Mini-review

Polyamines, peroxidase and proteins involved in the senescence process

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Senescence is the natural aging process at the cellular level or range of phenomena associated with this process. The objective of this review was to show the involvement of substances that may be related to senescence in plants, such as polyamines, peroxidase and proteins. These substances were related with the terminal stages of development of various plant organs (leaves, flowers, roots).

Key words: Flowers, putrescine, spermidine, spermine, reactive oxygen species.

The plants are generally classified as highly products perishable, the ephemeral nature of the different tissues that form, the high activity respiratory and the low-carbohydrate reserves (Nowak and Rudnicki, 1990; Skutnik et al., 2001). After harvesting occur flowers' biochemical, structural and physiological change processes leads to tissue disorganization and disintegration of organs which promote senescence. The polyamines [putrescine (PUT), spermidine (SPD) and spermine SPM)], classified as plant growth regulators, are related to different responses physiologically, such as senescence and stress. In plants, the diamine and putrescine synthesizes from arginine and ornithine. The putrescine is converted to spermidine and spermine by successive transfers of one or two aminopropyl groups via SAM (S-adenosyl methionine) (Lima et al., 1999).

Other substances related to senescence such as peroxidases, are enzymes that typically catalyze reactions using oxi-reduction hydrogen peroxide (H_2O_2) as electron acceptor to catalyze different oxidative reactions (Blokhina et al., 2003) and proteins (Sugawara et al., 2002; Azeez et al., 2007), whose synthesis is characterized by high and rapid adaptability molecular and physiologically functional with respect to the medium (Larcher et al., 2000).

POLYAMINES

The polyamines (PAs) are aliphatic molecules of low molecular weight present in all organisms. The main PAs found in the higher plants are PUT, SPD and SPM occurring in free form or conjugated to phenolic acids and low molecular weight molecules (Bouchereau et al., 1999;

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Kuznetsov et al., 2006). In addition to biosynthesis and conjugation, the oxidative degradation is one way of regulating the PAs levels (Kusano et al., 2008). In plants, the PAs are located not only in the cytosol but also in the organelles, such as mitochondria, chloroplasts and vacuoles (Kumar et al., 1997). Its synthesis is initiated by two different routes: the PUT is formed directly from ornithine or indirectly by arginine and the formation of SPM occurs by adding a decarboxylated S-adenosyl methionine (SAM) group added to PUT. The formation of SPM occurs by adding another decarboxylated SAM (Coruzzi and Last, 2000).

The presence of positive charges on the molecules of PAs allows their electrostatic binding to cellular macromolecules, including DNA, RNA, proteins, chromatin and may cause stabilization or destabilization of these macromolecules (Kusano et al., 2008). Thus, they are involved in fundamental cellular processes including gene expression regulation, signal modulation and cell proliferation and membrane stabilization (Tabor and Tabor, 1984; Cohen, 1998; Igarashi and Kashiwagi, 2000).

Recent studies show that PAs have function in modulating many physiological processes in plants, such as cell division and differentiation, organogenesis, embryogenesis and tolerance to biotic and abiotic stress (Bouchereau et al., 1999; Crozier et al., 2001; Martin-Tanguy, 2001; Baisand Ravishankar, 2002; Silveira et al., 2004; Silveira et al., 2006; Santa-Catarina et al., 2006; Groppa and Benavides, 2008).

Some plants use the mechanism called osmotic adjustment to tolerate the effects of abiotic stresses, which allows the cell to maintain its metabolic functions even in adverse environmental conditions, thus promoting tolerance to stress and maintain relatively high osmotic potential (Bayuelo-Jiménez et al., 2002). This mechanism enables the cell to accumulate substances called compatible osmolytes, which preserve cell integrity resulting in the continuation of vital activities to plant growth and development (Bray et al., 2001). There is evidence that the PAs stabilize the membrane and retard senescence (Smith, 1985). SAM can be turned successively into aminopropyl-carboxylic acid and ethylene (Slocum, 1984), once the PAs and ethylene compete for the same precursor (Bouchereau et al., 1999; Pandey et al., 2000).

The PAs inhibit the ethylene production by regulating the activity of synthase and oxidase of 1-aminocyclopropane-1-carboxylic acid (ACC) (Lee et al., 1997), while ethylene alters the PAs formation by reducing the arginine decarboxylase activity (ADC) and SAM decarboxylase (SAMDC) (Roustam et al., 1994). Changes in PAs levels and ethylene during senescence have been reported in some plants such as plum (de Dios et al., 2006) and *Hibiscus syriacus* L. (Seo et al., 2007). Li and Wang (2004) observed that between ethylene and polyamines, there is metabolic competition only under high stress conditions.

