Floral Lipoxygenase: Activity during Senescence and Inhibition by Phenidone

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Abstract. Analysis of petal extracts from various species revealed a wide range of lipoxygenase (LOX) activity among sources. A large increase in LOX activity was observed during senescence in both gladiolus (*Gladiolus* × *hortulanus* Bailey) and carnation (*Dianthus caryophyllus* L.) petal tissue. The increase began before the first visible signs of senescence were evident and activity peaked at early to mid-senescence stages. LOX activity of petal extracts from gladiolus was inhibited in vitro by phenidone, a known LOX inhibitor.

Lipoxygenase (linoleate : oxygen oxidoreductase, EC 1.13.11.12), which catalyzes the oxidation of unsaturated fatty acids containing a cis,cis-1,4-pentadiene system, has been implicated in the senescence and wounding response of plants (Galliard, 1978; Galliard and Chan, 1980; Lesham et al., 1981). LOX activity results in the formation of lipid peroxides that accumulate during aging of animal and plant cells (Dhindsa et al., 1981; Galliard, 1978; Pauls and Thompson, 1984). Lipid peroxide, levels have been correlated with senescence and increased membrane permeability (Dhindsa et al., 1981; Kockritz et al., 1985; Peterman and Siedow, 1985). LOX activity has been found to increase in apples during storage (Feys et al., 1980) and peak during the ripening of fruits of tomato (Bonnet and Crouzet, 1977; Frenkel and Eskin, 1977; Sekiya et al., 1983) and grape (Zamora et al., 1985).

Baker et al. (1985) reported that a vase solution containing phenidone, a known LOX inhibitor, greatly enhances the vase life of carnations. Their results suggested a possible involvement of lipoxygenase in carnation petal senescence.

The objectives of this research were to: 1) survey LOX activity in several floral species; 2) document changes in LOX during floral senescence of gladiolus and carnation; and 3) examine the effect of phenidone on LOX activity of petal extracts.

Materials and Methods

Lipoxygenase assay. Tissue was frozen in liquid N₂, ground to a powder with a prechilled mortar and pestle, and stored in sealed polyethylene bags at -20 to -15C for several days before analysis. Petal extracts were prepared with 5 g of powdered tissue in 15 ml of 0.1 M phosphate buffer (pH 6.5) at 0C. To this slurry, 0.5 g of dry polyvinylpyrrolidone (PVPP) was added and stirred intermittently for 5 min. The slurry was passed through eight layers of cheesecloth and centrifuged at 100,000× g for 15 min. The supernatant was kept on ice and assayed for O₂ consumption polarographically with an oxygen

electrode (Rank Brothers, Bottisham, Cambridge, U.K.) calibrated with 1 ml of air-saturated deionized double-distilled water and sodium dithionite.

A recirculating water bath at a constant 29C was used to maintain the reaction cell temperature. A 1-ml reaction volume was used with 0.9 ml of cold extract that was allowed to warm in the reaction cell while the recorder trace became steady and before 0.1 ml of cold substrate of blank was added. Linoleic acid (Sigma, St. Louis, Mo.) emulsified with 0.8% Tween 20 (Yoon and Klein, 1979) in N₂-saturated deionized double-distilled water was used as substrate (DuPont et al., 1982). Stock solution was stored at 2C in 3-ml vials flushed with N₂. The final concentration of substrate was 2.5 mM unless otherwise indicated. The control levels of O₂ consumption were determined using N₂-saturated deionized double-distilled water.

Species survey. Species selected for the survey were representative of flowering plants common in the floriculture industry. Potted or cut material was held on a laboratory bench at 20 to 25C until some early signs of petal senescence were visible, such as wilting or discoloration. Petal tissue from at least three flowers was then selected to yield 5 g fresh weight of powdered tissue for each of three replicates per species. The tissue was then assayed as described above.

