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Changes in Activities of Superoxide Dismutase, Catalase and Peroxidase during Senescence of Gladiolus Florets

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Summary

Treatments with sodium benzoate and n-propyl gallate as free radical scavengers slightly delayed the wilting of perianth in detached gladiolus florets. Superoxide dismutase (SOD) activity of the perianths sharply decreased in 2 days after their full unfolding (DAU) when they commenced to wilt. The decrease of SOD activity was alleviated by treatments with 300 μM cycloheximide (CHI) which prevented perianths from wilting. Specific activity of catalase (CAT) of perianths did not markedly change, but CAT activity on the basis of perianth decreased concomitant with their wilting. CHI slightly decreased specific activity of CAT. Peroxidase (POD) activity of the perianths sharply increased 1 DAU. This increase was almost completely suppressed by CHI treatments. These results indicate that the free radicals are involved with the decline of SOD activity, increase of POD activity, and in the wilting process of gladiolus perianths.

Key Words: superoxide dismutase, catalase, peroxidase, oxidative stress, perianth wilting.

Introduction

Senescence of flower petals is a complex process involving an increase of cell membrane permeability that results in wilting, pigment degradation, and ultimately, petal collapse (Jones and McConchie, 1995). Gladiolus spp. are iridaceous bulb plants and their florets are ethylene-insensitive after unfolding (Serek et al., 1994; Yamane et al., 1993). The ion leakage from gladiolus perianths starts to increase prior to the onset of wilting (Yamane et al., 1993) but the increase in membrane permeability and subsequent wilting are delayed by treatments with cycloheximide (CHI), a non-specific inhibitor of the peptidyl transferase activity of the 60 S ribosomal subunit in eukaryotes (Jones et al., 1994; Yamane and Ogata, 1995). While inhibition of protein synthesis extends vase life in some ethylene-insensitive flowers (Celikel and Van Doorn, 1995; Paulin, 1975; Lukaszewski and Reid, 1989), the key enzymes involved in membrane degradation are still unknown.

Free radicals are known to participate in plant senescence (Dhindsa et al., 1981). The superoxide radicals ($O_2^{\cdot -}$), or their derivatives, have been reported to induce degradation of membrane lipids in carnation petals (Mayak et al., 1983). Senescence of carnation flowers is delayed by free radical scavengers such as sodium benzoate (Baker et al., 1977), n-propyl gallate (Mayak et al., 1983), and 3,4,5-trichlorophenol (Paulin et al., 1986). Superoxide dismutase (SOD), which catalytically scavenges $O_2^{\cdot -}$ and produces hydrogen peroxide (H_2O_2), plays a key role in an antioxidative defence system for aerobic organisms (Monk et al., 1989). Catalase (CAT) and various peroxidases (POD) react with H_2O_2 (Monk et al., 1989). In carnation petals, SOD activity decreases and CAT activity increases during senescence (Droillard et al., 1987, 1989). Free radicals and antioxidative enzymes are involved in the wilting of daylily petals (Panavas and Rubinstein, 1998), but not in tepals of *Iris hollandica* (Celikel and Van Doorn, 1995). Thus, the role of oxidative stress in senescence of ethylene-insensitive flowers has not been elucidated.

In this study, we examined the activity of SOD, CAT and POD in the wilting process of gladiolus perianths treated with CHI. The hypothesis that membranes become leaky by lipid peroxidation was further tested by examining the effects of antioxidants.

Materials and Methods

Plant materials

Gladiolus (*Gladiolus grandiflorus* cv. Fujinoyuki) plants were grown in a greenhouse at Utsunomiya

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University. In August and September 1997, spikes with leaves were cut at the ground level when a perianth emerged from bracts of the florets on the spikes.

The stem ends of all cut spikes were recut under water, leaving three to four leaves; the spikes were immersed in distilled water of a 300 ml-glass bottle. The spikes were kept at 20 ± 1 ℃ under 20 μmol・m^{-2}・s^{-1} of continuous white fluorescent light and 60-80 % RH.

