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Growing conditions and preservatives affect longevity, soluble protein, H_2O_2 and MDA contents, activity of antioxidant enzymes and DNA degradation in cut lilacs

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ABSTRACT

Common lilac (*Syringa vulgaris* L.) is a popular ornamental shrub which under natural conditions of the temperate zone blooms for *ca* three weeks in May. Forcing procedures may extend the flowering period to 6 months in autumn and winter. Temperatures required to begin the forcing cycle range from 37 °C in November to 16 °C in March. Forcing lilac at 15 °C in November is also effective but longer than in the standard method, used by most growers. This study compared vase life and certain senescence-related processes in cut flowers of common lilac harvested from shrubs flowering under different conditions, i.e. outdoors under ambient temperature, forced in a greenhouse under 37 °C (the standard method) or 15 °C (the alternative method), and held in water or two vase solutions containing either nanosilver (NS) or 8-hydroxiquinoline citrate (8-HQC) as biocides and supplemented with 2% sucrose. The highest influence on vase life of cut lilacs had growing conditions. The vase life of cut lilacs obtained by the alternative forcing method – regardless of a holding solution – was significantly higher than those obtained by the standard forcing or blooming naturally. The highest H_2O_2 content and proteolytic activity were usually observed in flowers collected from stems held in water. MDA content varied depending on flowering conditions and holding solutions. Its relatively low content was observed in the alternatively forced lilacs held in both preservatives which also prolonged the vase life relative to water, decreased the H_2O_2 content and delayed nuclei degradation in petals. The activity of chosen antioxidant enzymes varied depending on the flowering conditions and generally remained unaffected by the preservatives. The effects of nanosilver with sucrose on cut lilac senescence were comparable to the action of the standard preservative containing 8-HQC, therefore this solution may also be recommended as an efficient preservative delaying senescence and prolonging vase life of cut lilacs.

1. Introduction

Cut flowers are the most important crops in the ornamental industry. Their quality, longevity and the dynamics of flower senescence may be affected by a range of factors such as plant maturity, the stage of flower bud development or the season of planting and harvesting (Manning, 1995; Pompodakis et al., 2005; Rafdi et al., 2014). To assure the best quality of plant material, several studies are undertaken to understand and control physiological and biochemical changes during the postharvest and post production life (Sylvestre et al., 1989; Paulin et al., 1986; Baker et al., 1978). One of the phenomena occurring during senescence of cut flowers is accumulation of the reactive oxygen species (ROS) and activation of the enzymatic antioxidant defense mechanism (Rubinstein, 2000; de Pinto et al., 2006). Under stress conditions, the balance between production and elimination of ROS is disturbed (Apel and Hirt, 2004; Munne-Bosch and Alegre, 2004; Karuppanapandian et al., 2011; Vellosillo et al., 2010). DNA fragmentation is a popular marker used in many plants to describe advances in the process of senescence (Green and Kroemer, 2004; Rogers, 2005). Common lilac (*Syringa vulgaris* L.) is a popular ornamental shrub which under natural conditions of the temperate zone blooms in May. Overcoming periodicity and prolonging flowering beyond the natural period has always been an important focus point for scientists and growers (Dale et al., 1999). Forcing is commonly used to induce flowering

Abbreviations: 8-HQC, 8-hydroxyquinoline; APX, ascorbate peroxidase; CAT, catalase; DAPI, 4′,6-diamidine-2′-phenylindole dihydrochloride; FW, fresh weight; H_2O_2, hydrogen peroxide; KI, potassium iodide; MDA, malondialdehyde; NS, nanosilver; PBS, phosphate-buffered saline; PCD, programmed cell death; POD, Peroxidase; ROS, reactive oxygen species; S, sucrose; SOD, superoxide dismutase; TBA, thiobarbituric acid; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling

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independently of a natural blooming date in many ornamental bulbous and woody plants but to be fully successful it requires overcoming endodormancy. Temperatures required to begin the forcing cycle of lilac depend on a season and range from 37 °C in November to 16 °C in March. Studies on the relationship between the temperature and the forcing cycle length have shown that forcing lilac at 15 °C in November is also effective, but it requires 49 days to be completed, as compared to 23 days for the standard method with 37 °C used by most growers. However, panicles produced at 15 °C are more decorative, while panicles produced by the standard high temperature treatment tend to be floppy and their flowers are not completely open (Jędrzejuk and Łukaszewska, 2008). Previous studies on intact lilac shrubs showed that poor quality of lilac flowers produced by high temperature was associated with high concentration of H_2O_2 and low CAT activity, opposite to flowers forced by the low temperature method (15 °C) (Jędrzejuk et al., 2016c).

Because of the detachment from the mother plant, cut flowers usually suffer from water stress that reduces their longevity (Mayak et al., 1974; Halevy and Mayak, 1981). This mostly results from xylem blockage by tyloses, gums, gels and/or bacteria. Biocides effectively reduce or delay xylem blockages formation. The most popular and effective biocides are esters of hydroxyquinoline (HQ) and silver ions. Sugar is another main constituent of flower preservatives used for decades (Mayak and Halevy, 1980). Endogenous and exogenous sugars not only provide a respiration substrate but also control water balance in cut flowers (Mayak and Halevy, 1980; Garcia Victoria et al., 2003). Observations in common lilac by Jędrzejuk et al. (2016b) showed that a preservative containing 2% sucrose and a biocide significantly prolonged its vase life as well as reduced the number of tyloses in xylem vessels.

