



Wound-induced increases in phenolic content of fresh-cut lettuce is reduced by a short immersion in aqueous hypertonic solutions

Ho-Min Kang, M.E. Saltveit*

Department of Vegetable Crops, Mann Laboratory, University of California, Davis, CA 95616-8631, USA

Received 19 September 2002; accepted 3 March 2003

Abstract

Wounding during the preparation of fresh-cut lettuce induces the synthesis of phenylalanine ammonia lyase (PAL, EC 4.3.1.5), and the synthesis and accumulation of specific phenolic compounds (e.g., chlorogenic acid) that contribute to subsequent tissue browning. Increased PAL activity and phenolic content were not induced in non-wounded lettuce that was kept in close proximity to wounded tissue through either a vapor or aqueous signal. The phenolic content of fresh-cut Iceberg and Romaine lettuce was reduced in tissue pieces immediately immersed for 2 h in hypertonic aqueous mannitol solutions (0.3–0.9 M) after cutting. Re-wounding tissue segments 1 day after the initial excision did not produce a second rise in PAL activity in those pieces in which wound-induced increases in PAL activity had been suppressed by a previous soak in hypertonic aqueous mannitol solutions. It appears that the hypertonic solution did not cause the loss of a portion of the wound signal through efflux of water from the tissue, but rather induced a general stress-related resistance to further abiotic stresses.

© 2003 Elsevier B.V. All rights reserved.

Keywords: Crisphead; Iceberg; PALphenylalanine ammonia lyase; Romainewound signal

1. Introduction

Preparing fresh-cut Iceberg lettuce (*Lactuca sativa* L.) entails wounding that induces the synthesis of enzymes of the phenylpropanoid pathway, the subsequent synthesis and accumula-

tion of phenolic compounds, and tissue browning that reduces quality (Bolin and Huxsoll, 1991; Lopez-Galvez et al., 1996; Tomás-Barberán et al., 1997). A wound signal appears to form at the site of mechanical injury (i.e. cuts, punctures, and abrasions) and moves into adjacent tissues at about 0.5 cm h^{-1} (Ke and Saltveit, 1989). This wound signal induces the transcription of specific mRNAs (e.g., wound-induced phenylalanine ammonia lyase (PAL) mRNA), the de novo synthesis of PAL (EC 4.3.1.5), the synthesis and accumula-

* Corresponding author. Tel.: +1-530-752-1815; fax: +1-530-752-4554.

E-mail address: mesaltveit@ucdavis.edu (M.E. Saltveit).

tion of phenolic compounds in cells up to 2 cm from the site of injury, and tissue browning near the site of injury (Peiser et al., 1998; Campos-Vargas and Saltveit, 2002).

The exact nature of the wound signal in lettuce is not known, but abscisic acid (ABA), ethylene, jasmonic acid (JA), methyl jasmonate (MeJA), and salicylic acid (SA) are some of the chemical wound signals identified in other plants (León et al. 2001). Exposure of harvested heads of Romaine lettuce to ABA, JA, MeJA, or SA did not induce significant changes in PAL activity, the concentration of phenolic compounds or browning in mature leaf tissue similar to that induced by wounding (Campos-Vargas and Saltveit, 2002). However, ethylene, JA, MeJA and SA did induce elevated levels of PAL activity, the accumulation of phenolic compounds and tissue browning in younger leaves, but the induced levels were far lower than those induced by wounding.

A number of abiotic shock treatments (e.g., chemical, osmotic, thermal) induce the synthesis and accumulation of a unique set of proteins exemplified by the family of heat shock proteins (hsps) (Linguist, 1986; Vierling, 1991). A 90 s 45 °C heat shock administered to fresh-cut lettuce induced the synthesis of hsps (Kang and Saltveit, 2002), reduced tissue browning, the accumulation of phenolic compounds and an increase in PAL activity (Loaiza-Velarde and Saltveit, 2001), or PAL protein, yet it did not reduce the transcription of wound-induced PAL mRNA (Campos-Vargas and Saltveit, 2002). Heat shock gene expression is controlled primarily at the translational level during cellular responses to stress (Apuya and Zimmerman, 1992; Brostrom and Brostrom, 1998). It appears that heat shock treatments that are effective in preventing wound-induced tissue browning interfered with the translation of wound-induced PAL mRNA (Saltveit, 2000).