Fully expanded and uniformly developed florets were excised at the bottom of the ovary from the spike and the cut ends were immersed in 300 μM CHI or distilled water. The fresh weight, protein content, activities of SOD, CAT and POD of the perianths were determined − 1, 0, 1, 2, 3 and 4 days after full unfolding (DAU).

Preparation of the enzymes

All perianths were frozen in liquid nitrogen and stored at - 30 ℃. Frozen perianths were ground in liquid nitrogen and extracted with 2 ml per 1 g tissue of 0.1 M Tris-HCl buffer (pH 8.0) containing 4 mM dithiothreitol and 2 mM EDTA. Homogenates were then centrifuged at 13,700 x g for 30 min. The supernatant was assayed for CAT and POD. For SOD assay, an aliquot of the supernatant was passed through a Sephadex G-25 column to remove salts and other low molecular weight compounds. Soluble protein was assayed by the method of Lowry et al. (1951) before and after the gel filtration.

SOD assay

SOD activity was determined with BIOXYTECH® SOD-525 kit (OXIS International, OR). The activity was expressed in units according to the kit.

CAT assay

CAT activity was assayed by measuring the initial rate of disappearance of H_2O_2 by the method of Chance and Maehly (1955). A 1.5 ml reaction mixture contained 30 μl water, 50 μl 1 M Tris-HCl buffer (pH 8.0) with 5 mM EDTA, and 900 μl 10 mM H_2O_2. To the mixture, which was preincubated at 37 ℃ for 10 min, 20 μl of the crude enzyme was added to start the reaction. The decline in A_{240} was recorded for 1 min. The activity is expressed as the number of μmols of H_2O_2 catalyzed by a unit of CAT per min (or mg of protein).

POD assay

POD activity was assayed by measuring spectrophotometerically the formation of tetraguaiacol (ΔA_{470} = 13.3 mM^{-1}・cm^{-1}) in a 1.5 ml reaction mixture of 750 μl 0.1 M K-phosphate buffer (pH 7.0), 300 μl 10 mM guaiacol, 400 μl 4 mM H_2O_2 and 50 μl crude enzyme. The activity is expressed as the μmols of guaiacol oxidized to tetraguaiacol by a unit of POD per min (or mg of protein).

Effects of antioxidants on senescence

The cut ends of detached florets were immersed in 0, 0.01, 0.1 and 1 mM of sodium benzoate, n-propyl gallate and phenidone as antioxidant solutions. The time from full unfolding to wilting of the florets was assessed twice daily. Five florets were used for each treatment except for sodium benzoate in which 10 florets were tested. Data were analyzed by contrast test using a software (SuperANOVA, Abacus Concepts, CA).

Results

Perianths of gladiolus florets held in distilled water commenced to wilt between 2 and 3 DAU (data not shown). CHI treatments prevented wilting throughout the duration of experiment.

Treatments with 0.01 and 0.1 mM sodium benzoate and 0.01, 0.1 and 1 mM n-propyl gallate prolonged the turgid state of the perianth by 0.4 to 0.6 days, whereas phenidone did not have a significant effect (Table 1).

Specific activity of SOD in the perianths of control florets was 0.75 units・mg $protein^{-1}$ at 0 DAU and decreased to 0.3 units・mg $protein^{-1}$ at 2 DAU (Fig. 1). SOD activity per perianth decreased by 4.0 units・$perianth^{-1}$ between 0 and 2 DAU. CHI treatments reduced the rate of decrease in SOD activities and maintained them above 0.5 units・mg $protein^{-1}$ and 2.5 units・$perianth^{-1}$, respectively.

Specific activity of CAT in the perianths of control florets increased to 22.4 units・mg $protein^{-1}$ at 1 DAU and remained unchanged until 3 DAU; it then decreased to 11.3 units・mg $protein^{-1}$ at 4 DAU (Fig. 2). CHI

Table 1. Effects of antioxidants on the vase life (days) of cut gladiolus florets. Cut gladiolus spikes were kept at 20 ℃ and 60-80% RH with their cut end in distilled water. When the perianths had unfolded fully, the florets were detached with their ovaries and held in 0, 0.01, 0.1 and 1 mM antioxidant solutions.