Lipoxygenase activity during senescence. To characterize the activity of LOX during gladiolus and carnation floral opening and senescence, flowers were harvested at predetermined stages of senescence and assayed for LOX activity. South American carnations ('Improved White Sim') were obtained free of any silver treatment and graded to a uniform stage with the outer petals at a 90° angle to the stem axis. Gladiolus spikes ('Manatee White') from Florida were graded so that only the initial bud showed 1 cm of color. The cut stems of both species were placed in deionized water in a room maintained at 21C with 60% to 80% RH under 12-hr days/12-hr nights with cool-white fluorescent lamps supplying 37 to 42 μmol·s⁻¹·m⁻². A maximum room ethylene concentration of 15 nl·liter⁻¹ was maintained through forced-air scrubbing. Flowers or florets were selected at random as they reached predetermined senescence stages. Gladiolus stages were: 1) petals not emerged from sepals; 2) petals emerged from sepals but not unfurled; 3) petals partly unfurled to 45° angles to flower's axis; 4) petals fully open, fully turgid, no visible signs of senescence; 5) petal loss of turgidity but no visible browning; 6) browning of wilted petals; and 7) marginal necrosis with papery petal edges. Carnation stages were: 1) petals emerged 2 to 3 cm from calyx; 2) outer petals at 90° angles to the stem axis; 3) flower fully open with

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outer petals folded back at 45° angles to stem axis; 4) loss of petal turgidity, no inrolling; 5) first visible petal inrolling; and 6) late inrolling, initial marginal necrosis. Samples were selected to yield 5 g fresh weight of the powdered tissue (four to eight gladiolus florets, three to four carnation flowers) for each of three replicate assays. LOX assay was as described above. To avoid artifacts from fresh weight changes during senescence, LOX activity was expressed on a per-milligram protein basis with protein content determined by the Coomassie brilliant blue standard assay procedure (Bio-Rad, 1984).

Inhibition of floral lipoxygenase by phenidone. Petal tissue from stage 5 gladiolus florets extracted as described above was reacted with linoleic acid substrate in the presence of phenidone. Phenidone stock solution was in deionized double-distilled water. The petal extract (0.8 ml) and 0.1 ml of phenidone stock solution (final concentration of 1 mM) were allowed to warm in the reaction cell before addition of 0.1 ml of linoleic acid emulsion. Deionized double-distilled water was used as control for comparison with phenidone. A substrate concentration series was used and was obtained by dilution of the linoleic acid stock solution described above.

Results

Species survey. A wide range of LOX activities from petal extracts was observed among species (Table 1). There was also considerable variation in activity within species. Several tissues showed little or no activity.

Lipoxygenase activity during senescence. The trend for gladiolus LOX levels is presented from two separate experiments (Fig. 1). The first experiment used stages 1 through 5. LOX activity was found to be still on the rise in the final stage selected, so a second experiment repeated stages 4 and 5 and extended the scale through mid-senescence (stage, 6) and late senescence (stage 7). The rise in activity began when the flower was still unfurling (stage 3) and rose dramatically when the flower was fully opened but not showing any visible signs of senescence (stage 4). The peak in activity coincided with early senescence (stage 5).

LOX activity in carnation petals followed a similar trend (Fig. 2). The activity was observed to be very low before opening of the flower, but rose when the flower was fully opened with no visible signs of senescence (stage 3). The peak in activity again

Fig. 1. Lipoxygenase activity of crude extracts of 'Manatee White' gladiolus petals at seven stages of development and senescence (see Materials and Methods). Vertical bars indicate ± 1 SD.

Fig. 2. Lipoxygenase activity of crude extracts of 'Improved White Sim' carnation petals at six stages of development and senescence (see Materials and Methods). Vertical bars indicate ± 1 SD.

occurred along with the visible signs of early (stage 4) and mid-senescence (stage 5). The peak of activity in carnation petals was about one-tenth the peak for gladiolus petals.

Inhibition of floral lipoxygenase by phenidone. At all substrate concentrations that had measurable activities, there was considerable inhibition by phenidone of LOX activity extracted from gladiolus petals (Fig. 3).

