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Effect of Silicon on Growth and Ornamental Traits of Salt-stressed Calendula (*Calendula officinalis* L.)

Hassan Bayat¹*, Morteza Alirezaie¹, Hossein Neamati¹ and Ali Abdollahi Saadabad²

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A pot experiment was carried out to determine the effect of foliage spraying of silicon (Si) on growth and ornamental characteristics of calendula grown under salt stress and greenhouse conditions. A factorial experiment based on completely randomized design was conducted with 3 levels of Si (0, 50 and 100 mg/l) and 3 levels of NaCl (0, 100 and 200 mM) with 4 replications. At flowering stage, Si was applied with spraying two times in two week intervals. NaCl was also applied as drench (200 ml per pot) in two days interval. The results showed that salinity decreased the growth, SPAD values, flower number per plant and flower diameter. However, foliar applications of Si resulted in greater root, shoot and total dry weight, plant height and leaf area of calendula plants under salt stress. The highest SPAD values were obtained under 100 mg/l Si application in all NaCl treatments. Salinity decreased number of flower per plant and flower diameter as ornamental characteristics; however Si increased them under salinity stress. Plants treated with 100 mg/l Si had the highest flower diameter at 100 mM of NaCl. Electrolyte leakage increased by salinity, however foliar application of Si significantly reduced electrolyte leakage under salt stress. These results suggest that the negative effects of salinity on the growth and ornamental characteristics of calendula plants can ameliorate by foliar application of Si treatments.

**Keywords:** Electrolyte leakage, Foliar application, Landscape, Salinity, SPAD value.
INTRODUCTION

One of the most important environmental factors limiting plant growth and productivity is salinity (Kaya et al., 2003). In most arid and semiarid areas, this problem is accentuated by competition for high quality water among agriculture, industry and landscape users has promoted the use of alternative water sources for irrigation. Thus, marginal quality water, somewhat saline, will become important in these areas (Chartzoulakis et al., 2002) and could be used for the irrigation of ornamental plants (Carter et al., 2005). However, the use of low quality water for irrigation affects plants in different ways, depending on the degree of salt tolerance of the species (Alarcon et al., 1994) and even within a given species (Sanchez-Blanco et al., 2003). Salt stress can affect plant survival, biomass, plant height and plant morphology and affect the capacity of a plant to collect water and nutrients (Parida and Das, 2005). Salinity can cause hyper ionic and hyper osmotic effects on plants leading to membrane disorganization, increase in reactive oxygen species (ROS) levels and metabolic toxicity (Jaleel et al., 2007a). The great effect of salinity is the inhibition of crop growth by the reduced hormone delivery from root to leaves (Jaleel et al., 2007b).

Calendula, mostly known as the pot marigold, is planted widely in gardens and landscapes. It is popular for the lush color and aromatic scent. It grows in sun or partial shade and is easy to grow requiring little cultivation (Dole and Wilkins, 2004).

Silicon (Si) is the second most abundant element on the surface of the earth, yet its role in plant biology has been poorly understood. Silicon has not been considered an essential element for the growth of higher plants; however, soluble Si has enhanced the growth, development and yield of several plant species including rice (Oryza sativa L.), sugarcane (Saccharum officinarum L.) and most other cereals and several dicotyledons (Jones and Handreck, 1967; Elawad and Green, 1979; Takahashi et al., 1990; Belanger et al., 1995; Savant et al., 1999), especially under biotic and abiotic stress conditions (Epstien, 1994). Silicon concentration in the soil solution is controlled by silicate minerals and ranges from 0.01 to 1.99 mM (Karathanasis, 2002). Silicon plays a significant role in imparting biotic and abiotic stress resistance and enhancing crop productivity (Okuda and Takahashi, 1965; Ma et al., 1989; Epstein, 1994; Liang et al., 1994). Improvement of salt tolerance has been reported by addition of Si in wheat (Ahmad et al., 1992), barley (Liang et al., 2003), rice (Yeo et al., 1999), maize (Wang et al., 2004), cucumber (Zhu et al., 2004), rose (Savvas et al., 2007) and zinnia (Zinnia elegans) (Kamenidou et al., 2009). Savvas et al. (2002) reported that quality and peduncle stem thickness in gerbera flower increased when potassium silicate was included in the hydroponic nutrient solution. Hwang et al. (2005) also reported that applications of potassium silicate improved the growth and quality of cut miniature rose ‘Pinocchio’ in the rock wool culture system.

Water deficiency is a serious problem in arid and semi-arid regions of Iran that characterized by little rainfall, high solar radiation and high temperatures in the summer. In recent years, the normal seasonal droughts that have occurred in Iran have caused local and state government to enact water conservation ordinances. Urbanization and increases in population, however, are seriously threatening sustainable natural resources. At present, non-renewable groundwater resources are being depleted to an alarming extent. As high-quality water supply becomes limited, the use of saline water with high salt levels for landscape irrigation is being encouraged. While crop tolerance to salinity has been given considerable attention; fewer studies have dealt specifically with ornamental plants. Therefore, the aim of this research was to investigate the effects of Si on growth and quality of calendula grown under normal and salinity stress conditions.

MATERIALS AND METHODS

Plant material and treatments application

For this experiment, the seeds of Calendula officinalis L. ‘Nana Bon Bon Naranja’ were selected and germinated in cocopeat in plastic germination trays under greenhouse conditions at
Ferdowsi University of Mashhad (36°17'44" N and 59°36'42" E), Iran. Uniform size seedlings (40-days old) were transplanted to plastic pots (15 cm top diameter) and filled with mixture of loam soil:sand:compost (1:1:1,v:v). The plants were grown in a naturally illuminated greenhouse with night/day set temperatures of 18/24 °C. A factorial experiment based on completely randomized design was conducted with 3 levels of Si (0, 50 and 100 mg/l) and 3 levels of NaCl (0, 100 and 200 mM) with 4 replications. Potassium silicate (K₂SiO₃) was used for silicon treatments. Thirty days after transplanting, Si was applied on the foliage of calendula plants with a hand sprayer. The volume of the spray was 30 ml per pot. NaCl was also applied as drench (200 ml per pot) in two days interval. A control group of plants was grown without NaCl and sprayed with deionized water. All plants were harvested 60 days after planting (30 days after treatments) and separated into leaves, stem, and root.

Measurements and data collection

Dry weights of separated roots and shoots were recorded on four randomly selected plants per treatment. Total leaf area was determined with a Delta-T Image Analysis System (Delta-T, LTD, Cambridge, UK). Plant height, flower diameter and number of flower per plant were also measured. Electrolyte leakage which is used to assess membrane permeability was determined according to Lutts et al. (1996). Leaf greenness or chlorophyll reading values (measured as the optical density, SPAD value) was recorded at the end of the experiment on three leaves per plant at similar middle positions of shoots for all plants in each treatment using a portable SPAD chlorophyll meter (SPAD 502, Minolta, Japan).