Immersion of tissue in hypertonic aqueous solutions causes cellular plasmolysis through an efflux of water from the cells. If the wound signal is a chemical that diffuses from the site of injury into adjacent cells, then immersing wounded tissue in a hypertonic solution might cause some of the signal to be carried out of the tissue with the efflux

of water. Research reported in this paper was undertaken to further characterize the wound signal by subjecting cut lettuce leaf tissue to aqueous solutions of various osmotic strengths. We show that immersion of cut lettuce in aqueous hypertonic solutions reduces the accumulation of phenolic compounds in a manner consistent with an induced tolerance to stress, rather a loss of a portion of the wound signal through efflux of water from the tissue.

2. Materials and methods

2.1. Plant tissue

Iceberg and Romaine lettuce (*Lactuca sativa* L.) was obtained from commercial sources within a few days of harvest. Mature heads free of visual damage or defects were stored in humidified ethylene-free air at 2.5 °C for no more than 2 days before use. Since wounding induces PAL activity in tissue up to 2.5 cm away from the site of wounding (Ke and Saltveit, 1989), only whole leaves free of injury were used. Outer leaves were removed and the next 4–6 whole leaves were cut at their bases and carefully separated from the head so that wounding (e.g., cracks, rips, etc.) did not occur within at least 3 cm of the tissue to be used for experimentation. In most experiments, leaves were cut into 2 × 2 cm pieces, rinsed in chlorinated water (50 µg ml⁻¹ NaOCl, pH 7.0) and manually centrifuged in a ‘salad spinner’ for 1 min.

2.2. PAL analysis

Midrib segments, 2 × 2 cm, were excised from treated leaves and used for PAL analysis as previously described by Ke and Saltveit (1986), and modified by Ritenour and Saltveit (1996).

2.3. Wound signal

Two series of experiments were done to see if the wound signal was an easily transmissible volatile or water-soluble compound. In the first series of experiments, four whole, fully expanded, non-injured Romaine lettuce leaves were enclosed in a

20-l glass jar with twice their weight of Romaine leaf tissue that had just been cut into 2×2 cm pieces. Two moistened paper towels were laid over the whole leaves to minimize water loss. The jar was capped and held at 10°C for 48 h. Levels of CO_2 were periodically monitored and never rose above 0.2%. In another series of experiments, the cut bases of the leaves were immersed in 500 ml of water containing a weight of 2×2 cm Romaine leaf tissue pieces equal to twice the weight of the four whole leaves. The containers were held at 10°C , and air flow past the leaves facilitated uptake and translocation of the water (Campos-Vargas and Saltveit, 2002).

2.4. Treatment solutions

Aqueous solutions were made by dissolving a weighed amount of mannitol in deionized water. Cut tissue was immediately immersed in 10 times its weight of aqueous solution for varying lengths of time, then rinsed twice with deionized water, and blotted dry with paper towels. After treatment, the fresh-cut lettuce segments were placed into containers through which a flow of humidified ethylene-free air was maintained to keep CO_2 levels below 0.2%. The tissue was stored at 10°C for various lengths of time.

2.5. Determination of phenolic compounds

The concentration of phenolic compounds was measured as described by Ke and Saltveit (1989): briefly, 10 g of mid-rib tissue was homogenized in 20 ml of HPLC grade methanol using an Ultra-Turrax[®] tissue homogenizer (Takmar[™], Cincinnati, OH) at moderate speed (setting of 60) for 30 s. Lettuce tissue is about 98% water, so the extract was about 67% methanol. The homogenate was filtered through four layers of cheesecloth and centrifuged at $15\,000 \times g$ for 15 min at 20°C . The absorbance of an aliquot of the supernatant was read at 320 nm using an UV-Vis spectrophotometer (Shimadzu UV-160A; Shimadzu Scientific Instrument, Columbia, MD) (Loiza-Velarde et al., 1997).

2.6. Statistical analysis

Results reported in this paper are the mean values accompanied by their standard deviations. Two leaves were used for each replicate and each treatment had three replications. All experiments were repeated at least twice.

