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Full Length Research Paper

Relation between catalase activity, salt stress and urban environments in *Citrus aurantium* L.

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In order to evaluate the degree of adaptation of urban *Citrus aurantium* L. trees to osmotic stress and to explore the effect of seed's origin on stress tolerance/adaptation, catalase activity levels were measured. Before carrying out the analysis, seedlings issued from high and low plant mother's vitality were grown on NaCl stressed media during 2 months. Catalase activities revealed different levels according to cities, plant mother vitality and salt dose. It increased in stressed medium for the same seedlings origin. Thus, tissues superoxide dismutase (SOD) activity seems to be initiated on plant mother trees and seedlings behaviour is correlated with seed formation environment.

Key words: Seedlings, origin, urban trees, salt stress, catalase.

INTRODUCTION

A remarkable diversity in trees' vitality is noticed between urban trees even at the level of the same row, thus demonstrating stress adaptation expressions with certain subjects rather than others (Rejeb et al., 1999). Osmotic stresses (draught, salinity and heat) are considered to be the most important abiotic factors responsible of urban trees heterogeneous vitality (Tomiczek, 2003; Ledoigt and Coudret, 1992). Stresses induce the production and the accumulation of toxic oxygen species (Foyer et al., 1994; Bowler and Fluhr, 2000) which can damage cellular components such as lipids, proteins, carbohydrates and nucleic acids (Monk et al., 1989). Cells have an enzymatic and non-enzymatic antioxidant system to neutralize these free radicals. In higher plants, three major enzymes are implicated in the AOS detoxification: Superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Dodet, 1991; Willekens et al., 1995; Scandalios, 2002). Stress tolerance/adaptation seems to be correlated with stimulation of antioxidant enzymes and the enhanced ability to remove AOS and a higher concentration of CAT is noted (Bettaieb et al., 2007; Xu and Huang, 2004; Vieira Santos et al., 2001). CATs and SOD are principle enzymes which scavenge active oxygen species and avoid lipid peroxidation, cell membranes damage and chlorophyll degradation (Smirnoff, 1993; Foyer et al., 1994). CATs control of H_2O_2 level in plant cells, reduce the germination rate of seeds (Wadsworth and Scandalios, 1990) and participate to photosynthetic process (Willekens et al., 1997).

Nevertheless, plant antioxidant response is dependent on exogenous parameters such as plant development environment leading to resistance or sensitivity (Xu and Huang, 2004; Alonzo et al., 2001). According to this, and considering the fact that citrus response to stress is correlated with seed formation conditions (Moya et al., 1999; Bedhioufi et al., 2008), CAT activity was analyzed for different urban origins seedling plants and submitted to three levels of salinity. The choice of low salt doses is based on the fact that SOD activity increased if initial plant material is exposed to moderate salt stress (Viera Santos et al., 2001). The aim of this research was to learn about the degree of adaptation of seedlings collected in different urban regions in Tunisia and to search for some elements in relation with stress tolerance

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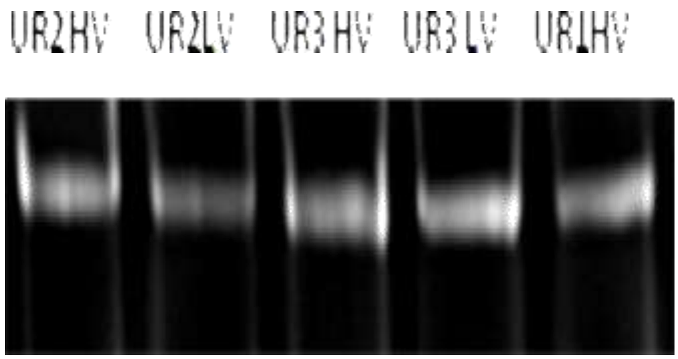

Figure 1. Zymogram analysis of catalase activity in different vitality *Citrus aurantium* urban trees (HV, high vitality ; LV, low vitality) in three urban regions (UR) of Tunisia.