The PAs concentration may vary by plant organ, maturi-

ty and post-harvest treatment (Carbonell et al., 2000; Chattopadhayay et al., 2002; Nayyar and Chander, 2004). Pomegranates show higher levels of PAs during cold storage; however, the main change occurred for SPM (Mirdehghan et al., 2007). PUT accumulation was also noted in pepper, cucumber, zucchini and citrus (orange and lemon) during exposure to cold chamber (Serrano et al., 1998; Martínez-Romero et al., 2003). In Faroe chrysanthemum stems Vieira et al. (2010) observed decreases in the levels of PAs throughout the cold storage. In several species, the PAs increase was correlated with the reduction of injuries caused by such stress (Kramer and Wang, 1989; Wang and Ji, 1989). It has been suggested that the accumulation of polyamines in tissues can confer more tolerance to various types of stresses due to these amines act in the removal of reactive oxygen species and also assist the stabilization of cell membranes (Larher et al., 2003; Groppa and Benavides, 2008).

High temperature also causes reduction in cell division and this effect may be linked to its action on the Pas. Under high temperatures would occur reduction in the concentration of these polyamines, affecting cell division (Poljakoff-Mayber and Lerner, 1994). In post-harvest of cloves kept at 21°C, Serrano et al. (1998) observed increase in PUT content and decrease in SPD. In addition to PAs, other substances may be related to senescence in plants such as peroxidase and proteins.

PEROXIDASE

Plants produce reactive oxygen species (ROS) in various metabolic processes, when they suffer some kind of stress (Jin et al., 2006). Thus, ROS may be generated during the post-harvest phase of flowers, which according to Shigeokaet al. (2002) would cause oxidative damage in plants. The accumulation of these ROS could promote damage to lipids, proteins, among others, forming toxic products (Pennycooke et al., 2005). Plants have antioxidative enzymes such as peroxidase (POD) that diminish the damage caused by excess peroxides (Scandalios, 1993). The POD's (EC 1.11.1.7) contain a heme prosthetic group (ferriprotoporphyrin IX) and in the catalytic process transiently oxidize the ferric ion (Fe^{3+}) to the higher valence states (FE^{4+} or FE^{5+}). In the reaction involving the POD, the electron donor can be ascorbate, amines and other organic compounds. The oxidation product show in many cases, intense staining (Richardson and Hyslop, 2000).

The POD can be considered a stress enzyme stimulated by low temperatures (El-Hilari et al., 2003). In baroapotato, Menolli et al. (2008) observed increased activity of the enzyme POD until the 7th day of storage at 5°C. Martinez-Téllez and Lafluente (1993) and El-Hilari et al. (2003) analyzing orange fruitsof Navelina cultivar stored at 1, 2.5, 5 and 10°C for 60 days and Mandarin' 'Fortune'' stored at 4 and 8°C for 4 weeks, verified changes in the

POD activity. Zauberman et al. (1985) did not detect variation in the POD activity in avocados 'Fuerte' stored at 0, 2 and 5°C for up to 18 days.According to Yang and Hoffman (1984), storage at low temperatures causes induction of various enzymes such as POD, which can convert the acid 1-aminocyclopropane-1-carboxylic (ACC) into ethylene. At higher temperatures, Costa (2009) did not observe a standard trend for POD activity in stems of *Heliconia Bilhai* at 12 and 19°C. Responses of this nature were also obtained by Vieira et al. (2010) in Faroe chrysanthemum stems at 10and 25.2°C.

The POD is also involved in various reactions such as oxidation of phenols, oxidation of indole-3-acetic acid (IAA), bonds of polysaccharides, wound healing, defense against pathogens in the hypersensitive response (HR), lignin and suberin synthesis to the cell wall thickening, regulation of cell elongation and senescence (Quiroga et al., 2000; Silva, 2000; Kao, 2003; Campos et al., 2004). Hossain et al. (2006) report that increased levels of peroxides could be a programmed regulation of the activity of POD enzymes which seems to be a prerequisite for the onset of petals senescence. Bartoli et al. (1997) observed increased POD activity in *Chrysanthemum morifolium* Ram petals during senescence and related to defense against oxidant molecules that promote membrane damage.

