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BRIEF COMMUNICATION

Net photosynthetic rate, ascorbate peroxidase and glutathione reductase activities of *Erythrina orientalis* in polluted and non-polluted areas

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Abstract

We investigated net photosynthetic rate and antioxidative enzyme activities in *Erythrina orientalis* grown in three different sites: Makati and Quezon (cities with high levels of air pollution, HP) and La Mesa (a non-polluted area, NP). Photosynthetic activity of *E. orientalis* was significantly reduced in the HP cities. In contrast, activities of the antioxidative enzymes ascorbate peroxidase and glutathione reductase were significantly higher in HP cities than in the NP area.

Additional key words: antioxidative enzymes; street tree.

Environmental stress, such as air pollution, is among the factors most limiting plant productivity and survivorship (Meloni *et al*. 2003). Air pollution in Metro Manila in the Philippines has become a serious problem during the last several decades. In particular, SO_2 and O_3 concentrations in the cities of Makati and Quezon continue to rise as a direct consequence of human activity, such as emissions from an increasing number of automobiles. Emissions have not been successfully restricted: ambient SO_2 and O_3 concentrations rose by 4–5 % by the end of 2004. Many studies have shown that the already decreased growth and photosynthetic rate observed in trees fumigated with ozone are further reduced when trees are exposed to additional air pollutants (Lawson *et al*. 2002, Grantz *et al*. 2003). However, the effects of air pollution on the physiological and biochemical changes in street trees in the Philippines have not yet been investigated. One of the biochemical changes which occur in trees subjected to environmental stress is the increased production of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals (Lascano *et al.* 2001, Fornazier *et al*. 2002, Meloni *et al*. 2003). Mitochondria and chloroplasts are important cellular generators of ROS, but trees regulate anti-oxidative enzymes to remove ROS. In fact, trees have developed a complex defence anti-oxidative system to reduce damage initiated by ROS, including low molecular mass antioxidants as well as anti-oxidative enzymes such as ascorbate peroxidase (APX, EC 1.11.1.1), superoxide dismutase (SOD, EC 1.15.1.1), and glutathione reductase (GR, EC 1.6.4.2). In particular, APX is distributed throughout the cell and catalyzes the reduction of H_2O_2 to H_2O. APX uses ascorbate as an electron donor in the first step of the ascorbate-glutathione cycle and is considered the most important peroxidase in H_2O_2 detoxification (Parida *et al*. 2004).

Erythrina orientalis is one of the most important tree species in the Philippines, both as a fast growing tree in urban areas as well as a component of natural ecosystems in rural areas. *E. orientalis* is a nitrogen fixing species that adapts well to harsh environments; therefore, it is commonly planted along streets in the Philippines. We surveyed differences in net photosynthetic rate (P_N) and APX and GR activities in three sampling sites in the

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Abbreviations: APX – ascorbate peroxidase; GR – glutathione reductase; HP – high polluted; NP – non-polluted; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; ROS – reactive oxygen species.
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Philippines: the cities of Makati and Quezon, which have high levels of air pollution (HP), and La Mesa, a non-polluted area (NP). The three areas are located latitudinally at 14°45'N and longitudinally at 121°05', with an elevation of 104 m above sea level. These areas have two pronounced seasons: the dry months of January to May and the wet months of June to December. The mean annual rainfall is 2 700 mm and the mean annual temperature is 23.8–30.0 °C. The objective of this study was to determine the effects of ambient air pollution on the antioxidant systems of *E. orientalis*. Therefore, in this study we compared P_N and APX and GR activities in *E. orientalis* grown along streets in different areas in the Philippines.

