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Symptoms of programmed cell death in intact and cut flowers of clematis and the effect of a standard preservative on petal senescence in two cultivars differing in flower longevity

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ABSTRACT

Clematis is visually a very attractive plant which can be used for cut flowers, provided that proper cultivars are chosen. In this study, the phenomenon of programmed cell death (PCD) was compared in petals of clematis blooming on mother plants in the open field and in cut flowers, in two cultivars with different flower longevity. The dynamics of PCD in petals of field grown plants in the two cultivars were different. In the long flower longevity cultivar the first symptoms of PCD were observed in the flower bud, and in the open flower the process was already advanced. On the other hand, PCD in petals of the short flower longevity cultivar was advanced already in the flower bud. In both cultivars the nuclear degradation started early, but the dynamics were different: in the long flower longevity cultivar 18.6% of the nuclei were degraded in the open flower *vs*. 68.3% of degraded nuclei already in the flower bud in the short flower longevity cultivar. Senescence processes are intensified in cut flowers. In cut flowers of both cultivars kept in water the process of DNA degradation was much faster than in flowers growing on mother plants. It could be slowed down by the addition of preservatives. The standard preservative of 8-hydroxyquinoline citrate (8-HQC) 200 mg L^{-1} with 2% sucrose prolonged the vase life of cut flowers of both cultivars as compared to water, but organelle degradation in petals was delayed only in the shorter-lived cultivar.

1. Introduction

Petal senescence is a highly regulated process that marks the final stage of a flower's lifespan. In general, senescence is viewed as a series of events, leading to the degradation and remobilization of proteins, lipids, and nucleic acids and, ultimately leading to cell death (Hoeberichts et al., 2005). Most studies on the regulation of flower senescence in ornamental plants have focused on herbaceous species (Azad et al., 2008; Jones, 2008); there are fewer reports on woody flowering plants (Zhang et al., 2012; Jędrzejuk et al., 2013; Zou et al., 2014).

It is not clear how exactly the petal cells die. The main site of the membrane and organelle degradation appears to be the vacuole (Winkenbach, 1970; Matile and Winkenbach,1971; Phillips and Kende, 1980; Smith et al., 1992). Petal cells in several genera, such as *Ipomoea* (Winkenbach, 1970), *Dianthus* (Smith et al., 1992), *Hemerocallis* (Stead and van Doorn, 1994), and *Iris* (van Doorn et al., 2003) show reduction in the number of small vacuoles, and an increase in size of the central vacuole. Autophagy is associated with plant senescence as the main mechanism of degradation and remobilization of macromolecules (Yamada et al., 2009). It involves the transfer of membranes and organelles to the vacuole, where degradation takes place. The increase in vacuole volume is usually accompanied by the loss of a considerable part of the cytoplasm and the disappearance of most organelles (Wagstaff et al., 2003; Thomas et al., 2003; Otegui et al., 2005; Zhou et al., 2005; Avila-Ospina et al., 2014). The involvement of programmed cell death (PCD) during petal senescence has been extensively studied showing that DNA fragmentation is a characteristic marker of the process (Green and Kroemer, 2004; Rogers, 2005). It has been observed in flowers of *Actinidia deliciosa* (Coimbra et al., 2004), in petals and ovary of *Pisum sativum* (Orzaez and Granell, 1997a, 1997b), petals of *Alstroemeria peruviensis* (Wagstaff et al., 2003) and *Petunia inflata* (Xu and Hanson, 2000; Azad et al., 2008). However, some plant cells show

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DNA degradation rather than classical DNA laddering (Yamada et al., 2006) and in this case the TUNEL (Terminal deoxynucleotidyl transferase dUTP Nick End Labeling) method, showing DNA degradation directly in the nucleus, appears to be more appropriate approach to study the phenomenon.

Owing to the detachment from the mother plant, cut flowers are usually under water stress (Mayak et al., 1974; Halevy and Mayak, 1981). This shortens their longevity, both directly and indirectly *via* PCD. During the postharvest life, probably as a consequence of mechanical injury at harvest, xylem vessels at the basal stem parts usually become obstructed by tyloses and microorganisms (van Doorn, 1999). These obstructions block water transport toward flowers leading to premature wilting. This process can be slowed down by addition of certain preservatives to vase solutions. The most popular and effective ones are the esters of hydroxyquinoline (HQ) and silver ions. The effectiveness of HQ as a biocide in cut flower vase solutions has been known for decades (van Doorn, 1997; Damunupola and Joyce, 2008), most commonly used in the sulphate (HQS) and citrate (HQC) forms. They may also increase flower longevity by acidifying the vase solution and by acting as antitranspirants limiting water losses (Halevy and Mayak, 1981; Damunupola and Joyce, 2008). An exogenous sugar is another main constituent of flower preservatives which significantly prolongs longevity of cut flowers and improves their development and coloration. Supplementation of sucrose delays flower senescence probably by increasing an endogenous sugar pool in petals which enhances respiration. Sucrose delays proteolysis and maintains proper water balance in cut flower (Mayak and Halevy, 1980).

