CHARACTERIZATION OF ANTIOXIDANT RESPONSES IN PLANTS OF IPOMOEA NIL CV. SCARLET

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INTRODUCTION

It is well known that antioxidants of the ascorbate - glutathione cycle play an important role on this defensive process of plants growing in the abiotic conditions, during both normal metabolic activities, as the photoreduction of the O2 into the thylakoids (Chernikova *et al.*, 2000) and by exogenous reasons, as the entrance of ozone to the plant (Iriti & Faoro 2008). Ascorbic acid (AA) is one of the most important antioxidants in plants. Hydrogen peroxide (H2O2) is decomposed while this antioxidant is oxidized to dehydroascorbic acid, a reaction that is mediated by ascorbate peroxidase. AA is also considered an important molecule against ozone due to its occurrence in cellular walls and membranes, once this pollutant enters the plant predominantly through the stomata and reacts immediately with water in the apoplast intensifying the ROS formation. The seasonality in the contents of leaf AA also affects ozone sensitivity of some plant species (Ferreira *et al.*, 2007). As scavenger of radical superoxide, the enzyme superoxide dismutase (SOD) is other important tool to defend plants against oxidative stress (Esposito *et al.*, 2009). Such antioxidants may have seasonality in their formation and activity due to exogenous factors, as for example, meteorological conditions, even in the absence of anthropic interference. This is a consequence of the fact that the seasonality in solar radiation, photoperiod, temperature and relative humidity, for example, beyond influencing the stomata aperture, regulates the processes of photosynthesis and respiration on chloroplasts and mitochondria and thus the natural production of ROS in the cells (Takac, 2004, Foyer & Noctor 2005). During the aging of the plant, variations in the levels of antioxidants may also occur (Iriti *et al.*, 2009). Therefore, it is hypothesized that variations in antioxidant responses throughout the life cycle occurs, taking the plant more susceptible to diverse environmental factors of stress, and that the seasonality in the climatic conditions can intervene with such variations, becoming the plant more or less vulnerable throughout the seasons of the year during their growth at the natural environment. In thesis, these hypotheses are also valid to the cultivar Scarlet O'Hara of Ipomoea nil, object of the present study. Besides its potential as an ornamental flowering climbing plant, it is sensitive to ozone and is indicated for biomonitoring of air quality (Nouchi & Aoki 1979). Therefore, the range of antioxidant responses to naturally varying meteorological conditions should be known before its usual application as ornamental or bioindicator plant in a determined environment.

OBJECTIVES

The aims of this work were to determine the variations in three antioxidative species in leaves of Ipomoea nil cv. Scarlet O'Hara, throughout their development through the four seasons of the year and to verify if such variations are related to the oscillations in temperature, relative humidity and solar radiation throughout the seasons.

MATERIAL AND METHODS

Plant cultivation and experimental campaigns

Seeds of Ipomoea nil cv. Scarlet O'Hara were commercially acquired (from CN Seeds LTD, www.cnseeds.co.uk) and derived from the same lot. They were germinated in a mixture of a commercial substrate mainly composed by barks of Pinus (Plantimax - Eucatex) and fine vermiculite, in the ratio of 3:1, respectively. Seedlings containing the cotyledonal leaves were transplanted to plastic vases with the same substratum mixture.

Four experimental campaigns were carried out, one in each season of 2006. The summer, autumn, winter and spring campaigns were performed in February/March, May/June, August/September and November/December respectively. Each campaign started when plants had at least seven expanded leaves including the cotyledonal ones, approximately one month after seedling transplantation, and lasted

28 days. It was initiated with 45 plants produced as described above. During the period of 28 days, in the time zero and in intervals between three or four days (totalizing nine sampling days), the concentrations of ascorbic acid (AA) and the activity of superoxide dismutase (SOD) and peroxidase (POD) were determined in the 5th, 6th and 7th older leaves of the main stem of five plants. During cultivation and experimental periods, the plants had the adequate irrigation guaranteed by capillarity thorough nylon strings and received periodic fertilization by aqueous nutrient solution prepared according to Epstein (1975). All the experimental campaigns, since the germination of the seeds to plant sampling, were carried out in a greenhouse supplied by filtered air located at the Institute of Botany. This is exactly in the Southeastern region of the São Paulo city, between' the 08" parallels 23º 38' 18" S and 23º 40 S and' the 48" meridians 46º 36' 00" W and 46º 38 W (Fernandes *et al.*, 002). Air conditioning regulated daily maximum temperatures of air so that it varied in a similar way to that in the external environment. Average values of temperature, humidity and radiation during each campaign indicate that the plants were exposed to acceptable range of these meteorological parameters and to similar environmental conditions observed in São Paulo.