3. Results and discussion

3.1. Transmittance of a wound signal in air and water

Being in close proximity to cut lettuce pieces did not induce the accumulation of higher levels of phenolic compounds in whole, non-wounded leaves (Fig. 1). Wounding Romaine lettuce by cutting the leaves into 2×2 cm pieces increased the phenolic content (absorbance of the methanol extract at 320 nm) of the mid-rib tissue by almost fivefold, from 0.28 ± 0.02 to 1.35 ± 0.07 Abs 320 nm. Removing 1 mm from the cut surface (i.e. the tissue that had turned brown) reduced the value for the inner white tissue to 1.00 ± 0.15 Abs 320 nm. A wound signal apparently originated at the

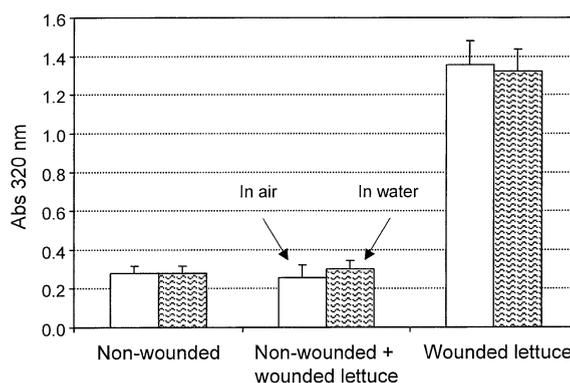


Fig. 1. Phenolic content (Abs 320 nm of a methanol extract) of Romaine mid-rib lettuce tissue. The mid-rib tissue was from non-wounded leaves, non-wounded leaves held with wounded leaves, and wounded leaves after 48 h at 10°C . The non-wounded leaves were either held in a 20-l glass jar with twice their weight of wounded leaves (i.e. in air), or the bases of the non-wounded leaves were immersed in 500 ml of water containing twice their weight of wounded leaves (in water). The lines on top of each bar represent the S.D. about that mean ($n = 6$).

cut surface and induced the synthesis and accumulation of phenolic compounds in non-injured tissue, even though only the tissue at the cut surface actually turned brown.

The initial phenolic content of the mid-rib of non-wounded whole leaves, and the phenolic content of non-wounded whole leaves held in the container with twice their weight of cut lettuce for 48 h at 10 °C were not significantly different from one another: 0.28 ± 0.02 vs. 0.26 ± 0.04 Abs 320 nm, respectively. Wounded Romaine leaf tissue apparently does not produce a volatile compound that can induce the synthesis and accumulation of higher levels of phenolic compounds in whole, non-wounded leaves held with wounded tissue for 2 days at 10 °C.

The phenolic content of the mid-rib of whole leaves of Romaine lettuce was not increased by having the cut end of the non-damaged leaf sit in water containing freshly cut lettuce for 2 days at 10 °C (Fig. 1). There was a slight (10%) increase from 0.27 ± 0.03 to 0.30 ± 0.05 Abs 320 nm for whole, non-wounded leaves held in water alone versus water containing cut lettuce leaves, respectively. However, this 10% increase was small compared to the almost fivefold increase that occurred in Romaine lettuce mid-rib tissue as the result of wounding.

3.2. Effect of hypertonic solutions

Cut tissue pieces steadily gained weight over 24 h when immersed in water (Fig. 2). In contrast, there was a 4, 10 and 15% loss in fresh weight after 2 h immersion in 0.3, 0.6 and 0.9 M mannitol, respectively. After the initial loss of weight, the cut tissue gained weight for the next 4 h, reaching a weight that was maintained in the 0.6 and 0.9 M mannitol for the next 18 h, but the increase in the 0.3 M solution continued for 18 h at a rate similar to that of tissue held in water.

The reduction in the accumulation of wound-induced phenolic compounds was greater as the osmotic concentration of the bathing solution increased from 0.0 to 0.9 M mannitol (Fig. 3). Lettuce mid-rib tissue soaked in water for 4 h gained about 8% in weight (Fig. 2) while the wound-induced accumulation of phenolic compounds decreased around 15% (Fig. 3). Part of the decrease may have resulted from the increase in fresh weight. The decline in phenolic accumulation was greater in higher osmotic solutions, with the 0.3, 0.6 and 0.9 M mannitol solutions producing a 34, 52 and 68% decline, respectively. The decline in the accumulation of wound-induced phenolic compounds was similar for the 3 and 4 h soaks in 0.3 to 0.9 M mannitol solutions.

