of local air pollution. These species were exposed for one month on the sampling sites of four land use classes (Parks, Roads, Industrial and residential areas) in Abidjan city. The activity of catalase and ascorbate peroxidase was performed by spectrophotometric methods. A higher level of catalase (EC 1.11.1.6) was measured in both plant samples collected in polluted areas (Roads, Industrial areas). However a lower level activity of ascorbate peroxidase (EC 1.11.1.11) was measured in both plant samples collected from less polluted classes (Residential areas and Parks). This study showed that catalase activity is more effective in stress conditions in *Jatropha integerrima* and *Cassia surattensis* and could be a valuable way in tropical countries to estimate the atmospheric pollution.
Key words: Urban air pollution, catalase activity, ascorbate peroxidase activity, Côte d'Ivoire.

INTRODUCTION

Air pollution is the alteration of atmospheric gases by human activity which has direct or indirect consequences on human health, plant physiology and growth, or ecosystem functions [1]. The urban air quality is continuously affected by emissions from both stationary and mobile combustion sources. Indeed, the alterations are mainly caused by unbalance of carbon monoxide (CO), carbon dioxide (CO_2), hazardous air pollutions (HAPs), nitrogen oxides (NOx), sulphur dioxide (SO_2) and tropospheric ozone (O_3) which all of them have a harmful effects on human health and environment. While plants can improve the air quality in some extent, air pollution may adversely influence the plant life. Air pollutants such as ozone may enter through plant tissues via stomata and elevate the level of reactive oxygen species (ROS) causing serious damage to the DNA, proteins and lipids [2, 3]. For controlling air pollution, air needs first to be monitored by such as physico-chemical methods, biological methods, bioindicators and mathematical models. Among these methods, the biological method is known to be inexpensive for prolonged surveys of air quality due to integration of the effect of all the environmental factors [4, 5]. It is well known that plant cells have several antioxidative defence mechanisms such as tocopherol, carotenoids, glutathione and glutathione reductase enzymes, superoxide dismutase, catalase, ascorbate peroxidase, polyphenol oxidase to protect plants against these oxidative stresses [6, 7, 8, 9]. However, the activation of antioxidative defence mechanisms requires a high consumption of energy which may consequently inhibit the plants growth [10]. In this study, the air pollution effects on the activity of antioxidant enzymes were investigated in *Jatropha integerrima* and *Cassia surattensis* in Abidjan city. The main feature for selecting *Jatropha integerrima* and *Cassia surattensis* species was their abundance in the study area. The main objective of this study is to evaluate air quality by determination of the activity of detoxification enzymes such as catalase (EC 1.11.1.6) and ascorbate peroxidase (EC 1.11.1.11) in *Jatropha integerrima* and *Cassia surattensis* plants in the polluted sites and less polluted areas.

MATERIALS & METHODS

Study area

The study was conducted in the city of Abidjan city, Côte d'Ivoire. Abidjan is the economic capital city with a human population of over 4 million on 422 km^2. The intensive development of industry and road traffic and the increasing number of automobiles in the city lead to a notable increase of air pollution. All transport vehicles (about 30,000 in 2010) have diesel engines [11]. The city was subdivided into 4 land use classes: Industrial (2 sites); Residential (2 sites); Parks (2 sites); Roads (4 sites). It is suggested that the air pollution is an important problem which should be resolved. First of all, effective technology of monitoring air pollution is needed. At each site three sampling leaf were selected to determine the activity of detoxification enzymes.

Sample collection

In this study, the air pollution effects on the activity of antioxidant enzymes were investigated in *Jatropha integerrima* and *Cassia surattensis*. The leaf samples of *Jatropha integerrima* and *Cassia surattensis* were collected in the 4 land use classes and kept in nitrogen liquid.

Preparation of extracts

The fresh leaves (200 mg) were grinded in powder using nitrogen liquid. The powder was homogenized in 1.2 mL phosphate potassium (pH 7.8 with 0.1 mM of EDTA). The homogenate were centrifuged to 15.000 x g for 20 min at 4°C. The supernatant were kept, and 0.8 ml phosphate potassium 0.2 M was added. The homogenate were centrifuged again to 15.000 x g during 15 min. The combined supernatants were stored on ice and used in order to determine the activity of detoxifying enzymes.

Determination the activity of antioxidant enzymes

Measurement of catalase enzymes (CAT)

CAT activity was performed using hydrogen peroxide consumption at 240 nm and an extinction coefficient 40 mm^{-1} cm^{-1} according to [12] method by spectrophotometer. The reaction buffer of 3 ml contained 2 ml enzyme extraction (diluted 200 times in 50 mM potassium phosphate buffer, pH 7.0) and 10 mM H_2O_2.

