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Protective effects of 1-methylcyclopropene and salicylic acid on senescence regulation of gladiolus cut spikes

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ABSTRACT

Reports indicate that senescence in cut flowers is accelerated. Therefore, the effects of 1-methylcyclopropene (1-MCP) or salicylic acid (SA) on the postharvest quality of gladiolus cut flowers and whether these treatments can regulate the flower senescence were investigated. Two concentrations of each 1-MCP (0.2 and 0.4 g m^{-3}) or SA (0.5 and 1 mM) were studied. The control spikes were kept in distilled water. 1-MCP or SA treatments significantly prolonged the vase life and minimized the weight loss of gladiolus spikes compared with the control. Both treatments enhanced the relative water content (RWC) of leaves and maintained chlorophyll content compared with the control values, which were decreased. Ethylene production, proline accumulation and malondialdehyde content were increased in florets of untreated spikes. 1-MCP or SA reduced ethylene production, decreased both proline content and malondialdehyde level and hence maintained membrane stability. An increase in floret antioxidant enzyme activities (CAT, SOD and POX) was observed in 1-MCP- or SA-treated spikes compared with the control. The effects of 1-MCP or SA on floret senescence seemed not entirely limited due to their effects on ethylene, but they most likely had a sustainable impact on the above-tested physiological parameters.

1. Introduction

Maintaining the quality of cut flowers is one of the main challenges of florists in the flower trade worldwide. In floriculture, delaying the onset of senescence in order to prolong the vase life of cut flowers is the focus of many researchers. The flower senescence and vase life are influenced by several factors including endogenous ethylene (Seglie et al., 2012). Moreover, unfavorable transport and storage conditions can reduce the flower quality as a result of endogenous ethylene production in response to postharvest stressful environment (Nowak and Rudnicki, 1990). Ethylene induces leaf yellowing, flower or petal drop, irregular opening and premature death (Nowak and Rudnicki, 1990). It also causes loss of cellular turgor, chlorophyll and pigment degradation and hence product quality such as vase life (Serek et al., 2006). Stimulation of ethylene biosynthesis has been suggested to involve the generation of reactive oxygen species (Pellinen et al., 1999). It has been observed that flower senescence is accompanied with increased permeability of petal cells and increased ROS production (Reezi et al., 2009). Ethylene control is, therefore, a critical factor in the flower maintaining quality after harvest.

1-MCP (C_4H_6) is a non-toxic inhibitor of ethylene action, which acts as a competitive and irreversible inhibitor of binding of ethylene to its receptor, thereby inhibiting flower senescence (Sisler et al., 1996; Sisler and Serek, 1997). Several reports have indicated that 1-MCP is a very potent inhibitor of ethylene action in different cut flowers (Hassan, 2009; Liou and Miller, 2011; Seglie et al., 2012), through retarding chlorophyll degradation and senescence (Hassan and Mahfouz, 2010). Even in an ethylene-free environment, treatment with 1-MCP significantly improved the vase life of some cut flowers (Serek et al., 1995; Sisler and Serek, 1997, 2001). Furthermore, 1-MCP is environment-friendly, safe to use and is appropriate for operations of any size (Liou and Miller, 2011). However, the role of 1-MCP to retard ethylene-dependent senescence processes is not studied yet, and little information is available about its role in mitigating the oxidative stress in gladiolus cut flowers.

Salicylic acid (SA) is a simple phenolic compound involved in the regulation of many processes in plant growth and development and can inhibit ACC-oxidase activity (ACC, a precursor for ethylene biosynthesis) (Zhang et al., 2003). SA lowers lipid peroxidation via

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increased antioxidant enzymes and thus retains membrane stability under various stresses (Ansari and Misra, 2007; Canakci, 2008; Kazemi et al., 2011; Hatamzadeh et al., 2012). SA plays therefore an important role in extending the vase life of gladiolus cut flowers, attributable to decreased ROS, maintained plasma membrane permeability of floret cells, overcome fresh weight loss and decreased ethylene production as well as increased antioxidant enzyme activities (Ezhilmathi et al., 2007; Marandi et al., 2011; Hatamzadeh et al., 2012). Most of the early works on the senescence of different cut flowers were focused on the effect of 1-MCP on the ethylene action (Sisler et al., 1996; Serek and Sisler, 2001; Celikel et al., 2002; Hassan, 2009). The present study was, therefore, undertaken to investigate whether 1-MCP can affect the postharvest quality of gladiolus as antioxidant rather than anti-ethylene and to compare its effects with SA as well. In order to achieve that, physiological and biochemical responses of gladiolus cut flowers to 1-MCP or SA were studied.

2. Materials and methods

2.1. Plant materials

Cut flowers used in the experiment were *Gladiolus grandiflorus* cv. "White Prosperity". The flowers were obtained from a commercial grower and directly transported to the Laboratory of Faculty of Science, Taif University, Saudi Arabia, during 2013 season. Cut spikes at tight bud stage, having 14–16 buds each and only the first floret was shown its color were selected. Lower leaves were removed and the spikes were trimmed to a uniform length of 75 cm and fresh weight (55 ± 2 g).