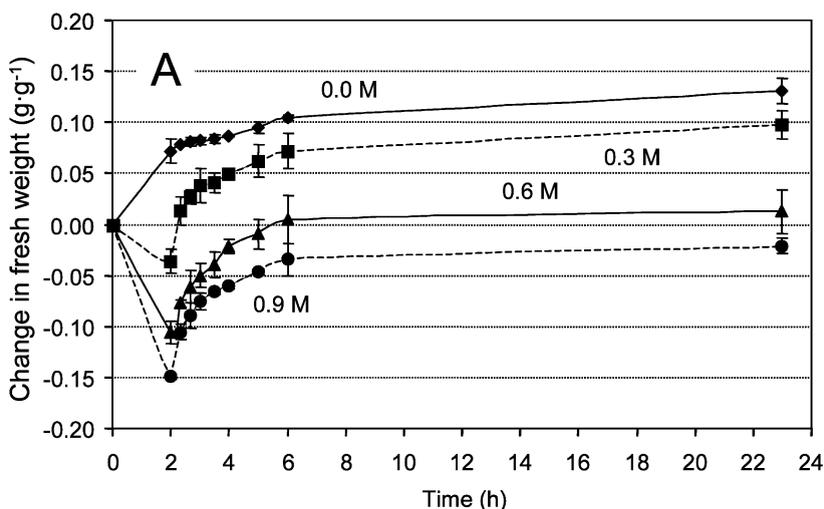


Fig. 2. Changes in fresh weight (g g^{-1}) of 2×2 cm pieces of mid-rib Romaine lettuce tissue held for 0–23 h in 0.0–0.9 M aqueous mannitol solutions. The vertical line at each data point represents the S.D. about that mean ($n = 6$).

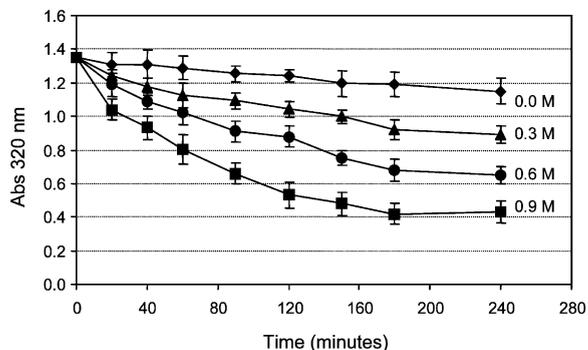


Fig. 3. Phenolic content (Abs 320 nm of a methanol extract) of 2×2 cm pieces of mid-rib Romaine lettuce tissue. The tissue was held for 0–240 min in 0.0–0.9 M aqueous mannitol solutions before being held at 10°C for 48 h. The vertical line at each data point represents the S.D. about that mean ($n = 6$).

Wound-induced increases in PAL activity were reduced by immersion in hypertonic mannitol solutions (Fig. 4). Cutting lettuce leaf tissue into 2×2 cm pieces stimulated a 6.9-fold increase in PAL activity from 0.08 ± 0.005 to 0.55 ± 0.007 $\mu\text{mol cinnamic acid g}^{-1}$ for Iceberg mid-rib tissue,

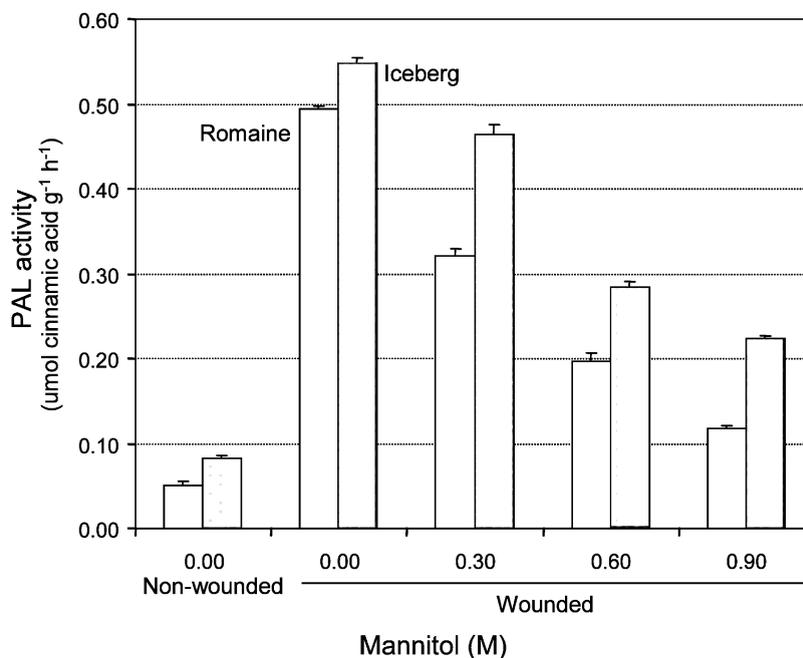


Fig. 4. PAL activity in Romaine and Iceberg 2×2 cm mid-rib lettuce tissue pieces. Tissue was either from non-wounded leaves, or from tissue pieces soaked in 0.0–0.9 M aqueous mannitol solutions for 3 h before being rinsed and stored at 10°C for 24 h. The lines on top of each bar represent the S.D. about that mean ($n = 6$).