E. orientalis specimens of the same age growing along streets were selected (n = 5 per site) for this study. P_N was measured on the fully expanded mature leaf number 4, counted from each shoot apex, on each tree (n = 5 per each tree) in the study in the three different sites. P_N was measured with a broad-leaf cuvette from the *Licor-6400* Portable Photosynthesis System (*Licor*, Lincoln, NE, USA), the leaf was sealed, and the CO_2 concentration was maintained at ambient levels. Measurements on each tree have five replications. Sample leaf pieces (0.02 g dry mass) were taken from a recently matured leaf of every individual in three sites (n = 5). Each sample piece was immediately plunged into liquid nitrogen, and was ground to a fine powder in a mortar with a tissue homogenizer containing 30 mg insoluble polyvinylpyrrolidone and 2 cm^3 CO_2-free extraction buffer. The extraction medium contained 100 mM *Bicine* (pH 8), 1 mM EDTA, 5 mM $MgCl_2$, 5 mM dithiothreitol, and 0.02 % bovine serum albumin (m/v). The crude solution was transferred to a 1.5 cm^3 micro-centrifuge tube, centrifuged for 30 s at 12 000×*g* (*Model Marathon* centrifuge *13 F/M*; *Fisher Scientific*, Pittsburgh, PA, USA), and supernatant was retained on ice for the measurement of activity. APX activity was assayed according to Nakano and Asada (1981). The reaction mixture (1.5 cm^3) contained 50 mM phosphate buffer (pH 6.0), 0.1 mM EDTA, 0.5 mM ascorbate, 1.0 mM H_2O_2, and 0.05 cm^3 enzyme extract. The reaction was started by the addition of H_2O_2, and ascorbate oxidation was measured at 290 nm for 1 min. Enzyme activity was quantified using molar extraction for acorbate (2.8 mol m^{-3}) and the results were expressed per protein unit (Neto *et al*. 2006). GR activity was determined at 25 ºC by measuring the rate of NADPH oxidation as the decrease in absorbance at 340 nm. The reaction mixture (1 cm^3) consisted of 100 mM Tris-HCl (pH 7.8), 21 mM EDTA, 0.5 mM NADPH, 0.5 mM oxidized glutathione (GSSG), and the enzyme. NADPH was added to start the reaction (Parida *et al*. 2004). Differences of the enzyme activities were determined by analysis of variance (ANOVA) and differences among means were tested using Duncan's multiple range test (p<0.05).

P_N in *E. orientalis* in both Makati and Quezon was significantly lower than in La Mesa (Fig. 1). P_N in *E. orientalis* in La Mesa remained high during the study

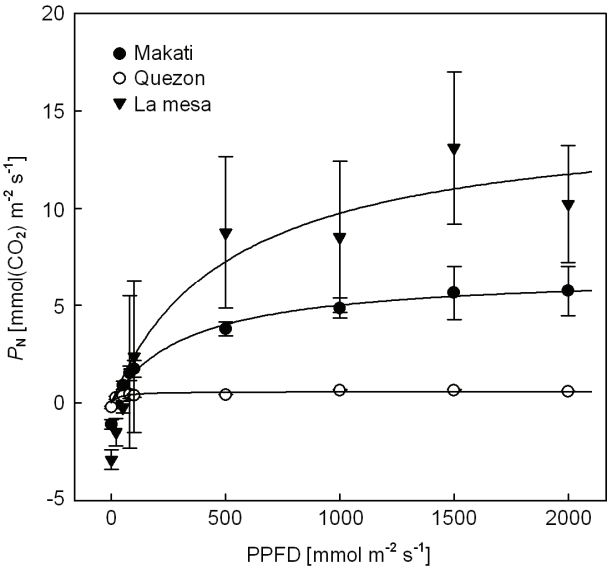

Fig.1. Photosynthetic photon flux density (PPFD) response curves of net photosynthetic rate (P_N) in *Erythrina orientalis* grown in three different sites: Makati (●), Quezon (○), and La Mesa area (▼). Means±S.D. expressed as a *bar*. n = 5.