Discussion

The results of the tissue survey agree with those previously published for other tissues in terms of level and variability (Pinsky et al., 1971; Rhee and Watts, 1966). The LOX activity observed in tulip petals was clearly higher than in any other source but with considerable variability. The trends in LOX activity from gladiolus and carnation petals, that were subsequently observed (Figs. 1 and 2) indicated that at the early stages of visible senescence the LOX activity is undergoing a rapid increase. Considerable variation in LOX activity at this stage with tulips and other species would therefore be expected. The wide range of activity detected certainly does not suggest a common role for LOX activity in floral senescence across species.

An increase in LOX activity, which was initiated before vis-

Table 1. Lipoxygenase activity in petal extracts² of selected species.

Species	$\mu\text{mol O}_2$ $\text{min}^{-1}\text{g}^{-1}$ fresh wt
<i>Alstromeria aurantiaca</i>	20 (11)
<i>Anthurium andreanum</i>	ND —
<i>Antirrhinum majus</i>	11 (14)
<i>Calceolaria</i> sp.	42 (15)
<i>Chrysanthemum × morifolium</i>	4 (16)
<i>Dianthus caryophyllus</i>	21 (45)
<i>Gladiolus</i> sp.	141 (76)
<i>Hyacinthus orientalis</i>	261 (135)
<i>Iris hollandica</i>	22 (13)
<i>Lilium longiflorum</i>	2 (2)
<i>Narcissus pseudonarcissus</i>	56 (26)
<i>Rosa × hybrida</i> 'Royalty'	3 (1)
<i>Sinningia speciosa</i>	ND —
<i>Tulipa gesneriana</i>	1007 (1501)

²Petals sampled at stage of first visible senescence symptom.

³Values are means of three replications with (SD), or were nondetectable (ND).

Fig. 3. Lipoxigenase activity in a crude extract of 'Manatee White' gladiolus petals at a range of substrate concentrations alone and in the presence of 1 mM phenidone.

ible signs of senescence for both gladiolus and carnation (Figs. 1 and 2), suggests that either LOX activity has a causal role in some degradative senescence processes or other early senescence processes only result in an increase in activity. Our observations agreed with others' in that LOX activity increased when tissue was at a pre-senescence stage, peaked during early senescence, and then declined (Bonnet and Crouzet, 1977; Frenkel and Eskin, 1977; Zamora et al., 1985). However, our data do not conclusively support a causal relationship between increased LOX activity and petal senescence.

The inhibition of gladiolus petal LOX activity by phenidone (Fig. 3) was more severe than that observed by Blackwell and Flower (1978). They reported 50% inhibition of platelet LOX by 3 mM phenidone. In our studies there was $\approx$ 50% inhibition of LOX activity with only 1 mM phenidone over a range of substrate concentrations. Miyazawa et al. (1985) reported 50% inhibition of soybean LOX by only 7 μ M phenidone. Thus, there appear to be differences in the effectiveness of phenidone in inhibiting LOX activity, dependent on the LOX source.

Our observed trends in LOX activity (Figs. 1 and 2) and inhibition (Fig. 3) support the possibility that phenidone or other LOX inhibitors could be useful as floral preservatives. Success with these compounds would require that they inhibit LOX activity in vivo and that LOX inhibition actually delay petal senescence, or that the compounds function by some other mechanism. We have been unable to duplicate the successful use of inhibitors as floral preservatives reported by Baker et al. (1985) with carnations (Peary and Prince, 1989). We have also failed to significantly enhance the vase life of roses and gladiolus (Peary and Prince, 1989) as well as excised flowers of *Lilium longiflorum* (Prince et al., 1987) by inhibitor application. The cause for this discrepancy in response is unknown.

