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ScienceDirect
Scientia Horticulturae 113 (2007) 49–51
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

Relation between the low temperature stress and catalase activity in gladiolus somaclones (*Gladiolus grandiflorus* Hort.)

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Received 3 April 2006; received in revised form 2 November 2006; accepted 12 January 2007

Abstract

In order to evaluate low temperature tolerance of five variable somaclones as well as the cv. Peter Pears of gladiolus (*Gladiolus grandiflorus* Hort.) from which they were originated, catalase (CAT) activity and hydrogen peroxide (H_2O_2) level were measured. Before carrying out the analyses, vitroplants of these clones were exposed, during 21 days, to 8 °C temperature. In response to the stress generated by this low temperature, catalase (CAT) activity revealed on native gel and quantified by spectrophotometry, showed variable levels, being all of them higher compared to cultivar control. For these clones, strong CAT activities were always accompanied by low H_2O_2 levels. Thus, at low temperatures the antioxidants system of adapted clones is more active compared to control.

Keywords: Low temperature tolerance; Catalase activity; Hydrogen peroxide

1. Introduction

Biotic and abiotic stresses such as drought, salinity, diseases and thermal stresses accelerate the production of active oxygen species (AOS) which then exceeds the capacity of the cells antioxidant system (Foyer et al., 1994; Bowler and Fluhr, 2000). 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 catalases (Dodet, 1991; Willekens et al., 1995; Scandalios, 2002). In the case of cold sensitive plants, catalases are more implicated than other enzymes (Volk and Feirabend, 1989).

Among AOS, hydrogen peroxide (H_2O_2) seems to be implicated in various stress situations (Bowler and Fluhr, 2000). In fact, H_2O_2 as well as after its transformation into radical $OH^{\cdot}$, is the origin of direct cellular damage. In addition to the main role of CAT in the control of H_2O_2 level in plant cells, significant functions in the stress responses are linked to these enzymes. Wadsworth and Scandalios (1990) indicated that a deficiency in CAT remarkably reduced the germination rate of barley and corn seeds. Willekens et al. (1997) indicated in their work on tobacco lines with partially limited CAT activity, that these enzymes belong to the normal process of the photosynthetic mechanism and are essential for the oxidative stress response. Shikanai et al. (1998) observed an improvement of the tolerance to light stress and drought that over express CAT activity.

Among environment limits, light deficiency and thermal shocks are regarded as the most significant factors that affect CAT activity (Volk and Feirabend, 1989; Willekens et al., 1997; Matsumura et al., 2002; Sairam et al., 2002). In order to establish a possible relation between cold tolerance and certain antioxidant enzymes, CAT activity was analyzed and H_2O_2 level was measured for five gladiolus vitrovariants (R_1, Rb_1, Rb_2, Rib_1, Rib_2) selected in a previous work (Bettaieb, 2003) for their tolerance to low temperatures and adapted to winter culture.

2. Materials and methods

The vitrovariants were regenerated from irradiated callus of 'Peter Pears' with gamma ray (50 Gy). The cultivar 'Peter Pears' was used as control before undertaking analysis, vitroplants were

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doi:10.1016/j.scienta.2007.01.007

placed in 1 l bottles containing a culture medium consisting of rock salt and vitamins of Murashige and Skoog (1962) with 30 g l^{-1} of sucrose and 0.5 mg l^{-1} of IBA (indole-3-butyric acid). The pH of culture medium was adjusted to 5.8 and this medium was solidified with agar (6 g l^{-1}). The bottles remained 21 days at 8 °C (to induce the thermal stress), with a photoperiod of 11 h at 36 μmol m^{-2} s^{-1}. Frozen plant tissues were ground to a fine powder in a pestle with liquid nitrogen and mixed with 1 volume 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* × 20 min at 4 °C. The supernatant was collected and the protein concentration was determined using Bradford's method (1976).

Soluble protein samples were subjected to non-denaturing PAGE 10% according to Laemmli (1970). Catalase activity was revealed on the 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, 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). 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 80 μg 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 μmol of H_2O_2 per minute.