Antioxidants	n	Concentration (mM)	Vase life (days)
Sodium benzoate	10	0	2.6
		0.01	3.0*
		0.1	3.1**
		1	2.8^{NS}
n-Propyl gallate	5	0	2.6
		0.01	3.1*
		0.1	3.2**
		1	3.2**
Phenidone	5	0	2.6
		0.01	2.9^{NS}
		0.1	2.9^{NS}
		1	2.8^{NS}

^{NS},*,** Nonsignificant or significant at P=0.05 or 0.01 by contrast test vs. control, respectively.

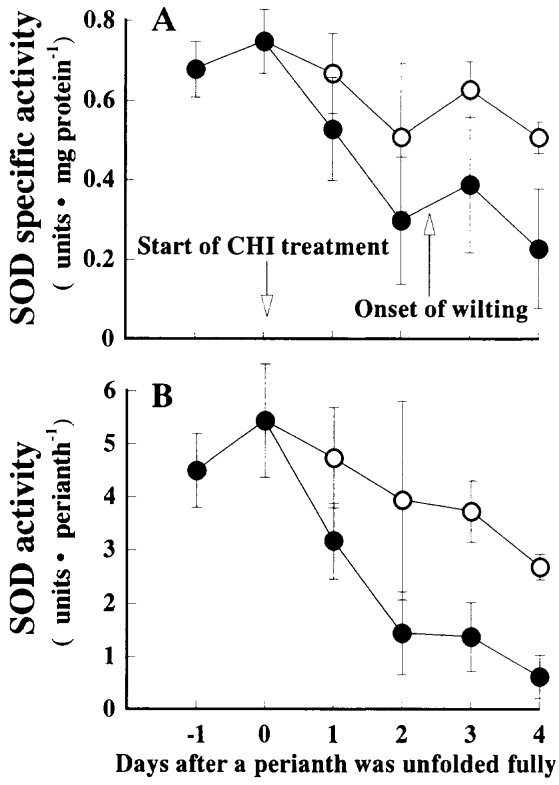

Fig. 1. Changes in superoxide dismutase (SOD) activity of perianths expressed on the bases of protein (A) or per perianth (B) in cv. Fujinoyuki gladiolus florets. Cut gladiolus spikes were put in distilled water, and kept at 20 ℃ and 60-80 % RH. When the perianths were fully unfolded, the florets with the ovaries intact were placed in 300 μM cycloheximide (CHI) solutions (○) or in distilled water (●). Vertical bars represent ± S.E. (n=5).

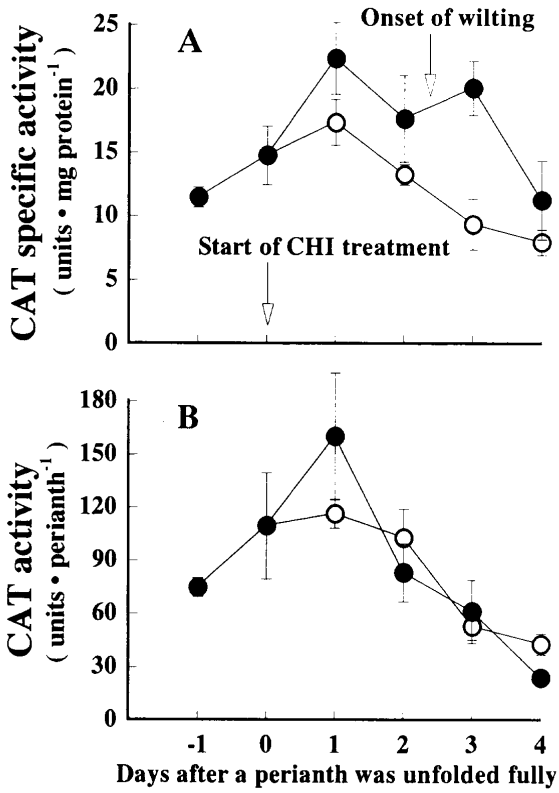

Fig. 2. Changes in catalase (CAT) activity of perianths expressed on the bases of protein (A) or per perianth (B) in cv. Fujinoyuki gladiolus florets. Gladiolus florets were treated as described in the legend to Fig. 1. ○, 300 μM CHI; ●, distilled water. Vertical bars represent ± S.E.

stimulated slightly the decrease in specific activity of CAT throughout the experiment. CAT activity per perianth peaked at 160 units • perianth^{-1} and continuously decreased to 24 units • perianth^{-1} at 4 DAU. CHI diminished the peak of CAT activity at 1 DAU.