Statistical analysis

Analysis of variance (ANOVA) for all the variables was carried out using the JMP8 software. Treatment means were compared using the protected Least Significant Difference (LSD) test at p<0.05 level.

RESULTS

Plant growth

Shoot, root and total dry weight were recorded as influenced by different levels of Si and NaCl. As shown in Fig. 1, shoot, root and total dry weight of calendula plants were lower at salt stress treatment as compared to non-saline conditions. However, foliar application of Si increased dry matter accumulation in all parts of calendula plants under salt stress. Application of Si (100 mg/l) under salt stress (100 mM NaCl) gave the higher values for these parameters than the other treatments. It indicated that foliar application of Si alleviated the growth inhibition induced by added NaCl. Exogenous application of Si also enhanced shoot, root and total plant dry weight under no salt stress (Fig. 1).

Fig. 1. The dry weight of shoot (A), root (B) and total plant (C) of calendula in response to foliar Si applications under salt stress. Different letters on top of bars indicate significantly differences according to LSD test (p < 0.05) at each salt level. Vertical bars indicate the mean ± SE.
Salt stress significantly decreased plant height; however, foliar application of Si improved plant height under salt stress (Fig. 2A). Treatment 100 mg/l Si + 100 mM NaCl had the highest plant height as compared to the other treatments under salt stress. Foliar application of Si (100 mg/l) increased plant height by 27% as compared to control in 100 mM NaCl (Fig. 2A).

Salt stress decreased leaf area as compared to the non-saline conditions. NaCl (200 mM) decreased leaf area by 58%, but the reduction was 41% when 100 mg/l Si treatment was applied to NaCl-treated plants, as compared to control. Under non-saline conditions, foliar application of Si (100 mg/l) significantly increased leaf area (1.6 fold) as compared to control (Fig. 2B).

Ornamental characteristics

Salt treatment (200 mM) significantly decreased number of flower per plant by 70% as compared to control, but the reduction was only 40% when Si (100 mg/l) was applied to NaCl-treated plants. Under non-salt stress condition, Si increased flower number by 35% as compared to control (Fig. 3A).

Flower diameter of calendula plants decreased dramatically with the increasing NaCl concentration. All Si treatments increased the flower diameter compared to non-treated plants both in absence and presence of salinity. Plants treated with 100 mg/l Si had the highest flower diameter at 100 mM of NaCl (Fig. 3B).
SPAD value and electrolyte leakage

SPAD values were significantly decreased with the increasing salinity stress. However, foliar Si applications were caused to the elevated SPAD values. Under saline conditions, the highest SPAD values were obtained from 50 mg/l Si application in all NaCl treatments (Fig. 4A).

Electrolyte leakage increased by salt treatment; however, Si significantly decreased electrolyte leakage under salt stress. The lowest electrolyte leakage was obtained by 100 mg/l Si in 100 mM NaCl concentration under salt stress condition. Under no salt stress, foliar application of Si only slightly decreased electrolyte leakage compared to control (Fig. 4B).

DISCUSSION

Under saline conditions, plant growth of calendula decreased significantly. Growth reduction under saline stress has been reported in various plants by many researchers (Senaratna et al., 2000; Alpaslan and Gunes, 2001; Kaya et al., 2003; Sivrittepe et al., 2003). As stated by Munns (2002), suppression of plant growth under saline conditions may either be due to decreased availability of water or to the toxicity of sodium chloride. Si treatments alleviated the deleterious effects of salinity on plant growth. Similar results were reported in rice (Matoh et al., 1986; Yeo et al., 1999), maize (Wang et al., 2004), cucumber (Zhu et al., 2004), rose (Savvas et al., 2007) and zinnia (Zinnia elegans) (Kamenidou et al., 2009) who observed exogenous Si treatments ameliorated the negative effects of salt stress on plant growth. It has been observed that GA1 and its precursor GA20 enhanced with N and Si application in rice cultivars (Hwang et al., 2008). Gibberellins affect cell enlargement and division which leads to internode elongation in stems and increases stem height. Under saline growth conditions, it has been reported that the benefits of Si are due to the reduction of Na content in the shoots of rice, P. juliflora, and barley (Matoh et al., 1986; Bradbury and Ahmad, 1990; Yeo et al., 1999; Liang et al., 2003), enhanced K uptake of barley (Liang et al., 1996) and improved photosynthesis rate in barley and tomato (Liang, 1998; Al-Aghabary et al., 2004). It has been also reported that Si increases the plant growth and yield of cucumber (Miyake and Takahashi, 1983; Voogt and Kreuzer, 1989).

Si increases rigidity of the mature leaves, which have a rougher texture and are held more horizontally, delays leaf senescence and increases chlorophyll content and ribulose, 1-5- bisphosphate carboxylase activity (Adatia and Besford, 1986). Moreover the induction of antioxidant enzymes and their protective role of membranes caused increasing the tolerance of plant to damages (Liang, 1999).

Salt stress decreased chlorophyll as compared to the non-saline conditions. These results are similar to those of Downton et al. (1985), Stepian and Klobus (2006) and Yildirim et al., (2008) who indicated that chlorophyll content significantly decreased in the leaves of spinach and...
cucumber plants with increasing NaCl concentration. In the present study, Si treated plants showed greater chlorophyll values than non-treated plants. These results are similar with Al-aghabary et al. (2004) that reported Si treatments caused to increase chlorophyll content of leaves of tomato under salt stress.

Salinity impairs membrane permeability and increases electrolyte leakage. However, application of Si partly maintained membrane permeability (Table 4B). Present study showed that Si reduced the amount of ion leakage in salt stressed calendula plants and Si protected the maintenance of membrane functions under stress conditions. Supporting evidence was shown when Si reduced electrolyte leakage salt stress condition in rice (Agarie et al., 1998) leaves.

Salt stress decreased flower number per plant and flower diameter as compared to the non-saline conditions. In this experiment, different concentrations of Si increased flower number per plant and flower diameter. This is in agreement with the works by Savvas et al. (2002) and Kamenidou et al. (2010) that previously reported an increased gerbera flower quality when potassium silicate was added to the hydroponic nutrient solution. Hwang et al. (2005) also reported that applications of potassium silicate improved the growth and quality of cut flower miniature rose ‘Pinocchio’ in the rockwool culture system in agreement with the present results. It is possible that higher chlorophyll contents in Si treatments resulted in photosynthetic activity improvement and higher productivity (Table 4A).

**CONCLUSION**

Based on the present results, Si alleviates the negative effect of salt stress on growth, chlorophyll reading values, electrolyte leakage and flower quality depending on the concentration of Si Maximum alleviation of salt stress was found with 100mg/l Si application.

**Literature Cited**


River, New Jersey.