Analytical procedures

AA was determined in fresh leaves (0.2 g), homogenized with 12 mL of EDTA - Na2 (0.07%) and oxalic acid (0.5%). The mixture was centrifuged at 40000 g for 30 minutes at 2ºC according to Bulbovas *et al.*, (2005). SOD and POD activity were determined according to Bulbovas *et al.*, (2005).

Statistical Analyses

Differences in antioxidant responses among leaves in each sampling day were searched by means of one way analyses of variance. Two way analyses of variance with two factors were performed in order to identify differences between the seasons (factor 1) and throughout the time in each season (factor 2). In all the cases, post - hoc multiple comparison test (Student - Newman Keuls) was applied if analysis of variance indicated significant differences. When necessary, the data were transformed to reach normal distribution and/or equal variances.

Analyses of correlation (Pearson) were carried out to determine the relations between and antioxidant responses in the leaves and meteorological conditions inside the greenhouse in each experimental campaign and between the antioxidant responses in plants analyzed during the overall experimental period.

RESULTS AND DISCUSSION

In the majority of the cases, no significant differences in the antioxidant responses were found among the leaves analyzed in each sampling day during each experimental campaign, which might characteristically indicate a leaf aging effect (data not shown), opposing the expectative that younger leaves should present more efficient capacity of defense than older leaves in the same. Moreover, the young leaf generally presents a high metabolic rhythm, due to its stage of development, which should intensify the formation of active oxygen species and demands a higher efficiency of the antioxidative system. Ohe *et al.*, (2005), for example, observed that under conditions of photoxidative stress older leaves of Nicotiana tabacum cv. Xanthi presented lower content of AA and lesser activity of ascorbate - peroxidase in the chloroplasts when compared with younger leaves. As a consequence of the absence of differential responses among leaves, the antioxidants measured in each sampling day were presented as average per plant. Such responses oscillated in their levels throughout the year.

The contents of AA in the plants of I. nil cv. Scarlet O'Hara exposed during the summer campaign gradually increased with time, reaching maximum values after seven days of experiment and then gradually decreased. The leaf AA concentration remained almost constant during plant growth in the autumn campaign. The winter campaign was characterized by a significant high concentration of the AA in plants taken in the last day of sampling. In the spring leaf AA varied throughout all 28 days of experiment, reaching maximum values after 11 to 14 days. On average, no significant variations in the leaf levels of AA were observed among seasons.

The enzyme SOD in plants of I. nil cv. Scarlet O'Hara showed an oscillatory profile during the summer campaign, with activities significantly higher after 04, 21 and 28 days of experiment. During the autumn, SOD kept a relatively constant activity in the plants, showing a gradual decrease in its levels from the 21st day on and presenting in the last day of analysis a significantly lower activity when compared to that measured in the other days. Similar to AA, activity of SOD was significantly low in plants sampled in the middle of the campaign and reached maximum values in plants sampled in the last day of sampling. The activity of SOD gradually increased with time during the spring campaign, reaching maximum values in the three last days of the sampling. On average, significantly higher value of SOD was observed in the plants analyzed during the spring campaign.

The activity of POD in plants of I. nil cv. Scarlet O'Hara did not significantly differ in plants sampled during the autumn campaign. During the winter campaign it was significantly low in plants taken after four days and high after 18 days of experiment. POD showed a clear oscillatory profile during the spring campaign. Its activity gradually increased during the first 11 days and then decreased until the 21st day. Similar oscillation was also observed at the end of this campaign. Significantly higher average value of POD was also observed in the plants analyzed during the spring campaign.

The highest values of antioxidants found in the spring campaign supposedly reflected ideal environmental conditions for growth of I. nil cv. Scarlet O'Hara. Meteorological conditions observed inside the greenhouse during that period seemed to promote high rates of photosynthesis in the plants. Consequently the formation of ROS should have been intensified, demanding increased antioxidant responses. In fact, according to Foyer & Noctor (2005), these seasonal oxidation - reduction relations may be part of how plants perceive and respond to environmental triggers.