and a 9.8-fold increase from 0.05 ± 0.004 to 0.49 ± 0.03 $\mu\text{mol cinnamic acid g}^{-1}$ for Romaine mid-rib tissue. A 3 h soak in 0.3, 0.6 and 0.9 M mannitol immediately after cutting, reduced these wound-induced increases in mid-rib tissue by 15, 48 and 60% for Iceberg, and by 35, 60 and 76% for Romaine lettuce, respectively.

The reduction in the wound-induced accumulation of phenolic compounds (Fig. 3) and the suppression of wound-induced PAL activity (Fig. 4) brought about by soaks in hypertonic mannitol solutions could have been caused by a loss of the wound signal in the water leaving the cut lettuce pieces (i.e. weight loss shown in Fig. 2). If this was the case, then re-wounding tissue previously soaked in hypertonic mannitol solutions to reduce the wound responses should have produced an increase in PAL activity, the standard wound response. However, when excised Romaine mid-rib tissue was re-wounded after being soaked in 0.3–0.9 M mannitol for 3 h, rinsed and held at 10°C for 24 h, the wound response continued to be suppressed (Fig. 5). Soaking cut lettuce in

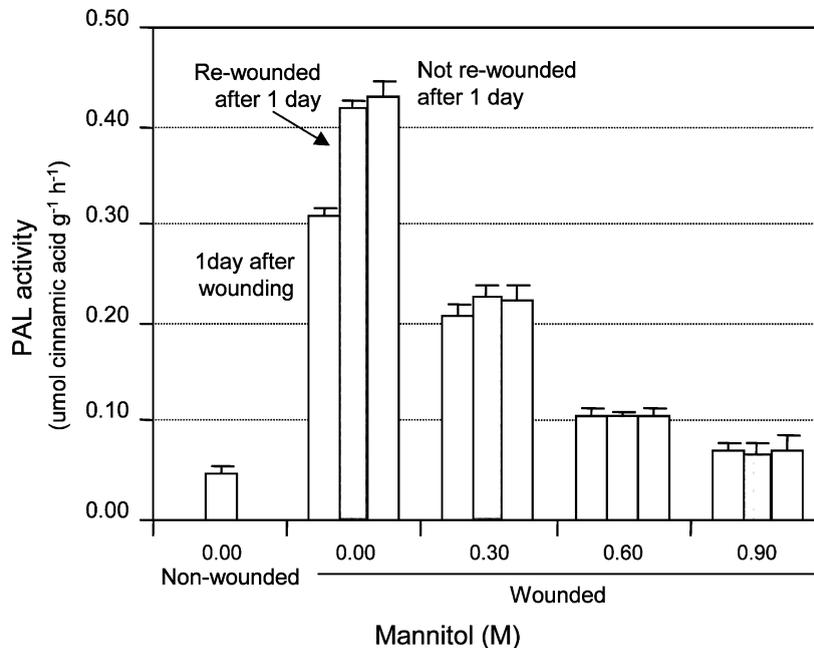


Fig. 5. PAL activity in Romaine 2×2 cm mid-rib lettuce tissue pieces. Tissue was either from non-wounded leaves, or from tissue pieces soaked in 0.0–0.9 M aqueous mannitol solutions for 3 h before being rinsed and stored at 10°C for 24 h. After 24 h, the tissue pieces that had been soaked in the mannitol solutions were either re-wounded or left alone and then held for another 24 h at 10°C . The lines on top of each bar represent the S.D. about that mean ($n = 6$).

hypertonic solutions appeared to render the tissue insensitive to the subsequent wound induction of PAL for at least 2 days. A similar protective effect resulted from subjecting cut lettuce to heat shock treatments (Loaiza-Velarde and Saltveit, 2001), another abiotic shock.