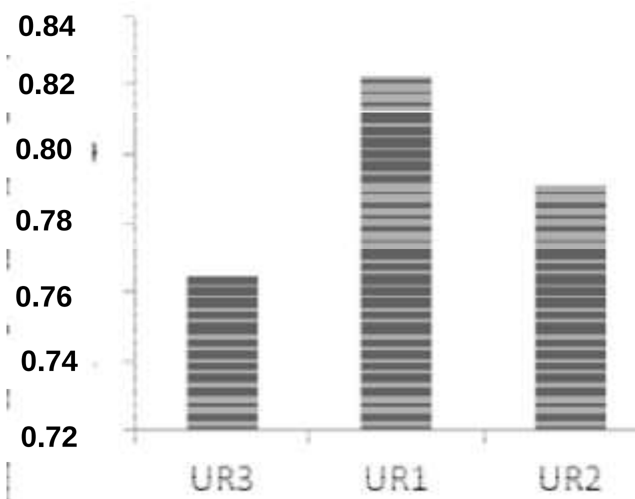

Figure 2. Fv/Fm ratio of urban Sour orange trees cultivated in three urban regions of Tunisia.

adaptation.

MATERIALS AND METHODS

Plant material and growth conditions

Seed *Citrus aurantium* L. trees showing heterogeneous vitality were selected one year in advance in different urban regions (UR) in Tunisia. Some ripe fruit were collected on hard high and low vitality *C. aurantium* L. Three cities in different bioclimatic stages were chosen for this study : UR1 a coastal humid area, UR2 a high bioclimatic semi-arid level and UR3 a typically arid region. Fruit were washed with some running water, left to soak for 10 min in bleach at 12°C, then for 15 min in a fungicide and thiophanate-methyl solution (250 g/hl). They were then left to dry in the shadow under natural temperature for 3 days and finally canned at 4°C in polyethylene bags. Seeds were grown under controlled environment with a photoperiod of 16°C and a darkness period of 8 h at 19°C. The nutrient solution was supplemented with increasing concentrations of NaCl (0 mM, 15 mM and 30 mM). Salt treatments were given for two months.

CAT analysis

Frozen fresh leaves were grounded to a fine powder in a pestle with liquid nitrogen and mixed with 1volume of extraction buffer (50 mM potassium phosphate pH 7.6, 10 mM sodium metabisulfite, 1mM ascorbic acid, 1 mM EDTA, 20% (w/v) sorbitol, 2% (w/v) polyvinylpolypyrrolidone and centrifuged at 12,000 g for 20 min at 4°C) . The supernatant was collected and the protein concentration was determined using Bradford's method (1976) . CAT activity was measured essentially as described by Clairbone (1985), with some modifications. The assay contained 15 mM H_2O_2 in 50 mM phosphate buffer (pH 7.0) and 50 mg of protein extract in total volume of 1 ml. CAT activity was estimated by a decrease of H_2O_2 absorbance at 240 nm and one unit of CAT was defined as the amount of enzyme dismounting 1 mM of H_2O_2 per min. Statistic analysis were carried out using statistical analysis system (SAS) program.

Catalase activity of mother trees showing high and low vitality was revealed on gel as follows ; the gel was washed three times (15 min each) with water, then incubated for 10 min in 0.88 mM H_2O_2 solution, which was rinsed again with water, and finally incubated with 1% (w/v) of ferric chloride and potassium ferricyanide solution until yellow bands appeared on a green background).

Chlorophyll fluorescence

Beside enzymatic measures, analysis of chlorophyll fluorescence was undertaken. The photosynthetic plant mother tree activity was estimated equal to the chlorophyll fluorescence of their leaves using a rotating system (FIM 1500) analytical development limited (ADC). The maximal measured fluorescence Fm, the initial fluorescence F0 and the variable fluorescence Fv (obtained by the difference between F0 and Fm of dark adapted leaves) were used to calculate the ratio Fv/Fm. These parameters were measured on a sample of 24 leaves for each urban region UR1, UR2 and UR3.