Specific activity of POD in the perianths of control florets was very low until 1 DAU, then significantly increased, reaching 21.7 units • mg protein^{-1} at 3 DAU (Fig. 3). POD activity per perianth significantly increased between 1 and 4 DAU. In CHI-treated perianths, the POD activities remained below 0.9 units • mg protein^{-1} and 5 units • perianth^{-1}, respectively.

Discussion

Treatments with sodium benzoate and n-propyl gallate as free radical scavengers delayed slightly the onset of wilting in perianths of gladiolus florets (Table 1) which agrees with the finding of Baker et al. (1977) with sodium benzoate, and Mayak et al. (1983) with n-propyl gallate in carnations. Sodium benzoate reduced H_2O_2 and inhibited senescence in daylily petals (Panavas and Rubinstein, 1998). Phenidone did not affect the vase life of gladiolus florets (Table 1), possibly because of its poor mobility in flower tissues (Jones and McConchie, 1995). Our results suggest that free radicals are involved in the wilting of ethylene-insensitive gladiolus perianths. The role of free radicals in petal wilting of gladiolus was similar to that of ethylene-sensitive carnation and ethylene-insensitive daylily but not to *Iris hollandica* (Celikel and Van Doorn, 1995).

Several enzymes such as SOD, CAT and POD are involved in the scavenging of free radicals in plant systems (Monk et al., 1989). In this experiment, SOD activities measured as specific activity or on a per perianth basis, decreased sharply between 0 and 2 DAU, after which the perianth wilted (Fig. 1). In carnation petals, specific activity of SOD decreased after the onset of withering (Droillard et al., 1987) but SOD activity on the basis of petal commenced to decrease after full blooming (Droillard et al., 1989). Panavas and Rubinstein (1998) reported that the specific activity of SOD increased simultaneously with senescence in daylily, but they attributed the increase to the decrease in total protein content. CHI treatments reduced the rate of decrease of SOD activity and prevented the wilting (Fig. 1) on account of its inhibition of protein synthesis and thereby suppress SOD activity. CHI prevented the increase of ion leakage from gladiolus perianth tissues (Yamane and Ogata, 1995) which indicates that decreasing SOD activity induced a loss of a defensive function against $O_2^{\cdot -}$ and in turn hastening membrane degradation in gladiolus perianth tissues and eventually

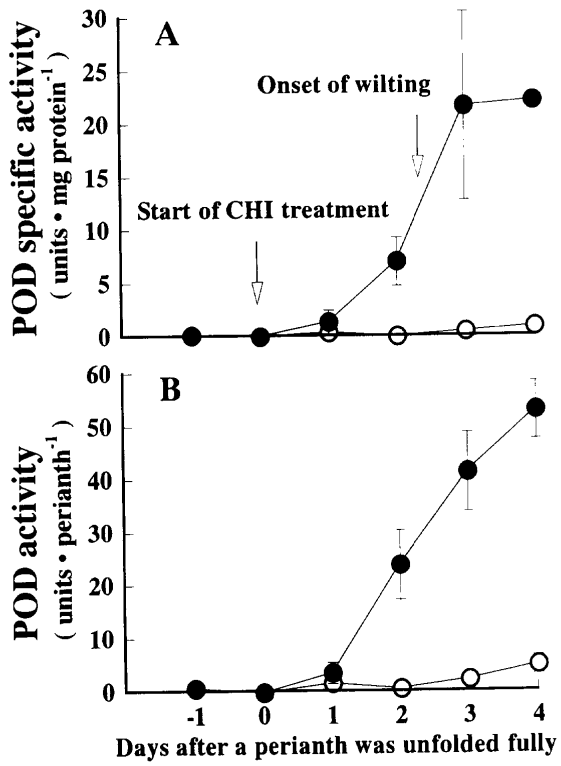

Fig. 3. Changes in peroxidase (POD) activity of perianths expressed on the bases of protein (A) or per perianth (B) in cv. Fujinoyuki gladiolus florets. Gladiolus florets were treated as described in the legend to Fig. 1. ○, 300 μM CHI; ●, distilled water. Vertical bars represent ± S.E.

wilting.