Shading Impact on Qualitative Characteristics and Chlorophyll Content of Cut Rose (Rosa hybrida cv. Avalanche)

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Light intensity is considered a limiting factor in greenhouse rose production. The main aim of this experiment was to evaluate the effect of shading treatments (0, 25, 50, and 65% shading) on quality and chlorophyll content of cut rose (Rosa hybrida cv. Avalanche), under greenhouse conditions. The experiment was planned in randomized completely block design with four replications. All shoots were bent downwards from above the second bud after removing the young flower bud. Shading significantly affected on bud sprouting, flowering stem fresh and dry weight and flowering stem diameter, so that earliest bud sprouting, highest flowering stem, fresh and dry weight and flowering stem diameter were observed in no shading treatment. However, shading had no significant effect on flowering stem length and leaf area, but specific leaf area increased with shading percentage increment at 65% shade. Results of total chlorophyll content as well as chlorophyll a and b showed a decrement with increasing of shading percentage. In general, shading could be a cause of low-quality in cut roses; therefore greenhouse roses growers should consider greenhouse architecture to maximize light deep penetration.

Keywords: Bending, Canopy, Chlorophyll, Greenhouse roses, Marketing quality, Shading.
INTRODUCTION

Roses are, undoubtedly, one of the world’s most favorite cut flowers (Dole and Wilkins, 1998). Rose growth, harvesting time, and flower quality are usually affected by light intensity (Zieslin and Mor, 1990). Light affects on the number of buds developing from the base of the plant and the remaining part of a branch after harvesting the rose flowers, also, it influences in flower development (Mass and Bakx, 1995). Although natural solar irradiance rate is ideal in Iran, the production rate of greenhouse roses is low per square meter in comparison to other countries (Hashemabadi and Zarchini, 2010). There are several reasons for the low yield; however the main reason is a lack of attention to canopy architecture related to maximizing light absorption (Matloobi, 2007). It has been shown that leaf distribution pattern within the canopy influences in plant photosynthesis by light penetration rate to the inner layers of the canopy (Baille et al., 2006). Also, at present, ‘heightened systems’ are increasingly used by modern rose growers. In these systems, natural shade caused by a dense leaf canopy, has a low Red: Far-red ratio and is deficient in photosynthetically active radiation (PAR) due to selective filtering by photosynthetic pigments (Smith, 1982). Research on changes of photosynthesis rate and photosynthetic parameters within a rose plant canopy showed that rose leaves growing at the top of the canopy had higher rates of photosynthesis and photosynthetic traits comparing to those at the bottom of the canopy (Gonzalez-Real and Baille, 2000; Matloobi et al., 2009). Therefore, it is critical for cut rose growers to optimize light interception in a plant canopy to gaining maximize yield, especially in temperate regions, where natural irradiance can be very low (Mortensen et al., 1992). Moreover, there are some reports of decreasing the light intensity by shading causes reduction in flower quality in Antirrhinum majus (Munir et al., 2004) and Eustoma grandiflorum (Lugassi-Ben-Hamo et al., 2010). Apart from flower quality, plants tend to respond to ambient light by adjusting their chlorophyll content and composition (Walters, 2005). It has been found that leaf chlorophyll content is increased and chlorophyll a/b ratio is decreased under shade conditions (Boardman, 1977).

The arching technique is an advanced method in cut rose cultivation developed in late 1980’s in Japan. In this system, bending non-productive shoots down into the canopy or towards the aisle instead of pruning, resulted in a canopy consisting of horizontally bent shoots in addition to upright shoots (Ohkawa and Suematsu, 1999; Kim and Lieth, 2004).

The main objective of the present study was to evaluate the effect of shading on qualitative characteristics and chlorophyll content of cut roses.

MATERIALS AND METHODS

Plant material

The experiment was conducted in a greenhouse at Research Station of Khalatpooshan, Faculty of Agriculture, Tabriz University, Tabriz, Iran (27°38’N, 27°46’E and 1360 m above sea level) from October to November 2011. Rooted cuttings of Rosa hybrida cv. ‘Avalanche’ were grown in 6 liter pots filled with a medium composed of 70% cocopeat and 30% perlite. The currently local growers nutrient solution was used (mM l⁻¹: NO₃⁻ 11.25; H₂PO₄⁻ 1.2; SO₄²⁻ 0.5; NH₄⁺ 1.25; K⁺ 4.25; Ca²⁺ 2.00; Mg²⁺ 1.25). The pH and electrical conductivity (EC) of the nutrient solution was maintained between 5.5 - 6.0 and 1.5 - 2 mS cm⁻¹. When the primary shoot reached the pea size stage, bending was done above the second bud after removing the young flower bud. Shade treatments were implemented using internal shading nets fixed at a height of 1 m above plants providing 25, 50, and 65% reduction in light intensity. Full sun light was considered as control.

Evaluated traits

After flowering stems appearance, traits including days to bud sprouting after bending, flowering stem length and diameter, fresh and dry weight of flowering stem, leaf area, specific leaf area and leaf chlorophyll content were measured. Length of flowering stems was measured
from the shoot base to the flower bud base before harvesting. After harvesting, flowering stems were weighed by a digital balance. To measure the dry weight of flowering shoots, the materials were put in an oven with the temperature of 80°C for 48 hr. Leaf area was measured by leaf area meter (Li-Cor, Li-1300, USA), and the leaves then dried at 80°C in order to determine specific leaf area (SLA). Chlorophyll was extracted with 80% acetone. Extracts were filtrated and chlorophyll a and b (mg/g⁻¹ FW) were determined by spectrophotometry at 645 and 663 nm, respectively (Arnon, 1949). Chlorophyll content was calculated using below equation:

\[
\begin{align*}
\text{Chlorophyll a} & = (9.93 \times A_{663} - 0.77 \times A_{645}) \times V/W \times 1000 \\
\text{Chlorophyll b} & = (17.6 \times A_{645} - 2.81 \times A_{663}) \times V/W \times 1000 \\
\text{Total chlorophyll} & = (7.12 \times A_{663} + 16.8 \times A_{645}) \times V/W \times 1000 \\
V & = \text{solution volume, } W = \text{leaf sample fresh weight (g)}
\end{align*}
\]

**Experimental design and statistical analysis**

The experiment was performed in a randomized completely block design with four replications (two plants per replication). Statistical analysis was undertaken using SPSS (version 16) and means comparisons were done by Tukey method (P≤0.05).

**RESULTS**

**Days to bud sprouting after bending**

Shading had a significant effect on days to bud sprouting, so that days to bud sprouting increased with increasing of shading percentage (Table 1). The earliest and the latest bud sprouting were related to control and 65% shade treatment, respectively (Table 2).

**Length and diameter of flowering stem**

Shading had a significant effect on diameter of flowering stems, but length of flowering stems was not affected significantly (Table 1). The highest (5.52 mm) and the lowest (4.22 mm) flower stem diameter were found in control and 65% shading, respectively (Table 2).

**Fresh and dry weight of flowering stem**

Effect of shading in this experiment was significant on fresh and dry weight of flowering stems (Table 1). On the basis, the highest and the lowest fresh and dry weight of flowering stem were obtained from control and 65% shade, respectively (Table 2). In general, shading reduced fresh and dry weight of flowering stem. Positive correlation was also observed between dry weights of flowering stem with fresh weight of flowering stem (Table 4).