The quantification of H_2O_2 was determined by chemiluminescence (Warm and Laties, 1982), with modifications (Noel, 2001). One gram of plant tissue was ground in liquid nitrogen, mixed to 250 mg of active charcoal and extraction was with 4 ml of ice cold 5% (w/v) trichloracetic acid. The crude extracts were centrifuged for 30 min at 12,000 × *g*. Two milliliters of supernatant were passed two times through a BioRad column AG1*8 (poly-prep prefilled chromatography columns), in the dark and cold room (8 °C). H_2O_2 content was measured by adding 50 μl of eluate to 50 μl of 0.5 mM luminol and 100 μl of 0.5 mM ferricyanide ($K_3Fe(CN)_6$). The luminol and the ferricyanide were solubilized in NH_4OH pH 9.5. Recovery estimates (which consisted of adding a known concentration of H_2O_2 to aliquots of the initial extracts that were processed in parallel). Average of 90% was recovered and used as a correction factor for each sample. The experiment was repeated three times and the average was considered. Trials were carried out according to a completely random block model. Each treatment was randomized on three blocks and each experimental unit related to a treatment in a block consisted of six homogeneous vitroplants. The Duncan test has been conducted to check difference between means at the 1% level.

3. Results

Staining gel showed superiority in the catalase activity at the vitrovariants selected for their cold tolerance as compared to the control. Differences between these genotypes were also observed (Fig. 1).

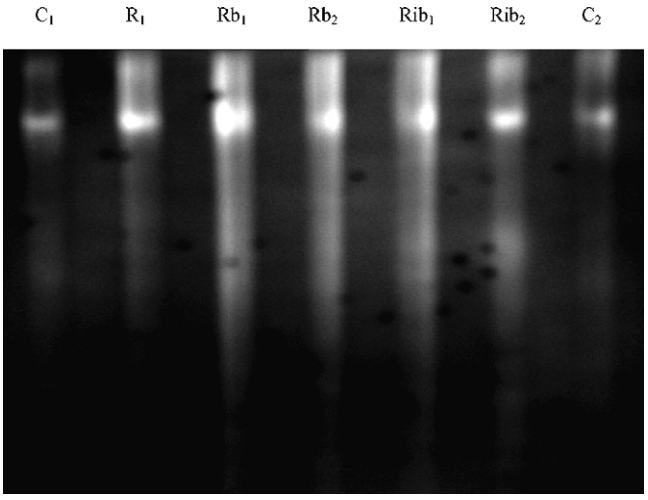

Fig. 1. Zymogram analysis of catalase activity in five clones (R_1, Rb_1, Rb_2, Rib_1 et Rib_2) of gladiolus (*Gladiolus grandiflorus* Hort.) and in two samples of the cv. control Peter Pears (C_1 and C_2) grown at 8 °C.