Leather-flower (*Clematis* sp.) is a garden vine, very popular because of its profuse blooming and decorative leaves and seed heads. Numerous species and cultivars have a long flowering periods. In the US, clematis is grown for cut flowers; in Europe it is mainly planted in gardens and on balconies/patios although recently florists also have begun to include its cut flowers in floral arrangements. Clematis can be difficult to harvest as in many cultivars stem are short and flowers poorly withstand transport (Greer and Dole, 2009). In cooperation with a clematis breeder, Dr. Szczepan Marczyński, the Dept. of Ornamental Plants of the Warsaw University of Life Sciences has undertaken studies on clematis to select accessions/cultivars suitable for cut flower production and to determine postharvest treatments capable of improving their keeping qualities.

In this study, the PCD in petals of two cultivars of clematis with different flower longevity was observed, both in undetached and detached flowers. The choice of the cultivars was based on earlier observations of their vase lives (Skutnik and Rabiza-Świder, 2005, 2006). They represent cultivars with different, genetically based longevity: a long-lived cultivar with cut flowers usually lasting for 10–12 d, and a short vase-life cultivar, usually lasting for 5–9 d. Degradation of petal cells in flowers blooming on mother plants in the open field ("undetached" flowers) was compared with that occurring in cut flowers – both – in those kept in water and in a preservative composed of 8-HQC and sucrose.

2. Material and methods

2.1. Plant material

All observations were done on flowers of two clematis (*Clematis* L.) cultivars, 'Popieluszko' and 'Andromeda'. Earlier experiments (Skutnik and Rabiza-Świder, 2005, 2006) have established that cut flowers of cv. Popieluszko usually last for 10–12 d while those of cv. Andromeda for 5–9 d. Consequently, cv. Popieluszko will be referred to in this article as a long-lasting cultivar and 'Andromeda' as a short-lived cultivar. Plants for the experiments were kindly provided by the Clematis Sz. Marczyński and W. Piotrowski Nursery in Duchnice near Warsaw, Poland. Flowers for the experiments were harvested from different plants, but growing under the same conditions, at the same developmental phases, *i.e.* open flowers with no visible symptoms of disease, pests or mechanical defects. Shoots were trimmed to 0.2 m with one pair of leaves left and placed individually into calibrated cylinders (volume 0.5 L) and treated as separate replications, with ten shoots in each treatment. The treatments were: distilled water (control) and a standard preservative (SP), *i.e.* the aqueous solution containing 200 mg L^{-1} 8-hydroxyquinoline citrate (8-HQC) and 20 g L^{-1} sucrose. The experiments were carried out under controlled conditions: temperature 20 °C, relative humidity 60%, 12 h photoperiod with light intensity of 35 μmol m^{-2} s^{-1} PAR. Vase life was recorded in days and regarded as terminated when wilting, drying or dropping of petals occurred. Under field conditions the time from flower opening to senescence was measured. The average night and day temperature and air humidity under the field conditions present during clematis flowering, were kindly provided from Warsaw Meteorological Station placed in Podkowa Leśna, and are given in Table 1. The trials on longevity of flowers was repeated 3-times during three years.

2.2. Microscopic observations

Samples for microscopy were collected from both flowers on mother plants ("uncut flowers") that bloomed naturally in the field, and from cut flowers. Samples from shrubs growing in the field were collected at four phenological phases: flower bud, open flower, fading, and completely wilted flower; samples from cut stems were collected at three phases: open flower, fading, and wilted flower. The apical fragments measuring 5 × 5 mm from ten petals were collected for observations. The apical fragments were chosen because it was easier to observe senescence symptoms in those parts. Collected samples were handled according to the Methods of Preparation for Electron Microscopy (Robinson et al., 1987): the material was fixed for 6 h in 5% glutaraldehyde and 4% formaldehyde (Sigma-Aldrich, Inc. Fluka, St. Louis, USA) in 0.1 M sodium cacodylate (Sigma-Aldrich, Inc. Fluka, St. Louis, USA) buffer, pH 7.2–7.3 at 0.8 atm at room temperature, rinsed with the same buffer, postfixed in 2% OsO_4 (Merck, Darmstadt, Germany) in the 0.1 M sodium cacodylate buffer for 2 h, and rinsed again with the same buffer. Fixed material was dehydrated gradually in

Table 1
The average day and night temperature and air humidity under field conditions in the years 2011–2013 (August–September).

Year/month	Average day temperature (°C)	Average night temperature (°C)	Relative humidity (%)
2011 August	31.2	18.2	92.2
2011 September	23.4	14.4	84.2
2012 August	30.9	19.9	88.2
2012 September	22.8	13.9	81.1
2013 August	32.1	19.7	87.9
2013 September	23.1	14.9	79.2