Effects of Arginine, Cysteine and 5-Sulfosalicylic Acid on of Vase Life of Tuberose Cut Flowers.

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ABSTRACT

Nitric oxide (NO), hydrogen sulfide (H2S) and salicylic acid (SA) are the main signaling molecules in plants. In this study, the effects of different concentrations of cysteine and arginine as precursor of H2S and NO respectively, and 5-SSA as SA donor on the postharvest physiology of Polianthes tuberosa cut flowers were investigated. Vase solution containing Arg, Cys or 5-SSA significantly increased vase life and opened flowers and besides that it decreased floret abscission compared to control. The increment in vase life concurred with a decrease in lipid peroxidation and electrolyte leakage. Tuberose flowers which were treated with vase solution containing Arg, Cys and 5-SSA had lower polyphenol oxidase (PPO) activity and higher phenylalanine ammonialyase (PAL) activity. Results of this experiment suggests that these compounds effects may be related to the induction of PAL activity, antioxidant compounds production, inhibition of PPO activity, reduced lipid peroxidation and consequently prolonged membrane integrity.

Keywords: Floral abscission; hydrogen sulfide; lipid peroxidation; nitric oxide; Polianthes tuberosa.

Abbreviations:
Arg: arginine; Cys: cysteine; H2S: hydrogen sulfide; MAD: Malondialdehyde; NO: Nitric oxide; PAL: phenylalanine ammonialyase; PAs: Polyamines; PPO: polyphenol oxidase; SA: salicylic acid.

INTRODUCTION

Flowers have long been admired with each civilization and culture in the world. Dating as far back, flowers were used to decorate graves and celebrate major life events. Numerous cultures have incorporated flowers in their everyday lives as expressions of beauty and art (Anderson, 2006). Floriculture is an emerging and fast expanding globalized market and consequently studies on postharvest handling of cut flowers occupy a pivotal position (Gul and Tahir, 2013). The high value of cut flowers has driven major increases in production in many developing countries. Production of cut flowers and foliage can be highly profitable in countries with ideal growing environment and low labor costs (Reid and Jiang, 2012).

Cut flowers are highly perishable and have short vase life, which limits efficient marketing of economically significant ornamental plants. The vase life of cut flowers is influenced by many internal factors such as plant hormones especially ethylene which triggers the senescence process (Kebenei et al., 2003; Wouter et al., 2008). Senescence of cut flower is associated with a series of highly regulated physiological and biochemical processes such as degradation of proteins, nucleic acid, membrane lipids and membrane leakage, floral abscission, color change, leaf yellowing or weight loss (Buchanan-Wollaston et al., 2003). It has been reported that application of compounds such as ethylene inhibitors, sugar, hormones and antimicrobial compounds were effective for increase the vase life of flowers (Huang and Chen, 2002; Shimizu and Ichimura, 2009).

Tuberose (Polianthes tuberosa L.) is an ornamental bulbous plants and one of the most important cut flowers in tropical and subtropical areas (Huang et al., 2001). This genus of Agavaceae family is native to Mexico and consists of about 12 species (Naz et al., 2012). This ornamental flower bulb is a prominent cut flower in Iran and is used for many events. Tuberose is one of the major cut flowers in Iran and it is extensively cultivated in many floricultural regions within the country (Jowkar and Salehi, 2005). Like other flowers with spike inflorescence, it is normally harvested with few open florets, and postharvest longevity and
opening of the remaining florets on the spike play a very important role in this flower’s postharvest physiology and value. Therefore, compounds which are able to retard senescence of this flower are very important from the commercial point of view (Ezhilmathi et al., 2007). In order to increase vase life, various compounds have been applied on tuberose. Jowkar and Salehi (2005, 2006) reported the beneficial effect of 450 mg/l citric acid as vase solution for cv. ‘Golderosht-e-Mahalat’. It has been reported that aluminum sulfate increased tuberose cut flower vase life by increasing solution absorption, protein and pigment content and reducing water loss (Mohammadi et al., 2012). This positive effect on vase-life was related to antimicrobial activity of this compound. Cobalt chloride also extended the vase-life of tuberose cut flowers (Mohammadi et al., 2012). Benzyl adenine at 100 ppm increased the vase life of Polianthes tuberosa L. cv. ‘Golderosht-Mahalat’ (Hassanpour Asil et al., 2011). Gibberellic acid (150 ppm) extended the vase life of tuberose (Shoor et al., 2007).