RESULTS

Staining gel showed superiority in the catalase activity of the adapted mother trees compared to sensitive ones even if it seems to be variable from one urban region to another (Figure 1). The higher CAT activity was observed for both, high and low *C. vitality*, cultivated in UR3. This tendency goes hand in hand with chlorophyll fluorescence values measured in each city. Infact, chlorophyll fluorescence values measured (Figure 2) showed that UR3 plant mother trees witnessed a low photosynthetic activity once compared to those of the UR1 and UR2 translated by a photochemical ratio (Fv, Fm) much lower. The weak values of Fv/Fm of urban sour orange photosynthetic system of the UR3 better reflect a lack of vitality as a result of a much higher level of stress. This superiority seems to be heritable and a highest CAT activity's intensity was observed for seedlings issued from trees showing high vitality compared to those issued from sensitive ones (Figure 3). The CAT activity averages for each NaCl level increased

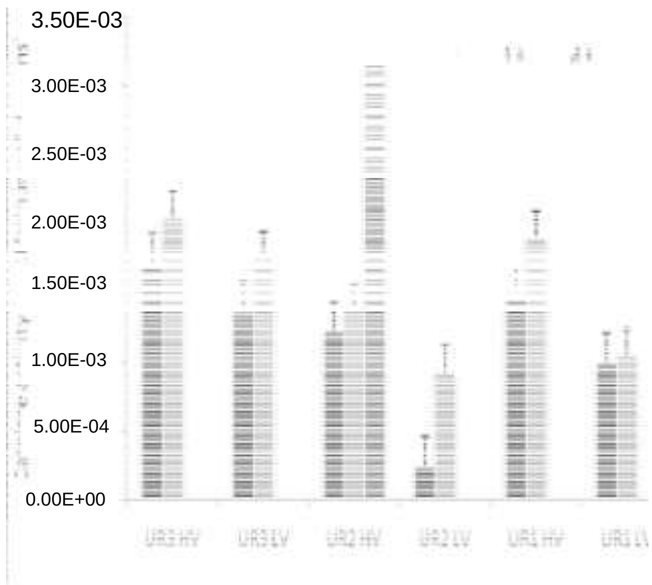

Figure 3. Catalase activity in leaves of urban *Citrus aurantium* seedlings collected in three cities of Tunisia (RU3, arid zone ; RU2, high bioclimatic semi-arid level; RU1, subhumid level) submitted to different NaCl doses (0, 15 mM and 30 mM).

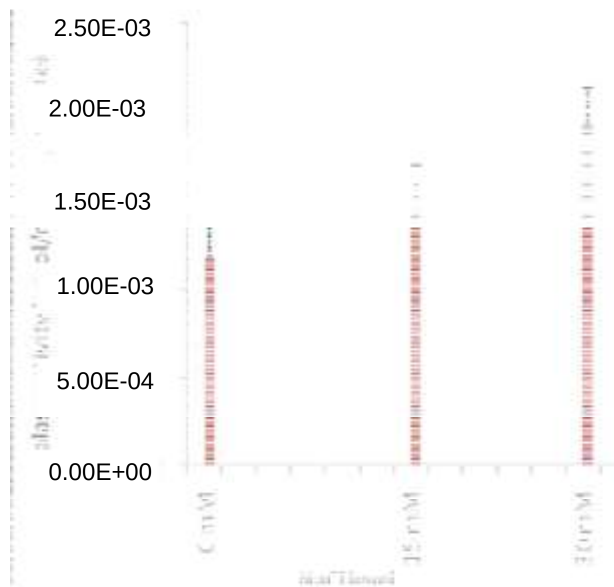

Figure 4. Averages of catalase activity in leaves of urban *Citrus aurantium* seedlings submitted to three salt doses.

significantly (p = 0.0006) with salt doses (Figure 4). A positive evolution of antioxidant capacity of urban seedlings for all tested NaCl doses was observed. This rule is verified for all origins but with different degrees (Figure 3) and the variable UR had hardly affect the CAT activity (p = 0.0005). Variability of catalase activity between cities could reveal different adaptation levels face to salt which increases from the less stressed plant