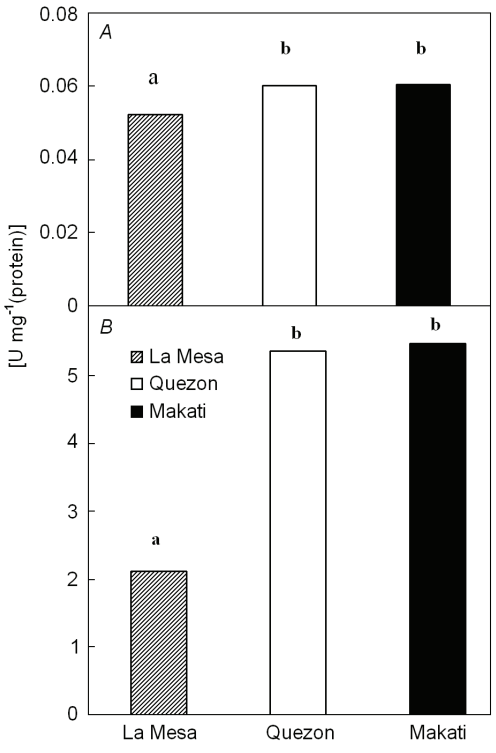

Fig. 2. Comparison of ascorbate peroxidise, APX (*A*) and glutathione reductase, GR (*B*) activities [unit mg^{-1}(protein)] in leaves of *Erythrina orientalis* grown in three different sites. *Different letters above bars* show significant differences among means at p<0.05. n = 5.

(Fig. 1). P_N in *E. orientalis* was probably decreased by chronic air pollution stress in Makati and Quezon. The mechanism by which air pollution inhibits P_N in trees is unclear, and many explanations are possible (Koch *et al*. 1998, 2000, Gravano *et al*. 2003). Air pollution stress leads to stomatal closure, which reduces CO_2 availability in leaves and inhibits carbon fixation (Robinson *et al*. 1998). P_N is a commonly used indicator of impact of increased air pollutants on tree growth. We considered P_N the most important of the variables evaluated in this study. First, P_N is regarded as a reliable predictor of plant function in response to stress. Second, high photosynthetic activity is one of the main characteristics selected for air-pollution resistant species in urban areas (Reich *et al*. 1983, 1984). Leaf APX and GR activities were higher in *E. orientalis* in Makati and Quezon than in trees in La Mesa (Fig. 2). High exposure to air pollutants forces chloroplasts into an excessive excitation energy level, which in turn increases the generation of ROS and induces oxidative stress (Meloni *et al*. 2003). Increased production of ROS in chloroplasts of trees grown under air pollution increases activities of anti-oxidative enzymes such as APX and GR (Lascano *et al*. 2001, Parida *et al*. 2004). Increased anti-oxidative enzyme activities induced by air pollution stress may indicate an enhanced protective mechanism to reduce oxidative damage triggered by pollution. In this study, increased APX and GR activities in *E. orientalis* growing in Makati and Quezon suggest that some environmental stresses affect plant growth. Plants commonly adjust physiologically to environmental stress. Reduction in the root-to-shoot ratio and an accelerated rate of leaf maturation are examples of these adjustments. Many changes in plant physiology and growth, such as those caused by air pollution, are biological compensatory responses to environmental stress (Mooney *et al*. 1988, Winner 1994). The main stress compensatory strategy in plants is to minimize damage from stress. Specifically, air pollution can reduce biomass and photosynthesis, but trees may increase their anti-oxidative enzyme activity in leaves in order to detoxify ROS. Resistance to air pollution is strongly associated with high activities of anti-oxidative enzymes (Parida *et al*. 2004). In tolerant trees, APX and GR activities are higher, enabling trees to protect themselves against oxidative stress. In this study, APX and GR activities were significantly increased in *E. orientalis* in Makati and Quezon. These higher APX and GR activities suggest a typical compensatory strategy (Neto *et al*. 2006). The relatively high APX and GR activities and low P_N in *E. orientalis* grown in HP environments (Figs. 1 and 2) may be a strategy to adjust or compensate for air pollution stress.

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