**Leaf area and specific leaf area**

There was no significant difference among treatments in respect of leaf area (Table 1). However, the highest leaf area was observed in 25% shade treatment. In addition, shade treatments had higher

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<th>flower shoot length (cm)</th>
<th>Flower shoot diameter (mm)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Leaf area (cm²)</th>
<th>Specific leaf area (cm² g⁻¹)</th>
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<td>2.04 *</td>
<td>1.04 **</td>
<td>0.193 **</td>
<td>0.044 **</td>
<td>0.006 **</td>
<td>43.715 **</td>
<td>188.87 **</td>
<td>0.0027</td>
<td></td>
<td></td>
<td>0.0009 **</td>
</tr>
<tr>
<td>Shading</td>
<td>3</td>
<td>3.12 *</td>
<td>1.45 **</td>
<td>2.28 **</td>
<td>58.67 **</td>
<td>6.37 **</td>
<td>40.397 **</td>
<td>0.260 **</td>
<td>0.061 **</td>
<td>0.566 **</td>
<td>0.072 **</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>0.486</td>
<td>3.70</td>
<td>0.110</td>
<td>1.17</td>
<td>0.058</td>
<td>73.6681</td>
<td>0.011</td>
<td>0.0021</td>
<td>0.023</td>
<td>0.0037</td>
<td></td>
</tr>
</tbody>
</table>

*: Significant at P<0.05. **: Significant at P<0.01. ns: not significant.
leaf area than control plants (Table 3). In case of specific leaf area, significant difference was observed between 65% shade and other treatments. The highest (220.87 cm² g⁻¹) and lowest (123.53 cm² g⁻¹) specific leaf area were shown in 65% shade plants and control treatment, respectively.

### Chlorophyll content

Shading significantly decreased total chlorophyll as well as chlorophyll a and b (Table 1). Plants exposed to full light showed higher total chlorophyll than the shaded plants. Obtained results revealed that severe shading increased chlorophyll a/b ratio. Moreover, total chlorophyll as well as chlorophyll a and b were decreased with increasing of shading percentage (Table 3).

### DISCUSSION

Light is a critical factor in bud sprouting and it is reported that high R: FR ratio promoted bud sprouting, but a low R: FR ratio showed a contrary effect (Zieslin and Mor, 1990). It has been found that in shade conditions, the fraction of blue light was increased but red light beam decreased (Li et al., 2010). Therefore, increase in number of days to bud sprouting in shade conditions can be related to decrease in red light (Zieslin and Mor, 1990).

Length and diameter of flowering stem are factors involving in economic value of cut-roses (Kim and Lieth, 2004; Steinmetz et al., 1994). In this research, although shading exhibited no effect on length of flower stems, but flowering stem diameter was affected significantly. In our experiment, positive significant correlation was observed between dry or fresh weight of flowering stem and diameter of flower stem; Thus, it could be concluded that the increase in diameter of flowering stems in control plants resulted from more assimilate production in full ambient light.

Shading significantly affected both fresh and dry weight of flowering stem, so the lowest fresh and dry weight of flowering stem were observed in the highest shade intensity. Similarly, decrease in cut stem weight for plants grown under a lower light integral has been reported in lisianthus (Islam et al., 2005). Fresh and dry weight decline of flowering stem seems to be attributed to low carbohydrate generation in shade condition due to reduced photosynthesis.

Lambers et al. (2008) believed that plants grown in a shade environment had higher leaf area than control plants (Table 3). It can be seen that significant difference was observed between 65% shade and other treatments. The highest (335.23 cm²) and lowest (25) specific leaf area were shown in 65% shade plants and control treatment, respectively.

### Table 2. Effect of shading on leaf area, specific leaf area and chlorophyll content of cut roses (Rosa hybrida cv. Avalanche).

<table>
<thead>
<tr>
<th>Shading (%)</th>
<th>Leaf area (cm²)</th>
<th>Specific leaf area (cm² g⁻¹)</th>
<th>Chl. a (mg g⁻¹ fresh weight)</th>
<th>Chl. b (mg g⁻¹ fresh weight)</th>
<th>Chl. (a+b) (mg g⁻¹ fresh weight)</th>
<th>Chl. a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>335.23 a</td>
<td>123.53 b</td>
<td>1.29 a</td>
<td>0.55 a</td>
<td>1.84 a</td>
<td>2.35 c</td>
</tr>
<tr>
<td>25</td>
<td>418.83 a</td>
<td>125.63 b</td>
<td>1.01 b</td>
<td>0.42 b</td>
<td>1.43 b</td>
<td>2.41 bc</td>
</tr>
<tr>
<td>50</td>
<td>413.51 a</td>
<td>156.81 b</td>
<td>0.96 b</td>
<td>0.37 b</td>
<td>1.34 b</td>
<td>2.52 ab</td>
</tr>
<tr>
<td>65</td>
<td>391.25 a</td>
<td>220.87 a</td>
<td>0.67 c</td>
<td>0.25 c</td>
<td>0.93 c</td>
<td>2.66 a</td>
</tr>
</tbody>
</table>

Different letters with in a column indicate significant difference according to Tukey’s test (P<0.05).
area and specific leaf area that is in agreement with our results. Although leaf area in shaded plants was higher than the control, it was not significant. As rose needs high light intensity, it seems that shade causes decrease in photosynthesis; consequently, invested relatively less of the photosynthesis products in leaf area. Moreover, with increasing intensity of shading, specific leaf area increased. In agreement with our experiment, decreasing the light intensity by shading has been reported to cause increase in specific leaf area in *Achillea millefolium* (Bourdot et al., 1984).

Results obtained on chlorophyll a and b contents as well as chlorophyll a/b ratio are inconsistent with more reports, but are in agreement with the finding of Matloobi *et al.* (2009) on *Rosa hybrida* cv. Habari, who indicated that leaves growing at the top of the canopy which received higher light, tended to have more chlorophyll a and b as well as total chlorophyll in comparison with lower parts. Since, shade leaves have lower N concentrations per leaf area unit than those exposed to the high light (Lambers *et al.*, 2008), it seems that under N limitations, plants may respond by reducing levels of all chloroplast components (Walters, 2005).

**CONCLUSION**

Shading could be a cause of low-quality in cut roses. In shade conditions, very low carbohydrate content reserves throughout the plant growth; consequently, natural shade following from a dense leaf canopy should be minimized in rose production.