Measurement of ascorbate peroxidase enzymes (APX)

APX was assayed following the procedure described by [13]. Enzymatic oxidation was performed by reduction in absorption at 290 nm in 3 minutes. APX activity was calculated using the extinction coefficient (2.8 mm^{-1} cm^{-1}). The reaction solution contained 0.5 mM ascorbate soluble in 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM H_2O_2 and 10 µl enzyme extraction.

Statistical analysis

The results are expressed as mean values and standard error or standard deviation (SD). The differences between the *C. surattensis* and *J. integerrima* extracts were analysed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test with a = 0.05. This treatment was carried out using Statistica 7.1 software.

RESULTS

Variation of ascorbate peroxidase (APX) in land use classes

The variation of APX activity in leaf samples of four land use classes is summarized (Figure 1). The average of activity of ascorbate peroxidase was increased in Park and Residential zones.

APX activity varied between (1.017 ± 0.176; OD) and (0.803 ± 0.303; OD), (0.937 ± 0.088; OD) and (0.785 ± 0.303; OD) in *J. integerrima* and *C. surattensis* respectively. The value of APX are decreased in Road and Industrial, and varies (0.410 ± 0.166; OD) with (0.473 ± 0.265; OD), of (0.390 ± 0.144; OD) with (0.437 ± 0.037; OD) for *J. integerrima* and *C. surattensis* respectively. These values are significantly higher in less polluted zones (Park and Residential zones) than in polluted zones (Student's Test t: $p<0.05$).

Variation of catalase enzyme activity (EC 1.11.1.6) in the land use classes

The variation of catalase activity in leaf samples of four land use classes is summarized in Figure 2. In both species, the activity of catalase is higher in Road and Industrial zone, lower in Park and Residential. The higher values of the polluted classes are 0.112 ± 0.093 OD to 0.048 ± 0.005 OD and lower values of the less polluted classes are 0.037 ± 0.03 OD to 0.021 ± 0.007 OD. The values of the enzymatic activity of catalase in *J. integerrima* are significantly higher than those of *C. surattensis* ($p <0.05$).

Correlation between catalase (CAT) and ascorbate peroxidase (APX) in *J. integerrima*

The linear regression of catalase and ascorbate peroxidase activity obtained starting from the slope of the models of regression (Figure 3) shows a negative but no significant correlation (P=0.44; r=-0.21).

Correlation between catalase (CAT) and ascorbate peroxidase (APX) in *C. surattensis*

The linear regression for enzymes activities of the catalase and ascorbate peroxidase obtained starting from the slope for the models regression (Figure 4) shows a negative but significant correlation (P=0.049; r=-0.51).

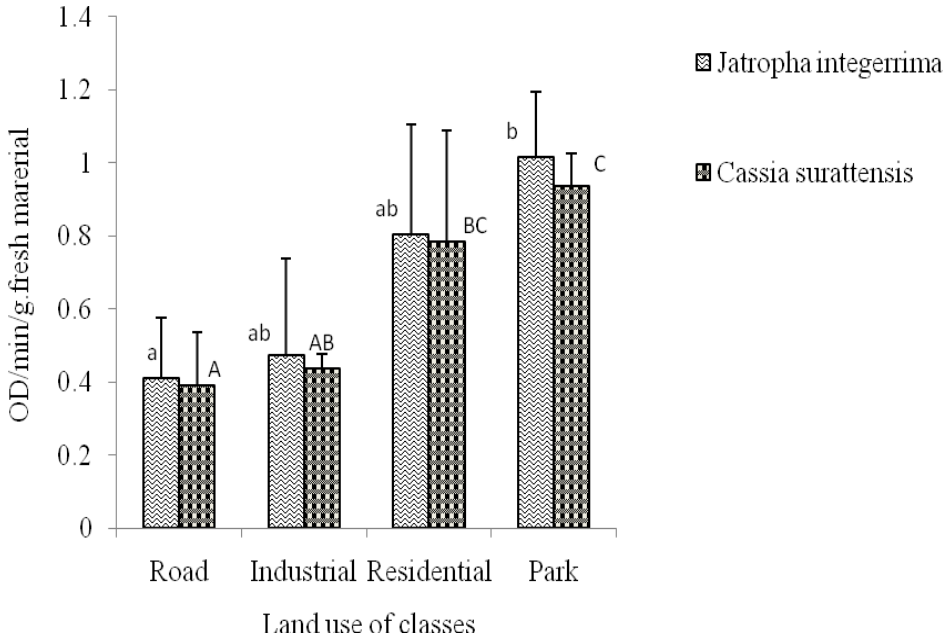

Figure 1: The variation of ascorbate peroxidase (EC 1.11.1.11) activity in *J. integerrima* and *C. surattensis* leaf samples. Capital letters indicate significant differences between land use classes in *C. surattensis*. Small letters indicate significant differences between land use classes in *J. integerrima*. Error bars are standard deviation. Significant $p < 0.05$.