The POD can occur in cells programmed for death and in flowers the deterioration is certainly programmed, it is not reversible and inevitably leads cells to death (Rogers, 2006). The substances deposited on the cut surface of flowers, as well as tyloses, can migrate into the xylem vessels, serving as barrier for microorganisms at the time they cause obstruction, preventing the water absorption. The formation of such substances, according to Vaslier and Van Doorn (2003), probably is involved in the ethylene synthesis and action of enzymes such as POD.

The POD enzyme is involved in vascular blockage of some species of flowers by oxidation of p-coumaryl alcohol, coniferyl and sinapil, which are precursors of lignin. Lignin is a compound that is part of the secondary metabolism of plants and, although providing support and structuring to water transport through the xylem, it may function as a protective mechanism against pathogen attack in case of stress by depositing on the cut surface preventing also the entry of water into vases (Boerjan et al., 2003). Loubaud and Van Doorn (2004) concluded that the blockage in *Astilbe* stems, since once developed in flowers kept in humid and dry storage, however, in roses cv. Red One and *Viburnum opulus*the blockage observed in these stems was apparently related to the presence of bacteria in the xylem.

PROTEINS

Proteins are linear polymers of amino acids (macromolecules) with molar mass ranging from hundreds to thousands of Daltons (1 Da = 1.661×10^{24} g). Only about 20

amino acids are found in animal and plant proteins, and these are combined in myriad ways to form a variety of different proteins (Magalhães, 2008). An amino acid has a carboxylic group and an amino group in protein molecules and they are responsible for peptide bonds formation, except the N and C terminals of the protein. Each amino acid has two ionization constants, which represent the ionization constants of 1-amino and 1-carboxylic acid groups of the free amino acid. However, some have more than two pKa values, once they have other ionizable groups in their chains. The net charge of a protein molecule in aqueous solution depends on the ionization constant of the constituent amino acid side chains and the solution pH (Melvin, 1987).

The proteins are divided into a plant cell among various organelles and have specific functions. As an example, a small amount of proteins may be found in the cell wall and part of them consists of enzymes that initiate formation reactions, remodeling or breakdown of the wall structure. The chloroplast has a double membrane and there are transport proteins in the outer layer of this membrane (Newton et al., 2004). The synthesis of proteins is the central function of all cells. In its absence, growth and maintenance of organs cease and this represents a limiting factor to the growth rate of plants (Porter and Lawlor, 1991). According to Larchjer et al. (2000), the protein synthesis is characterized by high and rapid capability of molecular, functional and physiologic adaptation relative to the medium.

Changes in the levels of proteins have been associated in part as a result of synthesis of "new" specific proteins during senescence (Woodson and Handa, 1987), such as the ribonucleases, the β-glucosidases and proteases (Sood et al., 2006).The knowledge of the synthesis and the nature of modifications that proteins regulate have important implications for successful handling of longevity of the flowers (Woodson and Handa, 1987). The percentage of total protein is a data that evaluates the plants conditions in the field and post-harvest. Sood et al. (2006) noted that the protein content was higher in young seedlings and lower in the stages of maximum development. Drevdahl and Thimann (1977) observed decrease in protein contents during development in oat plants. In *Sandersonia* petals (Eason et al., 2002) and *Dendrobium* cv. Khao Sanan (Lerslerwong et al., 2009), there was decreased protein content during senescence. This response was also observed in leaves and flowers of Faroe Chrysanthemum post-harvest (Vieira et al., 2010), while Laschi (2000) observed increased proteinsin rose stems. Souza (2008) reported decreases in protein levels between the third and tenth day of assessment in inflorescences of Heliconia Golden Torch. This author studied the percentage of proteins in heliconia bilhai and observed 7.0% increase from the harvest day and the four days of storage, followed by decreases until the tenth day.

The reduced proteins can be attributed to the action of

proteases. Elanchezhian and Srivastava (2001) suggested that the decreased protein contents in chrysanthemum petals during senescence was due to the synthesis inhibition and increased protein degradation by proteases, resulting in loss of functional capacity of the membranes, increased output of ions and finally senescence and death of tissues. Through studies on the programmed cell death (PCD), it was shown that proteases increased and their relationship with senescence as a possible regulator of programmed death in plant cells (Gerreiro et al., 1998; Gietl and Schimid, 2001).

Proteins play a critical role in modulating plant response to stress. Saliveit (2000) and Thomashow (2001) claim that the low temperature during storage induces changes in protein content. Ferguson et al. (1988) also mentions that high temperature can directly or indirectly injure plant proteins by inactivating enzymes, changing the conformation of peptides or disrupting membrane complexes. Within this field, important advances have been made in understanding the plant response to stress.

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