**Literature Cited**


| Table 4. Correlation coefficients of the measured traits within different shading treatments. |
|---|---|---|---|---|---|---|
| Property | Flower stem length | Flower stem diameter | Fresh weight | Dry weight | Leaf area | Specific leaf area |
| Flower stem length | 1 | | | | | |
| Flower stem diameter | 0.285 | 1 | | | | |
| Fresh weight | 0.214 | 0.696** | 1 | | | |
| Dry weight | 0.196 | 0.719** | 0.979** | 1 | | |
| Leaf area | 0.575* | .000 | 0.308 | 0.297 | 1 | |
| Specific leaf area | 0.269 | 0.771** | 0.648** | 0.705** | 0.028 | 1 |

*: In each row or column, indicates significant correlation between variable at the 1% level


The Effect of Gibberellic Acid and Stratification on Germination of Alstroemeria (*Alstroemeria ligtu* hybrid) Seed Under *In Vitro* and *In Vivo* Conditions

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The dormancy characteristics and optimum conditions for seed germination of *Alstroemeria ligtu* had not been explained. *In vitro* and *in vivo* alstroemeria (*A. ligtu* hybrid) seed germination tests were conducted in a Randomized Completely Design at two different treatments (gibberellic acid (GA₃) 0, 100, 200 and 400 mg/l with and without stratification in 5±1°C) in four replications. Seeds were planted in the soil mixture (peat/sand/perlite 1:1:1) or 1/2 MS media (1% sucrose, 0.7% agar and pH to 5.8). After 3-weeks keeping in the stratification conditions, transferred to the growth chamber (21°C and 16h photoperiod). Shoot and root length, number of root and leaf, root and shoot fresh and dry weight, seed germination percentage, germination rate and mean germination time were recorded during experiment. Stratification had a significant effect on seed germination (*p*<0.05). Soaking for 24 h in 100 mg/l GA₃ supplemented with stratification under *in vitro* and *in vivo* conditions increased germination up to 76.67% and 70.00%, respectively. Mean germination time (MGT) decreased with duration of stratification and concentration of GA₃. Seeds treated with 100 mg/l GA₃ plus 21 days of stratification produced the seedlings with the higher number of leaf, length of shoot, shoot and root dry weight in both *in vivo* and *in vitro* conditions. Non-stratified seeds without GA₃ application fail to germinate, whereas seeds chilled for 21 days had 36.6%, 40.0% germination under *in vivo* and *in vitro*, respectively. Stratification was successful in breaking seed dormancy; stratification at 5±1°C for 21 days or 100 mg/l GA₃+21 days of stratification overcame seed dormancy and increased the germination percentage of *A.ligtu* hybrid seeds. Thus, seeds of *A.ligtu* hybrid species probably exhibit a combination of physiological dormancy. In general, *In vivo* germination rates were lower than *in vitro* rates.

**Keywords:** *Alstroemeria*, Physiological dormancy, Pre-germination treatments.
INTRODUCTION

The genus *Alstroemeria* L. (Alstroemeriaceae) has been distributed in South America with two main centers; one Chile and the second throughout the eastern of Brazil and contiguous Paraguay and Argentina (Bayer, 1987; Aker and Healy, 1990). *Alstroemeria* is one of the important commercial cut flowers throughout the world (Gonzalez-Benito and Alderson, 1992). All species are herbaceous, perennial and rhizomatous plants with big flowers, living in a wide range of habitats from rainy forest to desert areas and from the mountains to the coast (Munoz and Moreira, 2003). This plant is planted in greenhouse for cut flower production and is propagated vegetative by rhizome division. Seed propagation is uncommon due to variability in the germination percent and the time required for the germination, may be caused by viability of seeds, seed dormancy or improper techniques (King and Bridgen, 1990). The dormancy characteristics and optimum conditions for seed germination of this species had not been explained. Thus, some information about effective factors on dormancy breaking and optimal conditions of seedling growth is necessary for recovery of seed germination in this plant. The erratic and unpredictable nature of *Alstroemeria* germination is undesirable for commercial growers who tend to higher and more synchronous germination. Also, determination of optimum germination conditions could aid breeding efforts and hybrid seed distribution.

Seed dormancy is a block to the completion of germination of an intact viable seed under favorable conditions (Hilhorst, 1995; Bewley, 1997). This block to germination has evolved differently across species through adaptation to the prevailing environment, so that germination occurs when conditions for establishing a new plant generation are likely to be suitable (Hilhorst, 1995; Bewley, 1997; Baskin and Baskin, 2004). Dormancy is an innate seed property that defines the environmental conditions which seed is able to germinate. It is determined by genetics with a substantial environmental influence which is mediated, at least in part, by the plant hormones such as abscisic acid and gibberellins (GAs) (Finch-Savage and Leubner-Metzger, 2006). Two major forms of physiological seed dormancy have been described, namely embryo and coat dormancy (Kucera et al., 2005). Physiological dormancy is the most abundant form and found in seeds of gymnosperms and all major angiosperm (Finch-Savage and Leubner-Metzger, 2006). Physiological dormancy can be divided in to three levels: deep, intermediate and slight dormancy (Baskin and Baskin, 2004). Genotypic and physical constraints, morphologically immature embryos, and may be physiological inhibitors in the seed coats appear to cause a combined dormancy in alstroemeria seeds (King and Bridgen, 1990).

In physiological dormant seed, it is thought that temperature and GAs can both release dormancy and promote germination (Kucerna et al., 2005; Baskin and Baskin, 2004). GAs plays a key role in dormancy release and promotion of germination (Kucerna et al., 2005; Cetinbas and Koyuncu, 2006). Gibberellic acid (GA$_3$) is widely used to break dormancy of seeds of various plant species. Dormant seeds which require stratification, dry storage after ripening and light as a germination stimulator, are often treated with GA$_3$ to overcome their dormancy (Gupta, 2003). Increased germination of alstroemeria seeds with a warm-cold treatment suggests that there are physiological factors in the seed coat of this species that are responsive to cold stratification or that time is required for softening of the seed coat (King and Bridgen, 1990). The embryo of many seeds fails to germinate because oxygen dose not diffuse through the seed coat. At low temperature more oxygen dissolves in water and therefore more oxygen is prepared for embryo (Young and Young, 1992). Dormancy and germination are complex phenomena that are controlled by both developmental and environmental factors (Bewley, 1997; Koornneef et al., 2002). When seeds released from dormancy, the receptors then initiate a signal transduction cascade, perhaps involving synthesis of or sensitization to germination-promoting GAs that lead to the completion of germination (Bewley, 1997). Imbibition stimulates GA secretion from embryo, secreted GA increases synthesis of hydrolytic enzymes located under aleuron layer. Synthesized enzymes are transported to endosperm.
via scutulum and are used for decomposing of stored food to supply the energy required for germination (Cirak et al., 2004).

The aim of the present study was to find a practical method to promote *A. ligtu* hybrid seed germination and dormancy breaking by means of stratification and GA$_3$ application. Therefore, we examine the effect of some treatments on *A. ligtu* hybrid seed germination.

