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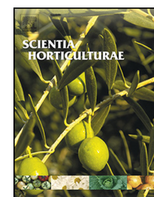

Antioxidative activities and qualitative changes in gladiolus cut flowers in response to salicylic acid application

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ABSTRACT

Extension in the display life of cut flowers is a pre-requisite of floral industry to meet with the demands of growers, wholesalers and consumers. Various chemical preservatives and growth regulators are currently in use to enhance the shelf and decorative life of cut flowers. In this study, a well-established plant growth regulator salicylic acid (SA) was supplemented in various concentrations, viz., 0, 50, 100, 150 and 200 mg SA L^{-1} in vase solution during two consecutive years 2010–11. Days taken to open floret, percent florets opened, vase life, percent fresh weight change, electrolyte leakage, activities of antioxidative enzymes SOD, POD, CAT, and free radicals scavenging activity were investigated in this study. Salicylic acid at 150 mg L^{-1} significantly increased the days to open florets, percent florets opened, retained higher fresh weight, and enhanced the SOD, POD, CAT and free radicals scavenging activity. The lowest electrolyte leakage and the highest free radical scavenging activity were recorded with SA levels of 200 and 150 mg L^{-1}, respectively. The study concludes that SA is helpful in extending the vase life of gladiolus cut flower, and the highest physiological and biochemical response was at an optimum level of 150 mg SA L^{-1} of vase solution.

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1. Introduction

Floral senescence is commonly accompanied by morphological, biophysical and biochemical deterioration. If proper postharvest handling and management is not exercised, cut flowers would rapidly lose freshness making them unattractive. Floral senescence is associated with highly controlled, genetic and physiological processes that include ultrastructural modifications, membrane leakage, oxidative stress and degradation of macromolecules (Rani and Singh, 2014). It is a major issue regarding the postharvest handling of cut flowers. Petal senescence is the final stage of display life that follows the physiological maturity, ultimately leading to the death of cells, organs or the whole plant. Flower longevity and quality are requisites for the cut flowers marketing (Bhattacharjee and De, 2005; Gebremedhin et al., 2013) which ensure that the stallholders, viz., wholesalers, retailers and end consumers will be satisfied and will return back to procure more flowers. Commercially, the customers need a guarantee for potential decorative life of cut flowers (Stead, 2004). Thus growers could fetch a better price through quality produce and consumer acceptability (Sudhagar, 2013).

Gladiolus, an imperative cut flower is reported to undergo senescence, independent of ethylene pathway (Woltering and Van Doorn, 1988), indicating that an alternate system exists for regulating the senescence process in gladiolus cut flowers, like oxidative stress (Saeed et al., 2013). Floral senescence is also associated with a series of highly regulated biochemical and physiological processes in cut flower (Nasibi et al., 2014). These processes include increased respiration rate, degradation of macromolecules, enhanced hydrolytic enzyme activity, and ultrastructural changes in various cell organelles, viz., tonoplast membrane invagination, rupturing of vacuole, chloroplast degradation and changes in mitochondrial ultra-structure (Rani and Singh, 2014).

In recent years, involvement of reactive oxygen species (ROS) in senescence mechanism has gained a considerable attention (Gill and Tuteja, 2010). These reactive oxygen species react with and degrade the lipids, proteins and nucleic acids consequently leading to cell death (Sen et al., 2010). Plants have well developed antioxidative defense system to scavenge free radicals. Antioxidant enzymes such as peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) are involved in scavenging ROS (Saeed et al., 2014). Oxidative stress and membrane degradation are major factors for quality deterioration, particularly in ethylene insensitive

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cut flowers. Therefore, there is dire need to work out the potential and cost effective agents, which could cope with oxidative stress during vase period.

Salicylic acid (SA) has been extensively studied both as a plant growth regulator and for its potential role in the maintenance of postharvest quality of vegetable, fruits and cut flowers (Ezhilmathi et al., 2007). It belongs to a plant phenolics group, widely distributed within plants and plays a vital role in plant growth metabolism and developmental phenomena. It has also been given more attention for the last two decades due its capability to persuade systemic acquired resistance (SAR) both for biotic and abiotic stress, and signaling mechanism in plants (Rejeb et al., 2014).

Salicylic acid influences cut flower quality and longevity, and involved in the regulation of several physiological processes. Its exogenous application may influence ion uptake, stomatal conductance and transpiration (Eraslan et al., 2007), and reduce the postharvest water loss (Hassan et al., 2007). It also has stimulatory effect on flowering, and acts as endogenous regulator for flower induction (Pacheco et al., 2013).

Activity of antioxidant enzymes, viz., CAT, SOD and lipid peroxidase (LOX) extends the vase period of cut flowers (Ezhilmathi et al., 2007). Salicylic acid is involved in eliminating the oxidative stress by promoting the activity of various antioxidant enzymes, e.g., LOX, CAT, SOD, POD, and ascorbate peroxidase (APX) etc. (Tareen et al., 2012), maintaining membrane integrity of cut flowers. Oxidative stress is a major problem of cut flowers in vase causing early wilting and loss in their aesthetic value. Keeping in view the perishable nature of cut flowers, response to exogenous salicylic acid application was investigated for extending the decorative life and quality attributes of gladiolus cut flowers during the vase period.