The specific activity of CAT in the perianths of control florets increased at 1 DAU and remained unchanged until 3 DAU, then decreased at 4 DAU (Fig. 2). In carnation petals (Droillard et al., 1987), the specific activity of CAT showed a similar pattern to that of gladiolus perianth (Fig. 2). In daylily petals, the specific activity of CAT gradually decreased after opening (Panavas and Rubinstein, 1998). In gladiolus perianths, the onset of wilting appeared between 2 and 3 DAU. The specific activity of CAT did not markedly change, whereas CAT activity per perianth decreased in association with the onset of wilting (Fig. 2). This difference is attributed to the decrease in total protein content. CHI seemed to suppress CAT synthesis between 0 and 1 DAU and then became ineffective in the anabolism or catabolism of CAT. It, however, prevented the wilting of perianths until 4 DAU (Fig. 2). Hence, CAT activity does not play a critical role in the wilting of gladiolus florets.

POD activity sharply increased after 1 DAU, accompanying floret senescence which was significantly delayed by CHI treatments (Fig. 3). POD activity increased in association with senescence of petals of daylily (Panavas and Rubinstein, 1998) and tulip (Carfantan and Daussant, 1975). These findings and ours indicate that POD is involved in the senescence of gladiolus perianths because it catalyzes the decomposition of H_2O_2. But the mode of action of POD differs from that of CAT in that POD liberates free radicals instead of oxygen (Burris, 1960). Moreover, decreasing CAT activity and increasing POD activity can lead to the slow removal of H_2O_2 in plant tissues (Wang, 1995). Therefore, an increase in the ratio of POD / CAT activity might promote oxidative stress in membranes of perianths, resulting in leakiness and consequently they wilted. On the other hand, various environmental stresses induce POD in plant tissues (Wang, 1995) so that membrane degradation in gladiolus perianths by $O_2^{\cdot -}$ could have been a consequence of one, albeit secondarily.

A role for POD in lignin synthesis was suggested (Burris, 1960). In daylily, POD activity appeared to be concentrated in vascular tissues of petals (Panavas and Rubinstein, 1998). Along with wilting of gladiolus perianths, some substances are translocated from wilting perianths to other parts of the spikes through vascular tissues (Yamane et al., 1995). An increase in POD activity in perianths may strengthen vascular cells, which remain functional during the later stage of senescence (Panavas and Rubinstein, 1998). Further study is necessary to clarify the role of POD in the wilting of gladiolus perianths.

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グラジオラス小花の老化にともなうスーパーオキシドディスムターゼ，カタラーゼおよびペルオキシダーゼ活性の変化

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摘　要

フリーラジカル捕捉剤である安息香酸ナトリウムおよび没食子酸n-プロピル処理により，切り離されたグラジオラス小花における花被のしおれの開始がわずかに遅れた．花被のスーパーオキシドディスムターゼ(SOD)活性は花被の完全展開後2日間で著しく低下し，その後，花被のしおれが開始した．300 μMシクロヘキシミド(CHI)処理により花被のSOD活性の低下は緩和され，しおれは抑制された．花被のしおれが開始したとき，カタラーゼ(CAT)の比活性はほぼ一定であり，花被当たりのCAT活性は低下した．CHI処理によってCAT活性はわずかに低下した．花被のペルオキシダーゼ(POD)活性は完全展開1日後から著しく上昇した．この上昇はCHI処理によりほぼ完全に抑制された．これらの結果から，フリーラジカル，SOD活性の低下およびPOD活性の上昇はグラジオラス花被の老化過程に関与することが示唆された．