Like heat shock treatments that confer resistance to subsequent thermal stress by inducing changes in protein synthesis (Zhang et al., 1984; Apuya and Zimmerman, 1992; Campos-Vargas and Saltveit, 2002) soaks in hypertonic mannitol solutions appeared to render the tissue resistant to subsequent abiotic stress, e.g., cutting. The wound signal that induced increased PAL activity does not appear to be easily transmitted to whole, non-injured leaves through either a gaseous or liquid medium. Further clarification of the nature of the wound signal is necessary to intelligently design treatments that minimize the deleterious effects of wounding that occur during routine postharvest handling, and the preparation of fresh-cut fruits and vegetables.

References

- Apuya, N.R., Zimmerman, J.L., 1992. Heat shock gene expression is controlled primarily at the translational level in carrot cells and somatic embryos. *Plant Cell* 4, 657–665.
- Bolin, H.R., Huxsoll, C.C., 1991. Effect of preparation procedures and storage parameters on quality retention of salad-cut lettuce. *J. Food Sci.* 56, 60–67.
- Brostrom, C.O., Brostrom, M.A., 1998. Regulation of translational initiation during cellular responses to stress. *Prog. Nucleic Acid Res. Mol. Biol.* 58, 79–125.
- Campos-Vargas, R., Saltveit, M.E., 2002. Involvement of putative chemical wound signals in the induction of phenolic metabolism in wounded lettuce. *Physiol. Plant.* 114, 73–84.
- Kang, Ho-Min, Saltveit, M.E. Wound-induced PAL activity is reduced in cut lettuce by heat-shock treatments that induce the synthesis of heat-shock proteins. *Physiol. Plant.*, in press.
- Ke, D., Saltveit, M.E., 1986. Effects of calcium and auxin on russet spotting and phenylalanine ammonia-lyase activity in Iceberg lettuce. *HortScience* 21, 1169–1171.
- Ke, D., Saltveit, M.E., 1989. Wound-induced ethylene production phenolic metabolism and susceptibility to russet spotting in Iceberg lettuce. *Physiol. Plant.* 76, 412–418.

- León, J., Rojo, E., Sánchez-Serrano, J.J., 2001. Wound signaling in plants. *J. Expt. Bot.* 52, 1–9.
- Linquist, S., 1986. The heat-shock response. *Annu. Rev. Biochem.* 55, 1151–1191.
- Loaiza-Velarde, J.G., Saltveit, M.E., 2001. Heat shocks applied either before or after wounding reduce browning of lettuce leaf tissue. *J. Am. Soc. Hortic. Sci.* 126, 227–234.
- Loaiza-Velarde, J.G., Tomás-Barberán, F.A., Saltveit, M.E., 1997. Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in Iceberg lettuce. *J. Am. Soc. Hortic. Sci.* 122, 873–877.
- Lopez-Galvez, G., Saltveit, M.E., Cantwell, M., 1996. Wound-induced phenylalanine ammonia lyase activity: factors affecting its induction and correlation with the quality of minimally processed lettuce. *Postharvest Biol. Technol.* 9, 223–233.
- Peiser, G., López-Gálvez, G., Cantwell, M., Saltveit, M.E., 1998. Phenylalanine ammonia-lyase inhibitors control browning of cut lettuce. *Postharvest Biol. Technol.* 14, 171–177.
- Ritenour, M.A., Saltveit, M.E., 1996. Identification of a phenylalanine ammonia-lyase inactivating factor in harvested Iceberg lettuce (*Lactuca sativa* L.). *Physiol. Plant.* 97, 327–331.
- Saltveit, M.E., 2000. Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. *Postharvest Biol. Technol.* 21, 61–69.
- Tomás-Barberán, F.A., Loaiza-Velarde, J., Bonfanti, A., Saltveit, M.E., 1997. Early wound- and ethylene-induced changes in phenylpropanoid metabolism in harvested lettuce. *J. Am. Soc. Hortic. Sci.* 122, 399–404.
- Vierling, E., 1991. The role of heat-shock proteins in plants. *Annu. Rev. Plant. Physiol. Plant Mol. Biol.* 42, 579–620.
- Zhang, H.Q., Croes, A.F., Linskens, H.F., 1984. Qualitative changes in protein synthesis in germinating pollen of *Lilium longiflorum* after a heat shock. *Plant Cell. Env.* 7, 689–691.