2. Materials and methods

2.1. Plant material and experimental conditions

Gladiolus cv. 'White Prosperity' was selected for the study during two consecutive years, 2010 and 2011, raised under greenhouse conditions. Spikes were harvested at 2–3 florets color break stage. Flower spikes were re-cut to the uniform size of 90 ($\pm$5) cm under water and were placed under controlled conditions (25 $\pm$ 2 °C, 70 $\pm$ 5% relative humidity and 14 h illumination period). Salicylic acid supplemented in vase solution was applied at various concentrations, viz., 0, 50, 100, 150 and 200 mg SA L^{-1}.

2.2. Morpho-physiological attributes

Days taken to open florets were counted from the tight bud stage to fully opened flower. Percentage of opened florets out of total number of florets on spike was worked out. Vase life of flower spike was noted from the D_0 (day of harvest) to the day of complete senescence. Percent change in fresh weight of flower spike was calculated by relative change in fresh weight at each 2 days interval with respect to D_0 until D_{10} (day 10 of vase life).

Membrane leakage (%) was assessed by following the method described by Singh et al. (2008) with some modifications. Five discs (1 cm diameter) from the bract leaf were put together in the test tube containing 10 mL distilled water (DW). Initial reading was taken by using conductivity meter after incubation of the test tubes at room temperature for 3 h. Then to liberate all electrolytes, the solution was boiled on a water bath for 10 min and the final conductivity was recorded. Membrane leakage percentage was calculated through the formula described by Singh et al. (2008) as in the followings:

$$\text{Electrolyteleakage}(\%) = C_1/C_2 \times 100$$

Where, C_1 is electrical conductivity of discs after 3 h incubation at room temperature and C_2 is the final conductivity of the solution.

2.3. Biochemical determinations

Cell-free enzyme extract was prepared from a leaf sample of 1.0 g, frozen in liquid nitrogen by grinding in pre-cold mortar and pestle for the determination of antioxidant enzymes activity. Leaf tissues were put in 5 mL of 0.1 M potassium-phosphate buffer (pH 7.8) containing 0.2 g of polyvinyl polypyrrolidone (PVPP) and 0.5% Triton and the mixture was centrifuged at 27000 $\times$ *g* at 4 °C for 30 min (Abassi et al., 1998).

2.3.1. Superoxide dismutase

Activity of SOD enzyme was estimated by following the method described by Dhindsa et al. (1981) with slight modification. Superoxide dismutase activity was determined by measuring the inhibition by 50% of photochemical reduction of nitro blue tetrazolium (NBT) under assay conditions. Two sets of five cuvettes (3 mL) were used; each containing 0, 100, 200, 300 or 400 mL of crude enzyme extract, and each were added with 50 mM potassium-phosphate buffer (pH 7.8). One set of reaction cuvettes was kept in dark served as blank, while the other set of five cuvettes was placed under fluorescent lamps for 10 min. Absorbance was taken at 560 nm wave length with a spectrophotometer (Optima® 3000 plus).

2.3.2. Peroxidase

Peroxidase activity was assayed by following the method used by Hassan et al. (2007) with some modifications. The assay mixture comprised of 1 mM H_2O_2, 0.1 mM guaiacol in 15 mM $NaKPO_4$ buffer (pH 6.0), added with 200 μL of crude enzyme extract. Activity of POD was recorded as a change in the optical density (OD) at 470 nm over a 3 min period.

2.3.3. Catalase

Catalase activity was estimated by following the method described by Abassi et al. (1998). Reaction for CAT activity was carried out by using two buffer solutions, one containing of 12.5 mM H_2O_2 and other consisting 50 mM potassium-phosphate buffer in 50 mM potassium-phosphate (pH 7.0). Reaction was triggered by adding 300 μL of crude enzyme extract to each buffer in 3 mL cuvettes, and OD was recorded at 240 nm.

2.3.4. Protein content

Total protein contents analysis was undertaken by using bovine serum albumin as standard (Bradford, 1976). For protein assay 100 μL of supernatant was added to 5 mL of protein reagent, based on the phenomena of proteins to bind the Coomassive Brilliant Blue G-250 (dye). Optical density was observed at 595 nm against protein reagent (blank).

2.3.5. Free radical scavenging activity

Free radical scavenging activity was determined by using a modified version of the method described by Brand-Williams et al. (1995). Scavenging activity of free radicals was estimated by scavenging the 2,2-diphenyl-L-picrylhydrazyl (DPPH) free radical, prepared in a methanol solution. Frozen leaf tissue (1 g) was grinded and extracted in 5 mL methanol. Optical density was noted at 515 nm at 0 and 30 min. Free radical scavenging activity was worked out as percent inhibition of DPPH radicals by following formula described by Tareen et al. (2012):

$$\text{Inhibition}(\%) = [(A_B - A_A)/A_B] \times 100$$

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