The aim of this study was to compare longevity of cut lilacs harvested from shrubs blooming under different conditions and to evaluate the effects of holding solutions on cut lilac vase life.

2. Material and methods

2.1. Plant material

Shrubs of common lilac (*S. vulgaris* L. 'Mme Florent Stepman') used in the current experiments were kindly provided by Mr. Michał Łyczko – the owner of nursery located in Grodzisk Mazowiecki, Poland. Shrubs were produced and maintained as semi – standard, having several strong flowering shoots and well developed root balls of 35–40 cm in diameter. During cultivation shrubs were fertilized according to the lilac's requirements. Shrubs were dug out in August and their root balls were exposed to low temperature during autumn. The control plants were left in soil and flowered in May in the open field (in a didactic garden of WULS), while other two batches of shrubs (10 shrubs per treatment) were transferred to a greenhouse (at WULS, Warsaw, Poland) and subjected to forcing procedures. Shrubs were planted into plastic pots (55 cm in diameter) filled with the standard peat substrate. Forcing started in the beginning of November. The standard procedure involved the initial temperature of 37 °C, which was falling gradually to 20 °C what led to flowering at the end of the month. During the alternative forcing 15 °C was maintained during the whole forcing process resulting in flowering in early January. At the beginning of forcing, shrubs were sprayed with 0.5% Rovral 500 FLO against fungal diseases. During the standard forcing shrubs were watered once a day and fogged once an hour for several seconds to increase air humidity. The latter procedure was repeated until breaking of inflorescence bud occurred. Shrubs from the alternative forcing were only watered as needed. Flower buds or petals collected from shrubs flowering under the above conditions were sampled at the developmental phases described by Jędrzejuk et al. (2013). The stems for vase life evaluation were harvested when *ca* 50% of the florets in panicles attained the phase of flower bud swelling. They were immediately transferred to the laboratory, trimmed to 50 cm and placed into solutions containing 2% sucrose with either 200 mg L^{-1} 8-HQC or 1 mg L^{-1} NS. All solutions were prepared in distilled water which also served as the control treatment. Water and solutions were not changed during experiments, but their depletions were supplemented. Ten shoots in each treatment were individually tagged and treated as separate replications. The experiments were conducted at a temperature of 18–20 °C and under a 12 h photoperiod, under illumination with the quantum irradiance of 25 μmol m^{-2} s^{-1}. The relative air humidity was maintained at 60%. Lilac vase life was regarded as terminated when 30% of the florets wilted, dried up and/or turned brown.

For biochemical and cytological analyses petals were collected from the basal parts of panicles in the following developmental phases (Jędrzejuk et al., 2013): flower bud swelling (after 1 d in November and 2 d in January and May since the stems were harvested), open flower (after 2 d in November and 5 or 3 d in January and May, respectively, since the stems were harvested), wilted flower (after 2–3 d in November, 9 or 5 d in January and May, respectively, since the stems were harvested), desiccated flower (after 3–4 d in November, 11 d in January and 7 d in May, since the stems were harvested).

2.2. Soluble protein content and proteolytic activity

The soluble protein content was determined by the method of Bradford (1976) and the total proteolytic activity was measured as described by Zagdańska and Wiśniewski (1996) with modifications of Jędrzejuk et al. (2016a). The protein content was expressed in mg per g fresh weight (FW) and proteolytic activity was determined in units (U) per mg based on protein content.

2.3. H_2O_2 content and lipid peroxidation

The hydrogen peroxide (H_2O_2) content of petals was measured spectrophotometrically after reaction with potassium iodide (KI) as described by Jędrzejuk et al. (2016b). The H_2O_2 content was expressed at 390 nm as μmol of hydrogen peroxide per g fresh weight.

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) produced by the thiobarbituric acid (TBA, Sigma-Aldrich, St. Louis, USA) reaction according to Hodges et al. (1999) with some modifications for common lilac petals as described by Jędrzejuk et al. (2016b). MDA content was expressed as μmol (MDA) per g FW.

2.4. Assays of antioxidant enzymes

Catalase (EC 1.11.1.6) activity was determined spectrophotometrically as the rate of H_2O_2 disappearance at 405 nm according to Goth (1991) and modified by Jędrzejuk et al. (2016b) for petals of common lilac. CAT activity was determined as units (U) per mg protein. 1 U of catalase deoxidizes 1 μmol of H_2O_2 in 1 min.

Peroxidase activity (EC 1.11.1.7) was determined as described formerly by Jędrzejuk et al. (2016b). The POD activity was estimated spectrophotometrically at 430 nm (Schimadzu UV-1800) and expressed as units (U) per mg protein.

Ascorbate peroxidase (EC 1.11.1.11) was measured according to Venisse et al. (2001). The absorbance was measured at 290 nm (Schimadzu UV-1800) immediately and after 4 min. The amount of oxidized ascorbate was calculated according to the formula: $(A_2 - A_1)/T$, where A_1 – is absorbance at the first measurement, A_2 – absorbance after 4 min, T – 4 min. The APX activity was measured in units (U) per APX activity (μmol ascorbate oxidized per min per mg protein).

The superoxide dismutase (EC 1.15.1.1) activity was determined as described by Beyer and Fridovich (1987). Test tubes containing the reaction mixture and control tubes without the enzyme extract, were kept in the dark, for 20 min at 25 °C. The increase in absorbance due to formazan formation was read at 560 nm (Schimadzu UV-1800). The increase in absorbance without the enzyme extract was taken as 100%