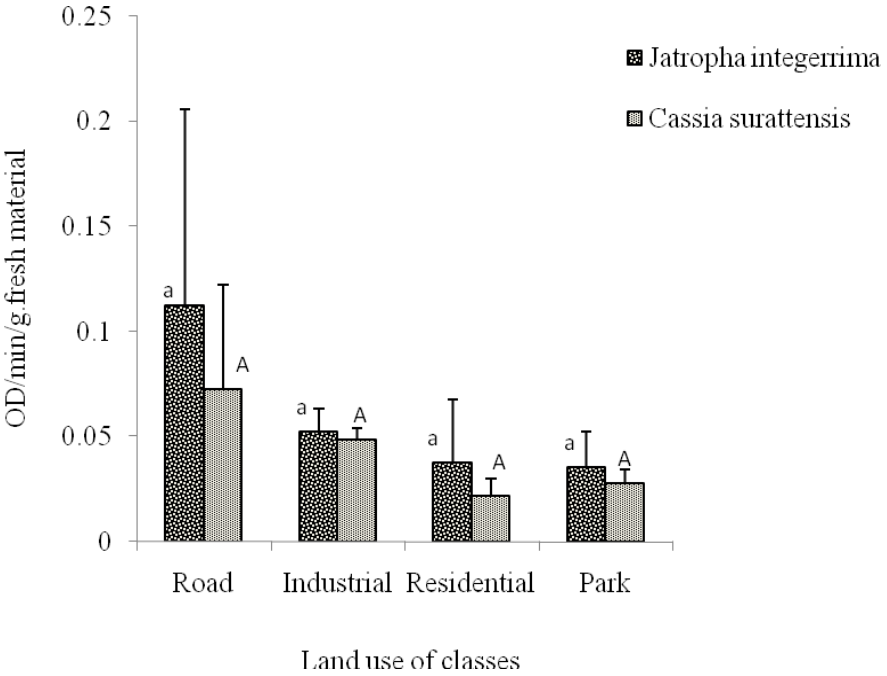

Figure 2: The variation of catalase activity (EC 1.11.1.6) in *J. integerrima* and *C. surattensis* leaf samples. Different capital letters indicate significant differences between land use classes in *C. surattensis*. Small letters indicate significant differences between land use classes in *J. integerrima*. Error bars are standard deviation. Significant $p < 0.05$.

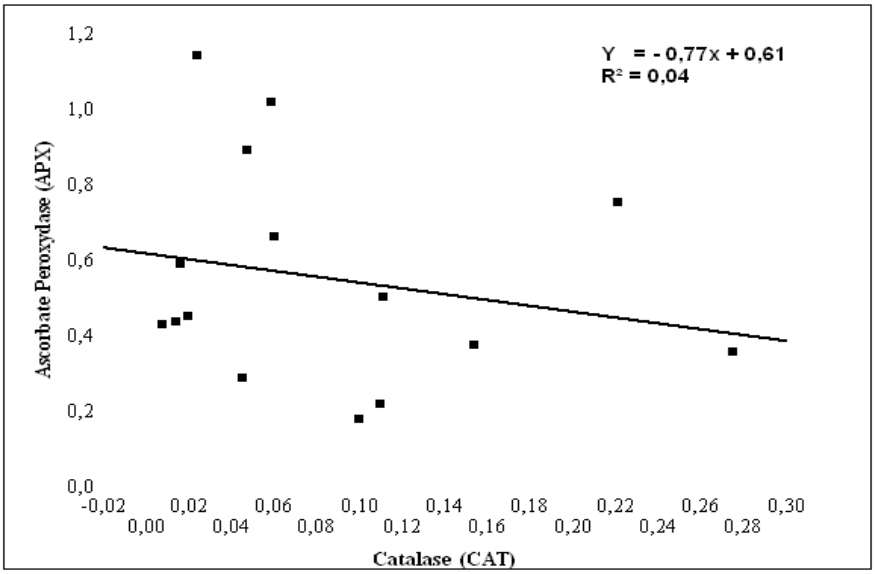

Figure 3: Correlation of ascorbate peroxidase and catalase activities in *J. integerrima* leaves