Literature Cited

- Baker, J.E., C.Y. Wang, and D.E. Terlizzi. 1985. Delay of senescence in carnations by pyrazon, phenidone analogues, and Tiron. *HortScience* 20:121-122.
- Bio-Rad. 1984. Bio-Rad protein assay. Bio-Rad Bul. 1069.
- Blackwell, G.J. and R.J. Flower. 1978. 1-Phenyl-3-pyrazolidone: an inhibitor of cycle-oxygenase and lipoxigenase pathways in lung and platelets. *Prostaglandins* 16(3):417-425.
- Bonnet, J.L. and J. Crouzet. 1977. Lipoxigenase from tomato fruit: partial purification and study of some properties. *J. Food Sci.* 42(3):625-628.
- Dhindsa, R., P. Plumb-Dhindsa, and T.A. Thorpe. 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Expt. Bot.* 32(126):93-101.
- DuPont, J., P. Rustin, and C. Lance. 1982. Interaction between mitochondrial cytochromes and linoleic acid hydroperoxide. *Plant Physiol.* 69:1308-1314.
- Feys, M., W. Naesens, P. Toback, and E. Maes. 1980. Lipoxigenase activity in apples in relation to storage and physiological disorders. *Phytochemistry* 19(6):1009-1011.
- Frenkel, C. and M. Eskin. 1977. Ethylene evolution as related to changes in hydroperoxides in ripening tomato fruit. *HortScience* 12:552-553.
- Galliard, T. 1978. Lipolytic and lipoxigenase enzymes in plants and their action in wounded tissues, p. 155-201. In: G. Kahl (ed.). *Biochemistry of wounded plant tissues*. De Gruyter, New York.
- Galliard, T. and H.W.S. Chan. 1980. Lipoxigenases, p. 131-161. In: P.K. Stumpf and E.E. Conn (eds.). *The biochemistry of plants*. vol. 4. Academic, New York.
- Kockritz, A., T. Schewe, B. Hieke, and W. Hass. 1985. The effects of soybean lipoxigenase-1 on chloroplasts from wheat. *Phytochemistry* 24(3):381-384.
- Leshem, Y.Y., Y. Liftman, S. Grossman, and A.A. Frimer. 1981. Free radicals and pea foliage senescence: increase of lipoxigenase and ESR signals and cytokinin-induced changes, p. 676-678. In: M.A.J. Rodgers and E.L. Powers (eds.). *Oxygen and oxy-radicals in chemistry and biology*. Academic, New York.
- Miyazawa, K., Y. Iimori, M. Makino, T. Mikami, and K. Miyasaka. 1985. Effects of some non-steroidal anti-inflammatory drugs and other agents on cyclooxygenase and lipoxigenase activities in some enzyme preparations. *Jpn. J. Pharmacol.* 38:199-205.
- Pauls, K.P. and J.E. Thompson. 1984. Evidence for the accumulation of peroxidized lipids in membranes of senescing cotyledons. *Plant Physiol.* 75:1152-1157.
- Peary, J.S. and T.A. Prince. 1988. Cut flower vase life is little-affected by lipoxigenase inhibitors. *HortScience* 24:808-809.
- Peterman, T.K. and J.N. Siedow. 1985. Behavior of lipoxigenase during establishment, senescence, and rejuvenation of soybean cotyledons. *Plant Physiol.* 78:690-695.
- Pinsky, A., S. Grossman, and M. Trop. 1971. Lipoxigenase content and antioxidant activity of some fruits and vegetables. *J. Food Sci.* 36:571-572.
- Prince, T.A., M.S. Cunningham, and J.S. Peary. 1987. Floral and foliar quality of potted Easter lilies after STS or phenidone application, refrigerated storage, and simulated shipment. *J. Amer. Soc. Hort. Sci.* 112:469-473.
- Rhee, K.S. and B.M. Watts. 1966. Evaluation of lipid oxidation in plant tissues. *J. Food Sci.* 31:664-668.
- Sekiya, J., T. Kajiwar, K. Munechika, and A. Hatanaka. 1983. Distribution of lipoxigenase and hydroperoxide lyase in the leaves of various plant species. *Phytochemistry* 22(9):1867-1869.
- Yoon, S. and B.P. Klein. 1979. Some properties of pea lipoxigenase isoenzymes. *J. Agr. Food Chem.* 27(5):955-962.
- Zamora, R., J.I. Maynar, and J.L. Mesias. 1985. Lipoxigenase activity in grapes (cv. Macabeo). I. Evidence and partial purification. *Amer. J. Enol. Viticult.* 36(4):316-318.