**MATERIALS AND METHODS**

This investigation was carried out in the Department of Horticultural Science, Agriculture Faculty, university of Zanjan, Iran. Seeds of *Alstromeria ligtu* hybrid were immediately washed with tap water and then divided to four groups (each group was divided to four replicates) and subjected to one of the following treatment: 1. soaking in tap water only for 24 h (control), 2. soaking in water for 24 h and then stratified at 5±1°C up to three weeks, 3. soaking in a GA$_3$ solution at 0, 100, 200 and 400 mg/l for 24 h supplemented with stratification at 5±1°C up to three weeks and 4. soaking in a GA$_3$ solution at 0, 100, 200 and 400 mg/l for 24 h without stratification. Seeds were sterilized by 70% ethanol (1 min), 3% sodium hypochlorite solution (20 min) either after soaking in water or GA$_3$ solutions and then rinsed with sterilized water (10 min each). Treatments were held at growth chamber (21°C, 16 h light) after sowing in either soil or MS media. For stratification treatments seed held at growth chamber (21°C, 16 h light) for one week before apply chilling.

The seeds were sow directly in the soil mixture (peat/sand/perlite 1:1:1) at a depth of approximately 0.5-0.7 cm in pot. Irrigation was done every 3 days. Each pot was containing 10 seeds. Germination of seeds was recorded at daily interval. After three-weeks keeping seeds in the stratification conditions, transferred to the growth chamber, and cultures were placed under 21°C and 16h photoperiod.

For in vitro study the seeds were incubated in 250 ml jars containing half strength MS medium (Murashige and Skoog, 1962), supplemented with 1% sucrose and 0.7% agar, and pH was adjusted to 5.8. Each jar was containing 10 seeds.

The progress of seed germination was recorded daily for a period of 30 days after treatment application. Radicle length of 2 mm was scored as germinated seed (Kaya et al., 2006). Mean Germination Time (MGT) was calculated to assess the rate of Germination (Ellis and Roberts, 1981). Shoot and root length, number of root and leaf, root and shoot fresh and dry weight, seed germination percentage, germination rate and mean germination time were recorded during experiment. The oven-dried weight was obtained by drying seedlings at 70°C to reach constant weight.

Experiment was randomized completely design with 4 replications. The statistical analysis was made using the ANOVA procedure of SAS. The difference between the means was compared using the Duncan’s multiple test (p < 0.05).

**RESULTS**

Stratification had a significant effect on seed germination of *A. ligtu* hybrid (p < 0.05). Seed germination percentage in stratified seeds without GA$_3$ treatments was 36.67 and 40% in vivo and in vitro conditions, respectively. Application of GA$_3$ (supplemented with or without stratification) affected total germination in these experiments (Table 1). Seeds treated with 100 mg/l GA$_3$ without stratification gave 13.33% and 16.67% germination, whereas seeds treated with 100 mg/l GA$_3$ + 21 day stratification gave 70% and 76.66% germination in vivo and in vitro conditions respectively. The germination percentage in half strength MS medium was higher than in vivo rates (Table 1). The difference between in vivo and in vitro seed germination under stratification was 5.02%. Therefore, the application of GA$_3$ (100 mg/l) resulted in higher germination percentage and rate, shoot and root fresh weight, shoot and root dry weight than those of seeds treated with 200 and 400 mg/l GA$_3$.

MGT decreased with duration of stratification and concentration of GA$_3$ (Fig. 1). MGT in
in vivo and in vitro conditions were reduced to 16.44 and 15.05 days by 100 mg/l GA3 + stratification treatment. In the other hand seeds treated with GA3, germinated after 51, 49 and 45 days at 100, 200 and 400 mg/l GA3 in vitro conditions (Fig. 1). These results showed that stratification treatment was more benefit for A. ligtu hybrid seed germination.

GA3 treatments increased germination percentage and rate in compare to those did not treated with GA3. In the current study, seed germination rate was depended to GA3 concentration. In higher concentration (200 and 400 mg/l) germination rate decreased. Further, MGT decreased in stratified compared to non-chilled treatments.

Stratification alone or in combination with GA3 improved seedling characteristics including seedling length, root dry weight, shoot dry weight significantly and has a larger effects than the GA3 treatments applied (Table 1). The highest root number was observed in 100 mg/l GA3 + stratification treatment (Fig. 2). Although all GA3 treatments had positive effects on seedlings length either in vivo or in vitro condition, but 100 mg/l was better than the other concentrations. Therefore, maximum seedling fresh and dry weights (roots and shoots) were recorded from seed treatment with 100 mg/l GA3 (Table 1).

Table 1. Effect of gibberellic acid (GA3) and stratification treatments on germination parameters in Alstroemeria ligtu hybrid seeds.