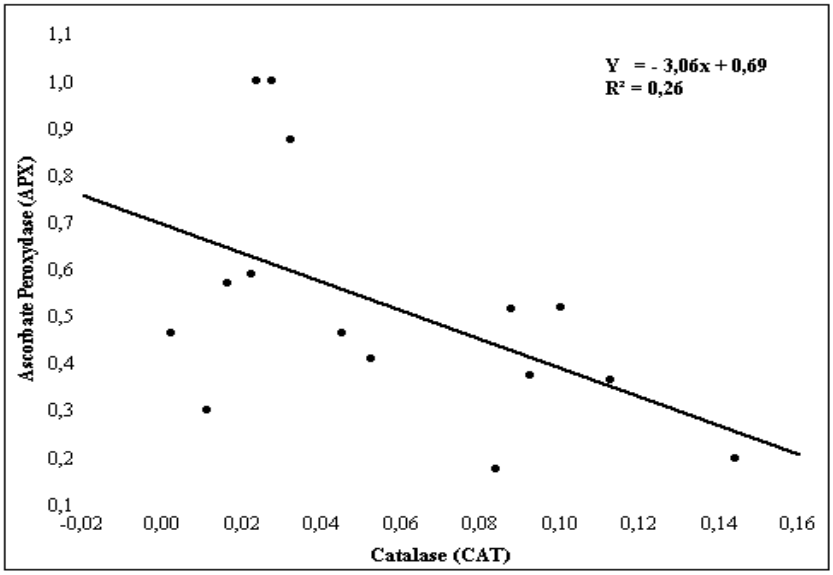

Figure 4: Correlation of enzyme activity in *C. surattensis* leaves

DISCUSSION

APX is the enzyme related in the elimination of hydrogen peroxide in the oxidative stress, in higher plants, algae and several cyanobacteria's [14]. It is a component of the ascorbate-glutathion cycle which functions like a street sweeper of active oxygen shapes and seems to be significant for the protection of the plants against the active shapes of oxygen generated (hydrogen peroxide) during the exposure of plants to pollutants [15]. The activity of the ascorbate peroxidase in *Cassia surattensis* and *Jatropha integerrima* in Residential and Park present a higher level activity, less in Road and Industrial classes ($p<0, 05$). The higher activity of the ascorbate peroxidase obtained in the less polluted zones could be justified by environmental factors such as high temperature, raised light intensity and lack of water. These results could be also explained by the toxic effects of the pollutants, which can overpower the antioxidant systems of defences [16]. Indeed, the ascorbate peroxidase in the less polluted sites shows a reduced activity of cells to trap hydrogen peroxide [17]. Our determination method of enzyme activity showed results which are in agreement with those of [10, 18]. Their work showed an inhibition of the activity of the ascorbate peroxidase in polluted zones.

Moreover catalase is implied in the removal of hydrogen peroxide (H_2O_2) [19]. Catalase plays a role in plant photorespiratory metabolism [20], it allows the cleaning of hydrogen peroxide (H_2O_2), β-oxidation of the fatty acids in germination of seeds [21] and also in stress abiotic conditions. This line of defence made up of catalase [22] depends on the type of leaf and pollutants. The results of our study show a higher activity level of catalase in Road and Industrial classes, a lower one in Residential and Park in *Jatropha integerrima* and *Cassia surattensis*. The higher level of the catalase activity in the polluted site could be explained by the presence of environmental pollutants which would cause a strong production of H_2O_2 at these species under oxidative stress [23, 24]. The system superoxide dismutase-catalase (Sod-cat) represents the first line of defence against the oxidative stress [25]. This massive production of hydrogen peroxide (H_2O_2) in plants could involve an increase in the content of catalase. Similar observations have been reported by [10, 26]. These authors explain these results by the increase of the sensitivity of plants to environmental stress. It has been identified that the level of ascorbate peroxidase and catalase in plants with larger leaves was higher than plants with smaller leaves.

The negative correlation obtained between catalase and ascorbate peroxidase in both species shows a more effective mechanism of defence of catalase through an increase in this one. Catalase is the most effective enzyme known; it plays a significant role in the elimination of hydrogen peroxide to strong concentration [28].The increase of catalase activity followed by the reduction of ascorbate peroxidase in more polluted sites could be explained by the high percentages of hydrogen peroxide which would stimulate an expression of the catalase in the detoxification of the plants in order to survive this stress, involving its decomposition out of water and oxygen.

CONCLUSION

The higher level of catalase activity was found in Roads and Industrial areas. Catalase activity in Park and Residential areas is lower than the polluted sites. However, ascorbate peroxidase activity is higher in Park and Residential areas and lower in Roads and Industrial areas. This study showed that catalase activity is more effective in stress conditions in *Jatropha integerrima* and *Cassia surattensis* and could be a valuable way in tropical countries to estimate the atmospheric pollution.

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