However, the highest levels of antioxidants in plants of I. nil grown during spring may have reflected environmental stressing conditions instead of ideal conditions for growth.

According to Larcher (2000), extreme amounts of radiation and an increase of radiation absorption of UV create a situation of stress that may firstly affect the photosystem II reaction center. This fact may have occurred in the present study during spring, considering that it was characterized by the highest values of solar radiation and temperature inside de greenhouse in comparison to the meteorological conditions observed in the other seasons. Other authors also observed such seasonality in antioxidants that had been associated to the meteorological characteristics of each season of the year. This was the case of Gilham & Dodge (1987), who showed that the ascorbate levels, ascorbate - peroxidase and glutationa - redutase in leaves of Pisum satium (L.) presented an outstanding seasonal variation. The winter was marked by low activity and concentration of antioxidants and the summer by high levels of them. The authors related these results to differences in the density of light flow between both seasons that is lower in the winter.

During the hottest seasons of the year the plant respiratory rates are higher and can also reflect an increasing antioxidant response due to a consequent higher ROS production in mitochondria. According to Dizengremel (2001), the respiration increase is associated with an increase in the NADH synthesis that is related to enzyme formation such as SOD. For example, Bowler *et al.*, (1989) found a significant SOD - manganese increase in Nicotiana plumbagifolia during the enhancement of respiration rates induced by some factors of stress.

Analyses of Pearson correlation indicated that the variations in antioxidants were more strongly stimulated by daily oscillations in climatic factors as temperature, humidity and radiation five days before plant sampling throughout the different seasons of the year, evidencing that the relation between the changes in the environment and the plant antioxidant responses is time dependent. These correlations showed to be the most explicative among those tested between leaf antioxidants and environmental conditions zero to ten days before the leaf sampling (data not shown).

No significant relations were found between antioxidants and meteorological conditions during the summer campaign. Air temperature influenced positively the content of AA during autumn and winter and the activity of both enzymes during spring. Relative humidity (RH) influenced positively the levels of AA in autumn, the activity of SOD and POD in the winter and of SOD in the spring. Significant and negative relation was observed between RH and AA in the spring period. Solar radiation was negatively related to the concentrations of AA during autumn and winter and positively related to AA and SOD in the spring. Thus, such no uniform relations in autumn, winter and spring reflected differential intensity of effects of a gradient of meteorological conditions on the prooxidant/antioxidant equilibrium in the cells, as pointed by Muggli (1993). Bulbovas *et al.*, (2005) also observed no uniform relations between antioxidant responses in plants of Caesalpina echinata Lam. cultivated in the same greenhouse used in the present study and oscillations in air temperature and relative humidity, which in turn regulate the stomata movement. The authors could similarly attribute the varying antioxidant responses to greater or minor rates of plant photosynthesis, transpiration and respiration.

The antioxidant profile observed in plants of I. nil cv. Scarlet O'Hara during the summer campaign seemed to mark more characteristically the rhythm of growth and aging of the whole plant than the influence of daily meteorological conditions, as commented Ohe *et al.*, (2005) and Foyer & Noctor (2005). Maximum leaf AA concentrations and SOD activity after seven days of experiment might have indicated maximum cell division and growth rate of plants, followed by an evident aging process.

Analyses of Pearson correlation finally showed that the antioxidant responses of I. nil 'Scarlet O' Hara' were themselves positively related throughout the four campaigns [AA x SOD ($r = 0.37$, $p = 0.03$); AA x POD ($r = 0.44$, $p = 0.02$) and SOD x POD ($r = 0.44$, $p = 0.02$)]. This fact suggests that integrated plant responses to ambient variations occurred, reflecting the good capacity against oxidative stress, as found by Foyer *et al.*, (1997).

CONCLUSION

The results retrieved in this study revealed an evident seasonality in the antioxidant responses of plants of Ipomoea nil 'Scarlet O' Hara' growing throughout the four seasons of the year. These oscillating responses could be associated to a gradient of environmental conditions imposed by temperature, relative humidity and solar radiation conditions the action of determined ambient stimulations, as that can demand greater or minor efficiency of the antioxidative system.

Acknowledgements

To CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for offering a MSc scholarship to the first author.

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