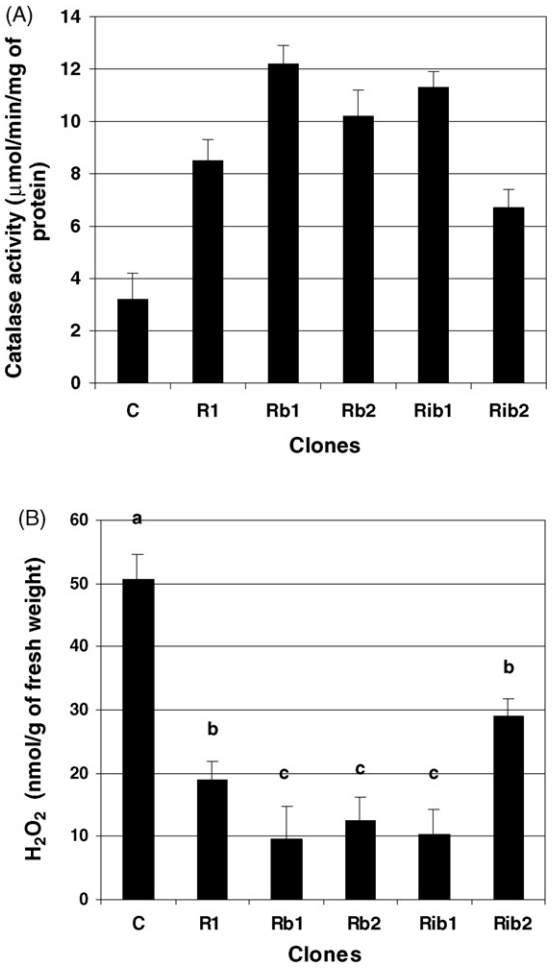

Fig. 2. Catalase activity (A) and hydrogen peroxide content (B) in tissue for five clones (R_1, Rb_1, Rb_2, Rib_1 et Rib_2) of gladiolus (*Gladiolus grandiflorus* Hort.) and of the control cv. Peter Pears grown at 8 °C. Indicated values represent the averages of the three repetitions. Bars represent the average of their estimated standard deviation. Means with the same letters are not significant at $p = 0.01$ probability level of Duncan test.

The spectrophotometry quantification of total catalase activity gave results comparable to those observed on non-denaturing gel PAGE with a superiority in all selected genotypes compared to the control (Fig. 2A). The statistical analysis showed highly significant differences between the control and genotypes for total CAT activity. Differences were also observed among genotypes with a superiority of Rb_1, Rb_2 and Rib_1. The H_2O_2 content showed highly significant differences between the control and selected genotypes for their tolerance to low temperatures (Fig. 2B). The control had the lowest level of CAT activity and the highest H_2O_2 content. The lowest level of H_2O_2 was observed in the genotypes Rb_1 which shows the highest level of CAT activity. Indeed, a high correlation ($r^2 = 0.95$) exists between these two parameters.

4. Discussion

The results of our experiments demonstrate that low temperatures induce an accumulation of AOS—who play a role in the photosystem II (PSII) operation (Willekens et al., 1997). The H_2O_2 accumulation was observed in the control (cv. Peter Pears) characterized by its sensitivity to cold (Vidalie, 1978). For the selected genotypes, the low level of H_2O_2 is explained by the important CAT activity that these genotypes exhibit.

Indeed CAT are the first enzymes which take place in H_2O_2 neutralization and thus could avoid cellular damage caused by excessive accumulation of the substrate (Willekens et al., 1994). James et al. (2001) pointed out, in their work on the deficient tobacco plant, the importance of these enzymes under stress conditions and enumerated several roles of H_2O_2 in molecular and biochemical plant mechanisms in response to stress conditions (Foyer and Noctor, 2005).

Feirabend et al. (1992) observed a reduction of 28% of catalase activity and an accumulation of H_2O_2 in rice seedlings exposed to 4 °C. After 24 h of seedlings transfer to 25 °C, these enzyme activity is restored. In addition, Volk and Feirabend (1989) suggested that, among the environmental constraints, thermal shocks are regarded as factors reducing catalase activity more than other enzymes. Puntarol et al. (1988) showed that the catalases are responsible for H_2O_2 elimination and that an over activity exhibition of these enzymes is able to induce hydrogen peroxide detoxification and is considered as a strategy for improving tolerance to the stress for plant cells.

5. Conclusion

The evaluation of the catalase activity and hydrogen peroxide measured in tissues of various selected vitrovariants for their tolerance to low temperatures showed different activities of somaclones. All somaclonal variants obtained in this work exhibited a catalase activity more significant than that of the control. Rb_1 Rb2 clones and Rib_1 have the most significantly catalase activities and consequently the lowest H_2O_2 contents. These results allow conclude that in the presence of low temperatures, the antioxidant system of adapted clones is more active compared to control. The difference in activity between vitrovariants was also highlighted. Considering this behavior, it may be inferred that these clones are becoming tolerant to the low temperature.

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