2.2. 1-MCP and SA treatments

Pre-treatment with 1-MCP was released from a commercial powdered formulation (Smartfresh™, Rohm and Haas Italy, Inc.) by adding distilled water, according to the manufacturer's instructions. The spikes of each treatment were placed in a glassy chamber and 1-MCP was applied at a concentration of 0.2 or 0.4 g m^{-3} for 6 h. The treatment of 1-MCP was conducted at 16 °C. For SA treatments, aqueous solutions of 0.5 and 1.0 mM SA were prepared and applied as holding solutions in 500 mL beakers. Control spikes were not treated with 1-MCP or SA and were kept in 500 mL beakers containing distilled water. After the treatment of 1-MCP gladiolus spikes were placed in the same containers for the vase life evaluation. Vase solutions for all treatments were changed every 24 h and all SA solutions were prepared with distilled water. Five treatments with four replicates were applied and each replicate consists of five flowers.

2.3. Vase life evaluation

The postharvest life of gladiolus cut spikes was evaluated at 20 °C, 70 ± 5% RH and 14 h photoperiod with 10 μmol m^{-2} s^{-1} irradiance from cool-white fluorescence lamps. The vase life was defined as the number of days in vase life required for 50% of the florets of each spike to lose its ornamental value (lost turgor and wilted).

2.4. Number of opened and unopened florets

On each spike, the number of opened and unopened florets was recorded from the beginning of the experiment until day 16.

2.5. Fresh weight measurements

The cut spikes were initially weighed at the beginning of the experiment. The fresh weight was repeated again daily until the end of vase life of control flowers. The change in fresh weight was determined. The spikes were analyzed subsequently for fresh weight changes during vase life.

2.6. Relative water content (RWC)

Leaf RWC was measured according to Weatherley (1950) as follows: $\frac{W_{fresh}-W_{dry}}{W_{turgid}-W_{dry}} \times 100$, where W_{fresh} is the sample fresh weight, W_{turgid} is the sample turgid weight after being saturated with distilled water for 24 h at 4 °C and W_{dry} is the oven-dry (at 70 °C for 48 h) weight of the sample. The third leaf from the base of inflorescence was used for measurements and the samples were taken on days 1, 3, 5, 7 and 9 from the beginning of the experiment.

2.7. Chlorophyll determination

Total chlorophyll content in leaves was determined by using chlorophyll meter (SPAD-502, Minolta Co., Japan) and represented by the SPAD value. Samples of leaves were taken from the third leaf from the base of inflorescence for chlorophyll determination at days 1, 3, 5, 7 and 9 from the beginning of the experiment.

2.8. Ethylene production determination

The floret samples for ethylene production were taken from the third floret at the base of spike at days 0, 1, 2, 3 and 4. The samples were put in 50 mL airtight jars sealed and fitted with gas sampling ports. The jars were kept at 20 °C and 70–75% RH for 2 h. Gas samples (1 mL) were withdrawn from the headspace of jars for ethylene determination. Ethylene content of the samples was quantitatively analyzed by gas chromatography using a Varian GC CP-3800 and MS Saturn 2200 equipped with a Factor Four capillary column (VF-5 ms 30×0.25 mm ID and film thickness 0.25 μm). The injector, column and detector temperatures were 80, 100 and 220 °C, respectively (Heiser et al., 1998). Ethylene values were indicated as nL g^{-1} h^{-1} FW.

2.9. Membrane stability index (MSI)

Floret samples from each treatment were taken from the third floret at the base of the spike on days 0, 1, 2, 3 and 4 for determining ions leakage by using the method of Sairam et al. (1997). Two floret samples (0.2 g) were taken and placed in 20 mL of double-distilled water in two different 50 mL flasks. The first one was kept at 40 °C for 30 min, while the second one was kept at 100 °C in boiling water bath for 15 min. The electric conductivity of the first (C_1) and second (C_2) samples were measured with a conductivity meter. The leakage of ions was expressed as the membrane stability index according to the following formula $MSI = [1 - (C_1/C_2)] \times 100$.

2.10. Proline determination

Proline content was determined in floret samples from the third floret at the base of the spike on days 0, 1, 2, 3 and 4. Frozen floret tissue (0.2 g) was homogenized with 10 mL of 3% sulfosalicylic acid at 4 °C. Then, the obtained extract was filtered. Two milliliters of filtrate plus 2 mL of acid-ninhydrin, and 2 mL of glacial acetic acid were mixed in a test tube and incubated at 100 °C for 1 h. The reaction was terminated on ice, and the reaction mixture was then extracted with 4 mL of toluene. The chromophore-containing toluene was separated from the hydrated phase. The absorbance