<table>
<thead>
<tr>
<th>Culture Type</th>
<th>Treatments</th>
<th>G.P (%)</th>
<th>G.R (per day)</th>
<th>No. of Leaves</th>
<th>Shoot Length (cm)</th>
<th>Root Length (cm)</th>
<th>F.W.S (mg)</th>
<th>F.W.R (mg)</th>
<th>D.W.S (mg)</th>
<th>D.W.R (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo</td>
<td>0</td>
<td>36.66c</td>
<td>0.17g</td>
<td>2.47 cde</td>
<td>3.81d</td>
<td>2.91d</td>
<td>82d</td>
<td>34cd</td>
<td>6.43cd</td>
<td>3.4ed</td>
</tr>
<tr>
<td>In vivo</td>
<td>0</td>
<td>0.0e</td>
<td>0m</td>
<td>0.1</td>
<td>0i</td>
<td>0h</td>
<td>0i</td>
<td>0i</td>
<td>0i</td>
<td>0i</td>
</tr>
<tr>
<td>In vitro</td>
<td>0</td>
<td>40c</td>
<td>0.17g</td>
<td>1.55jhi</td>
<td>2.32e</td>
<td>0.65g</td>
<td>27fg</td>
<td>14.67f</td>
<td>3.39efg</td>
<td>1.6ef</td>
</tr>
<tr>
<td>In vitro</td>
<td>0</td>
<td>0.0e</td>
<td>0m</td>
<td>0.1</td>
<td>0i</td>
<td>0h</td>
<td>0i</td>
<td>0i</td>
<td>0i</td>
<td>0i</td>
</tr>
<tr>
<td>In vivo</td>
<td>100</td>
<td>70ab</td>
<td>0.31e</td>
<td>5.04a</td>
<td>8.89a</td>
<td>5.17a</td>
<td>339.3a</td>
<td>63.5a</td>
<td>17.77a</td>
<td>8.8a</td>
</tr>
<tr>
<td>In vitro</td>
<td>100</td>
<td>13.33d</td>
<td>0.025k</td>
<td>1.97defgh</td>
<td>1.85efg</td>
<td>0.95fg</td>
<td>28fg</td>
<td>9.16fh</td>
<td>1.4hi</td>
<td>1.16ghi</td>
</tr>
<tr>
<td>In vitro</td>
<td>100</td>
<td>76.67a</td>
<td>0.37a</td>
<td>3.34b</td>
<td>5.5c</td>
<td>40.3c</td>
<td>71de</td>
<td>31.67d</td>
<td>6.3cd</td>
<td>3.4ed</td>
</tr>
<tr>
<td>In vitro</td>
<td>100</td>
<td>16.67d</td>
<td>0.027k</td>
<td>0.97i</td>
<td>0.91h</td>
<td>0.57g</td>
<td>9.2g</td>
<td>5.6hi</td>
<td>0.45i</td>
<td>0.52hi</td>
</tr>
<tr>
<td>In vitro</td>
<td>200</td>
<td>67b</td>
<td>0.35c</td>
<td>2.71bcd</td>
<td>7.97b</td>
<td>4.81ab</td>
<td>237b</td>
<td>42.67d</td>
<td>13.8b</td>
<td>4.57b</td>
</tr>
<tr>
<td>In vitro</td>
<td>200</td>
<td>10d</td>
<td>0.019l</td>
<td>1.82efgh</td>
<td>1.97ef</td>
<td>1.2ef</td>
<td>27fg</td>
<td>11.5g</td>
<td>1.9ghi</td>
<td>1.4efg</td>
</tr>
<tr>
<td>In vitro</td>
<td>200</td>
<td>73ab</td>
<td>0.36b</td>
<td>2.30cdef</td>
<td>4.96c</td>
<td>4c</td>
<td>58.67de</td>
<td>38bc</td>
<td>4.87de</td>
<td>2.97d</td>
</tr>
<tr>
<td>In vitro</td>
<td>200</td>
<td>15d</td>
<td>0.034j</td>
<td>1.22h</td>
<td>1.1gh</td>
<td>0.91fg</td>
<td>7.1g</td>
<td>6.9gh</td>
<td>0.41i</td>
<td>0.58hi</td>
</tr>
<tr>
<td>In vitro</td>
<td>400</td>
<td>66.67b</td>
<td>0.30f</td>
<td>3bc</td>
<td>5.6c</td>
<td>4.61b</td>
<td>152c</td>
<td>33cd</td>
<td>8.03c</td>
<td>4bc</td>
</tr>
<tr>
<td>In vitro</td>
<td>400</td>
<td>16.67d</td>
<td>0.032j</td>
<td>2.1defg</td>
<td>2.1e</td>
<td>1.4e</td>
<td>32fg</td>
<td>14f</td>
<td>2.5fg</td>
<td>1.6ef</td>
</tr>
<tr>
<td>In vitro</td>
<td>400</td>
<td>70ab</td>
<td>0.32d</td>
<td>2.41cede</td>
<td>3.97d</td>
<td>3.86c</td>
<td>51ef</td>
<td>21e</td>
<td>3.9ef</td>
<td>2.1e</td>
</tr>
<tr>
<td>In vitro</td>
<td>400</td>
<td>17d</td>
<td>0.044i</td>
<td>1.33gh</td>
<td>1.36gh</td>
<td>0.86fg</td>
<td>11g</td>
<td>5.3ghi</td>
<td>0.66hi</td>
<td>0.62hi</td>
</tr>
</tbody>
</table>

Means in a column followed by the same letter are not significantly different at the 5% level as determined by Duncan’s.

*: with stratification - : without stratification.

DISCUSSION

Stratification at 5±1°C for 21 days or in combination with 100 mg/l GA3 overcame seed dormancy and increased the germination percentage of *A. liglu* hybrid seeds. This results showed that these treatments were effective in inducing metabolic activity in the embryo required for the initiation of germination process (Al-Menaie *et al.*, 2007). Releasing dormancy can also be associated with an increasing gibberellin biosynthesis during stratification (Yamauchi *et al.*, 2004). The germination percentage higher than *in vivo* rates could have been due to the effect of various elements used in the medium. In vitro germination condition is a nutrient medium containing macro and micro elements and sucrose (1/2MS) that had a positive effect on *A. liglu* hybrid seeds germination. Control treatment had no germination which indicating a high level of dormancy. In current study, seeds were able to absorb water, which indicates no physical dormancy as postulated by Willan, 1987 and Schmidt, 2000 for other plant seeds. Results showed that stratification was successful in breaking seed dormancy. Raisi *et al.* (2013) investigated dormancy break of *Ferula assa-foetida* seed and demonstrated that one period of stratification treatment could increase germination of the seed. GA3 is effective in breaking the slight physiological dormancy, but it does not overcome the deep physiological dormancy (Baskin and Baskin, 1990). Application of GA3 during and after stratification on *Pistachio* seed increased the length, shoot diameter, internodes length, leaf area and fresh and dry weight of seedlings (Rahemi and Baninasab, 2000). It has been reported that germination and dormancy breaking can be induced by GA3 in many plant species, e.g., *Trichocereus terscheckii* (Baes and Rojas-Arechiga, 2007), *Rubia inctorum* L. (Sadeghi *et al.*, 2009), *Pedicularis olympica* (Kirmizi *et al.*, 2010), *Amaranthus retroflexus* L. (Ke, pczyn´ski and Szniñir, 2013) and *Aclypha indica* L. (Gupta and Bandopadhya, 2013). According to the results found in this study, *A. liglu* hybrid species probably exhibits a combination of physiological dormancy. Improvement of germination percentage by GA3 could indicate the presence of chemical dormancy as well, as application of gibberellic acid has shown effect on overcoming dormancy caused by inhibitors (Bewley and Black, 1994). Physiological dormancy in seeds is dependent on the ratio and the levels of abscisic acid (a growth inhibitor) and GA (a growth stimulator) (Hilhorst and Karssen, 1992). GAs are known to obviate the requirement of seeds for various environmental cues, promote germination, and counteract the inhibitory effects of ABA, frequently in combination with cytokines (Bewley and Black, 1994). Giba *et al.* (1993) reported that the inhibitory effect of retardants was overcome by GA3. Stratification might act simply to lower the rate of enzymatic reactions taking place in the seed, and might cause differential changes in enzyme concentrations or in enzyme production (Bewley and Black, 1994).

Incidence of abnormal seedling growth observed in seeds treated with only GA3 treatment. It is suggested that the onset of embryo dormancy is associated with accumulation of growth inhibitors and breaking of dormancy with a shift in the balance of growth regulators towards growth promoters to overcome the effect of inhibitors (Khan, 1971).