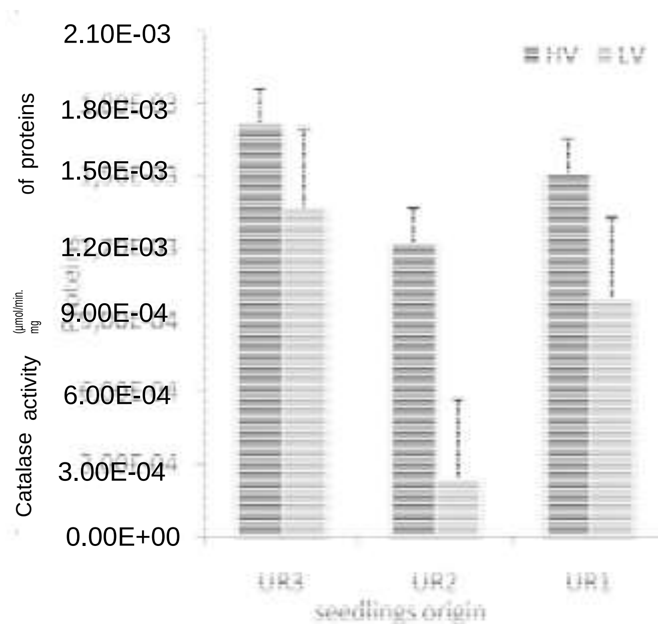

Figure 5. Catalase activity in leaves of *Citrus aurantium* control seedlings issued from high vitality plant mother trees collected in three cities of Tunisia (Gafsa : arid zone, Testour : high bioclimatic semi-arid level and Nefza : subhumid level).

mother environment UR1 to the highest one UR3 (Figure 5).

DISCUSSION

The results of this study experiments demonstrate that salt exposure induce an accumulation of CAT that play a role in the photosystem II (PSII) operation (Willekens et al., 1997) . It is probable that seedlings increased CAT activity in order to neutralize H_2O_2 and thus avoid cellular damage caused by accumulation of the substrate (Willekens et al., 1994). The increase of CAT activity for all stress levels is predicable according to the fact that SOD activity increased if the initial plant material is exposed to moderate salt stress (Viera Santos et al., 2001). Despite the importance of the antioxidant systems in stress tolerance and their sensibility to exogenous conditions, no study explored the physiologic effect of the mother plant disturbance on seedling response to osmotic stress. It seems likely that seeds origin act hardly on tolerance/adaptation capacity of seedlings and involve stimulation of antioxidant enzymes. This result converge with previous researches where stress tolerance were correlated with a higher concentration of CAT and the ability to remove AOS (Bettaieb et al., 2007; Xu and Huang, 2004; Vieira Santos et al., 2001), but, it is in contradiction with others (Jiang and Huang, 2001). Effectively, plant antioxidant response is dependent on exogenous parameters, such as plant development conditions leading to resistance or sensitivity (Xu and Huang, 2004; Alonzo et al., 2001).

The increase of CAT activity for all salt doses deduce that urban environments are stressful and that seed formation conditions involved different tolerance levels to stress according to previous works (Moya et al., 1999; Bedhioufi et al., 2008). UR1 is a costal city located at a humid climatic stage whereas UR2 is located at a high bioclimatic semi-arid level and UR3 is situated in atypically arid region. The hypothesis of acclimation of seeds which confer *Citrus vitro* plants the capacity to tolerate salt stress can be deduced. In mandarin, cold acclimation induce physiological and biochemical changes that confer tolerance to photo-oxidative event (Pietrini et al., 2005). These data support the idea that salt tolerance/adaptation may be correlated with a stimulation of antioxidant enzymes (Viera et al., 2001). Effectively, seedlings collected in the UR3 (arid region) are the most tolerant to NaCl, which is shown by the higher specific activity of CAT for both high and low mother trees vitality.

Conclusion

The evaluation of the catalase activity measured in leaves of various *Cirus aurantium* urban seedlings showed different values of specific activity from a city to another. All young plants noted an increase of CAT activity when NaCl stress increase. The increase of antioxidant activity values could deduce a tolerance/adaptation embryo's cells stimulation, on plant mother, induced by urban environment. This hypothesis is supported by the fact that UR3 seedlings noted the highest antioxidant activity for both high and low mother tree vitalities. Thus, this study suppose that antioxidant enzyme system is more stimulated. Thus, these results concluded that seedlings behaviour is correlated with seed formation environment and have learnt to question if urban stressed trees could be used to produce rootstock for *Citrus orchards*.

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