Nitric oxide (NO) and hydrogen sulfide are two gaseous signaling molecules in animals and recently there is accumulating evidence that shows these signaling molecules act as a gaseous growth regulator in plants (Yang et al., 2008; Papenbrock et al., 2007). It has been found that NO and H₂S at low concentration can ameliorate the negative effects of environmental stresses such as drought, salinity, and heavy metal toxicity (Zhang et al., 2009; Wang et al., 2010) and also improve the postharvest behavior of cut Polianthes tuberosa flowers for the first time. Therefore some morphological and physiological parameters which probably associated with vase life of flower were evaluated.

**MATERIALS AND METHODS**

**Plant Material and Treatment:**

Cut flowers of Polianthes tuberosa L. were purchased from a local commercial greenhouse. Flowers were placed in solution containing 0, 5, 10 and 20 µM of arginine, cysteine and 5-SSA in laboratory for 1–12 days at 25±1 °C with a 16 h photoperiod. Water, arginine, cysteine and 5-SSA solution were refreshed at 3-days intervals. Three cut flowers were placed in a 300 ml flask flowers with 250 ml of each mentioned solutions as a replication.

**Studied Characteristics:**

1. **Vase Life**

The vase life was determined based on petal wilting and vase life termination of each floret was considered when the first symptom of wilting was observed.

2. **Lipid Peroxidation**

The level of lipid peroxidation in petal tissue was measured by determination of Malondialdehyde (MDA) which is known to be breakdown products of lipid peroxidation. The MDA content was determined with the thiobarbituric acid (TBA) reaction. Briefly, 0.2 g of sample tissue was homogenized in 5 ml 0.1% TCA. The homogenate was centrifuged at 10000 g for 5 min. 4 ml of 20% TCA containing 0.5% TBA were added to 1 ml aliquot of the obtained supernatant. The mixture was heated at 95 °C for 15 min and cooled immediately on ice. The absorbance was measured at 532 nm by a spectrophotometer. The value for the non-specific absorption at 600 nm was subtracted from the above value. The level of lipid peroxidation was expressed as mmol of MDA formed using an extinction coefficient of 155 mmol⁻¹ Cm⁻¹ (Heath and Packer, 1968).

3. **Electrolyte Leakage**

Electrolyte leakage of the petals was determined by recording the electrical conductivity
of leachates in de-ionized water at 40 and 100 °C according to the method of Sairam et al. (1997).

4. Soluble Sugar Content

Frozen samples (0.1 g) were ground and extracted with 2.5 ml of 80% (v/v) ethanol at 90 °C for 60 min, followed by centrifugation at 10000 g at 4 °C for 10 min. The process was repeated for complete extraction. Total soluble sugar content was determined using anthrone reagent and glucose as standard (Roe, 1955). Results are expressed as mg soluble sugar per g FW.

5. Enzyme Extraction and Activity Determination

500 mg of petal tissue were homogenized in an ice-cold mortar using 50 mM potassium phosphate buffer (pH = 7.0) containing 1 mM EDTA, 1% (w/v) soluble PVP, and 1 mM PMSF. After centrifugation at 20000 g for 20 min, the supernatant was used for determination of PAL and PPO activities.

Phenylalanine ammonia-lyase activity

PAL (Phenylalanine ammonia-lyase) (EC 4.3.1.5) activity was assayed according to the method of Dcunha et al. (1996). The reaction mixture contained 100 mM Tris-HCl buffer (pH = 8.5), 1 mM 2-mercaptoethanol, 50 mM L-Phenylalanine and 100 µl of enzyme extract. The mixture was incubated at 30 °C for 15 min. The reaction was terminated by the addition of 0.5 ml 6 M HCl and absorbance of the supernatant was measured at 290 nm by spectrophotometer. One unit of enzyme represents the conversion of 1 µmol substrate to cinammic acid per min.

Polyphenol oxidase (PPO) activity

Polyphenol oxidase (PPO) (EC 1. 14. 18. 1) activity was determined according to the method of Nicoli et al. (1991). The reaction solution contained 50mM potassium phosphate buffer (pH = 7.0), 20 mM pyrogallol and 100 µl enzyme extract. Solution absorbance was recorded at 420 nm after 3 min and the activity was determined by using an extinction coefficient of 6.2 mM−1 cm−1.

6. Total Soluble Proteins

Protein content was determined according to the method of Bradford (1976) using Bovine serum albumin as standard.

Statistical Analysis:

The experiments were performed in a completely randomized design. Data were expressed as mean of three replicates with standard error. Statistic assays were carried out by one-way ANOVA using MSTATC software. Significant differences between means were determined by Duncan’s multiple range tests. P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Cut flower senescence is associated with a series of regulated physiological and biochemical processes such as lipid peroxidation and loss of membrane integrity, degradation of macromolecules, and cellular decompartmentalization (Buchanan-Wollaston, 1997). Results showed that the vase solution containing Arg, Cys and 5-SSA, significantly increased the vase life of flowers compared to the control solution (distilled water) (Table 1).

### Table 1: Effect of different concentrations of Arg, Cys and 5-SSA on vase life, flower opening and floral abscission in Polianthes tuberosa cut flowers.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vase Life (days)</th>
<th>Fully opened flower (%)</th>
<th>Floral abscission (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.00</td>
<td>5.623</td>
<td>10.77</td>
</tr>
<tr>
<td>Arginine 5 µM</td>
<td>8.66</td>
<td>16.67</td>
<td>2.93</td>
</tr>
<tr>
<td>Arginine 10 µM</td>
<td>10.66</td>
<td>19.34</td>
<td>2.89</td>
</tr>
<tr>
<td>Arginine 20 µM</td>
<td>10.00</td>
<td>20.62</td>
<td>2.05</td>
</tr>
<tr>
<td>5-SSA 5 µM</td>
<td>10.00</td>
<td>15.44</td>
<td>3.17</td>
</tr>
<tr>
<td>5-SSA 10 µM</td>
<td>9.33</td>
<td>13.87</td>
<td>1.50</td>
</tr>
<tr>
<td>5-SSA 20 µM</td>
<td>10.33</td>
<td>12.91</td>
<td>3.83</td>
</tr>
<tr>
<td>Cysteine 5 µM</td>
<td>10.00</td>
<td>9.27</td>
<td>5.00</td>
</tr>
<tr>
<td>Cysteine 10 µM</td>
<td>10.00</td>
<td>14.32</td>
<td>6.22</td>
</tr>
<tr>
<td>Cysteine 20 µM</td>
<td>9.66</td>
<td>10.38</td>
<td>6.28</td>
</tr>
</tbody>
</table>

* Values followed by the same letter within a column indicate they are not significantly different (P < 0.05) by Duncan’s multiple range test (DMRT).

The extended vase life in treated flowers was associated with increase in floral opening and decrease in flower abscission and lipid peroxidation (Fig. 1). The number of fully opened florets increased in all treatment except in 5 and 20 µM Cys. Measurement of MDA as the indicator of lipid peroxidation, showed that all of the treatments except 10 µM 5-SSA could decrease the amount of MDA in Polianthes tuberosa flowers.

![Fig. 1. Effect of Arg, Cys and 5-SSA](image)

Electrolyte leakage measurement also indicated that this parameter decreased in many treatment solutions; however 10 µM 5-SSA, 20 µM Arg and 20 µM Cys solutions had no significant effects on the decline of electrolyte leakage (Fig. 2). It has recently reported that delayed senescence of cut flowers is accompanied by higher membrane stability (Liao et al. 2012). The main products of
Arg catabolism are nitric oxide (NO), polyamines (PAs) and proline (Wu and Morris, 1998). The effects of NO, PAs or proline on delaying of senescence and enhancing the longevity of some flowers or fruits have been reported earlier (Bowyer et al., 2003; Khan et al., 2007; Kumar et al., 2010). For example, it has been reported that polyamines and NO can decrease ethylene production and delay senescence in strawberry fruit through inhibiting synthesis of ethylene (Khosroshahi et al., 2007; Zhu and Zhou, 2007). Exogenous application of proline reduces free radical damages and increases vase life of flower (Kumar et al., 2010). SNP application decreased lipid peroxidation and electrolyte leakage and increased the vase life of chrysanthemum flowers (Mansoori, 2012). It has been reported that the role of NO in prevention of lipid peroxidation is related to the ability of NO to react with lipid alcoxyl (LO•) and lipid peroxyl (LOO•) radicals and stop the chain of peroxidation in a direct fashion (Beligni and Lamatina, 1999).

In this research, cysteine solution also reduced the MDA content and electrolyte leakage (Fig. 1 and 2). These results are in agreement with those reported earlier by Zhang et al., (2011), who suggested that H2S donor prolonged the vase life of flowers, which was accompanied by increasing in the activation of antioxidant enzymes and decreasing in the levels of MDA and lipid peroxidation. The effects of Cys in this experiment may also be associated with the production of thiol groups such as glutathione (GSH) which is an important cellular antioxidant. The antioxidant activity of GSH has been reported earlier (Youssefian et al., 2001). 5-SSA solution except in 10 µM concentration decreased the lipid peroxidation and maintained membrane integrity. The effect of SA in extending the vase life of flowers through improving membrane integrity and decreasing lipid peroxidation was reported in previous researches (Zamani et al., 2011, Alaey et al., 2011). Ezhilmathi et al., (2007), showed that treatment of gladiolus cut flowers with 5-SSA, delayed the senescence of flowers and improved the flowers opening and induction of antioxidant enzymes. Since lipid peroxidation is mediated by ROS (Kellogg, 1975), SA may either be directly scavenging ROS and thus decreasing lipid peroxidation, or it may be modulating the activity of antioxidant enzymes.

Results of this research indicated that vase solutions had no significant effects on soluble sugar content and in some treatments (such as 10 and 20 µM Arg or 5 and 10 µM 5-SSA), the vase solution decreased the soluble sugar content in comparison with control flowers (Fig. 3).
some treatments surprisingly was observed a decrease in soluble sugar content but with an increase in vase life.

In this investigation, the PAL activity increased in all of the treated cut flowers (Fig. 4). The phenylpropanoid pathway is one of the important pathways of plant secondary metabolism, which yields a variety of phenolics with structural and defense-related functions. These phenolic compounds include phenolic acid, anthocyanin and flavonoids, which act as scavengers of free radicals and other oxidative species through their hydrogen donating (antioxidant) potential (Syvacy and Sokmen, 2004). Phenylalanine ammonia-lyase (PAL) is the first and crucial enzyme of phenylpropanoid metabolism, catalyzing the formation of trans-cinnamic acid by L-de amination of phenylalanine. This enzyme was induced by various biotic and abiotic stresses, which resulted in the accumulation of phenolic compounds such as phenolic acids and flavonoids (Solecka, 1997). There is controversial result in some researches which showed that SNP inhibited the PAL activity in fruits during storage (Duan et al., 2007). In the present study, it appears that Arg, Cys and 5-SSA may insert their antioxidant properties through induction of PAL activity and synthesis of a variety of phenolic compounds.

In contrast to PAL activity, PPO activity decreased in vase solutions except in 5 µM 5-SSA and 10 and 20 µM Cys solutions (Fig. 5). The browning phenomenon in cut flowers is regarded as indicative of oxidative stress. Polyphenol oxidase or peroxidase catalyzes the browning reaction and results in the formation of quinine, which is subsequently polymerized to varying degree leading to production of brown pigments (Dubravina et al., 2005). It has been reported that H₂S was effective in preventing browning of explants of rose and pomegranate through antioxidant role in this process (Zhang et al., 2011). In this investigation, vase solution may maintain the quality and color of cut flowers, through PPO activity decrease.

CONCLUSION

Results of the present experiment on the effects of Arg, Cys and 5-SSA and their effect on preventing senescence of cut *Polianthes tuberosa* flowers, suggest that the effects of these compounds may be related to the induction of antioxidant compounds production (especially phenolic compounds), inhibition of lipid peroxidation and maintenance of membrane integrity. Future work needs to focus on the role of these compounds on antioxidant enzyme activity and in crosstalk with other hormones such as ethylene and their molecular mechanism in cut flower senescence.

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