Seeds of treated with 100 mg/l GA3 + 21 days of stratification produced the seedlings with yielded higher number of leaf, length of shoot, shoot and root dry weight as compared with other treatments. Similar results were observed by Mostafa and Abou-Alhamd *et al.* (2011) and Dhupper (2013) where they found that application of GA3 showed remarkable increase in the number of leaves, length of shoots and dry weight of seedlings. da Silva Vieira *et al.* (2010) reported that the increase in height of the plant can be attributed to auxin, since it can cause the synthesis of gibberellins and thereby induce cell elongations. The increase in the dry weight of seedling due to treatment with GA3 might be attributed to increase in cell elongation, cell division and accumulation of building units that accompanied by greater sacharids content than those of untread plants (Akhtar *et al.*, 2008; Abdel- Latef *et al.*, 2009).}

These findings, except for the scarce response to GA3, firmly support the hypothesis that
A.ligtu hybrid seeds fit the characteristics a non-deep physiological dormancy according to the dormancy classification of Baskin and Baskin (2004). Further, physiological barriers to germination in embryos have been overcome by cold stratification in a number of rose species (Zhou et al., 2009). Results obtained in this study present strong evidence that the pericarp, the testa, and the embryo play important roles in regulating seed dormancy. The negative effect of the testa on germination can be attributed to some inhibitory substances in the testa and not to its role as a mechanical barrier or in restricting access to water (Bo et al., 1995). King and Bridgen (1990) reported that may be physiological inhibitors in the seed coats appear to cause a combined dormancy in Alstroemeria seeds. El-Refaey and El-Dengawy (2005), shown that stratification of seeds at 4-5°C or treatment of seeds with GA3 was successfully overcome dormancy in Eriobotrya japonica seeds. Cold stratification increased the germination percentage and rate of A.ligtu hybrid, as generally is known for a number of other species (Bewley and Black, 1994).

CONCLUSION

The current study demonstrated that the A.ligtu hybrid seeds were in a dormant state, which suggests that stratification at 5±1°C for 21 days or 100 mg/l GA3 plus 21 days of stratification overcame seed dormancy and increased the germination percentage of A.ligtu hybrid seeds.

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of gibberellin biosynthesis and response pathways by low temperature during imbibition of A. thaliana seeds. Plant Cell. 16: 367–378


An Efficient and Cost Effective Protocol for In Vitro Propagation of Pineapple

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Abstract

An efficient and cost effective protocol for in vitro propagation of Pineapple (Ananas comosus var. Queen) has been developed. In the proliferation stage, agar based Murashige and Skoog (MS) media was supplemented with 3.0 mg/l benzyleaminopurine (BAP), 0.5 mg/l indole acetic acid (IAA) and 50 mg/l adenine sulphate as RBC design experiment. Two approaches were taken to reduce the chemical cost of micropropagation media. Analytical grade sucrose was successfully replaced by commercial sugar, completely during proliferation stage and up to 66% during rooting stage. Again during the rooting stage, agar based solid media was replaced by liquid media (MS-media). Bio-degradable Coir and Luffa were used as supporting matrix. As supporting matrix in rooting media, Luffa was found to be more effective. The clonal fidelity of in vitro raised plantlets was confirmed by RAPD technique.

Keywords: Coir, Commercial sugar, Luffa, Micropropagation, Pineapple (Ananas comosus).
INTRODUCTION

Pineapple is one of the major economically important fruit crop in tropical zone. Comparing its annual world production which exceeds 15 billion kg per year, India produces nearly 1.3 billion kg per year (Economic Research Service, United State Department of Agriculture, 2012). But the seeds of these plants are very slow to germinate and therefore are not used for commercial purposes. For vegetative propagation of the plants, crowns and slips have been successfully used over years (United State Department of Agriculture, 2013). In case of industrial scale production suckers and heaps are also used (Firoozabady and Gutterson, 2003).

The tissue culture method of pineapple comes into the play to increase the selectivity of the desired traits coupled with a high multiplication rate. Following the standard tissue culture method (Mathews and Rangan, 1979; Zepeda and Segawa, 1981; Fitchet, 1990; Fitchet-Purnell, 1993; Kiss et al., 1995; Gangopadhyay et al., 2005) much higher rate of multiplication (40 to 85 fold in a 13 month period) was obtained. But the major requirement for an economical crop to remain industrially viable is that it should have a considerably low production cost; the condition which is not supported by the current tissue culture method of pineapple. Hence there has been a continuous effort among researchers to decrease the production cost of culture. But even a 3000-4000 fold multiplication in a 6 month period in vitro method, both solid and liquid culture approach (Escalona et al., 1999; Firoozabady and Gutterson, 2003) did not provide a considerable cost reduction when compared to field-propagation method.

The present investigation involves approaches to reduce the cost of pineapple micropropagation by replacing some of the costly components by readily available and much cheaper substitutes. Two attempts had been taken. Sucrose, a major and yet costly ingredient of the media was attempted to be replaced by commercial sugar. Again liquid media is commercially cheaper than solid media as it eliminates the cost of agar, though the major problem there is vitrification (Bhojwani and Razdan, 2005). In earlier attempts, enhanced rate of multiplication and rooting were obtained through the use of Coir and Luffa sponge in Gladiolus and Philodendron respectively (Roy et al., 2006; Gangopadhyay et al., 2004). In the current project both ‘Luffa’ and ‘Coir’ had been tested as supporting bases in liquid media during rooting stage. Luffa was found to be more suitable as supporting matrix in this approach. Commercial sugar may contain some toxic material that can cause some genetic (somaclonal) variation. Then RAPD was performed to test the clonal fidelity of the tissue culture raised plantlets.

MATERIALS AND METHODS

Plant material

Aseptic culture of the ‘Queen’ variety of pineapple, originally procured from Manipur, was collected from the experimental garden, Department of Agricultural and Food Engineering, Indian Institute of Technology, Kharagpur. This particular variety of pineapples is small in size, but has its unique taste and flavor.

Aseptic techniques

The shoot apical meristems were first washed in tap water for at least 15 minutes followed by stirring with 20% Bavistin (20 minutes). After that, the explants were disinfected with Tween 20 and 0.5% Sodium hypochlorite solution (15 minutes) followed by five times washing in CA water (solution of 0.25% citric acid and 0.5% ascorbic acid).

Culture media and growth condition

The sterilized shoot apical meristems were established in MS media (Murashige and Skoog, 1962) with BAP (6-benzylaminopurine) 3 mg l⁻¹, IAA (indole acetic acid) 0.5 mg/l and adenine sulphate 50 mg/l supplementation (Datta et al., paper communicated). The competency of sucrose supplementation...
by commercial sugar (at 16.67% (PA-0.5), 33% (PA-1.0), 50% (PA-1.5), 66% (PA-2.0) and 100% (PA-3.0)) in solid media was examined (Table1). The media was gelled with 0.8% agar and emerging of multiple shoots from the cut ends was observed within one month. The shoot buds from the matured plantlets were separated and placed in the same media for four more subcultures.

In rooting media, MS composition along with kinetin, 0.5 mg/l and IBA (indole butyric acid) and 2 mg/l were used (Gangopadhyay et al., 2002). The plantlets were subsequently placed in culture tubes containing liquid media with sterile coir as supporting matrix or in culture jars containing either liquid media with sterile luffa or solid agar media. The effectiveness of sucrose supplementation by commercial sugar (at percentages, 16.67%, 33%, 50%, 66%, 100%) in rooting media (both solid and liquid), was tested (Fig. 1A, Table 1). After the rooting stage, the plantlets were transferred to soilrite for hardening.

RAPD

DNA was extracted from the explants by CTAB method (Rogers and Bendich, 1998). To amplify the obtained amount, PCR was done by adjusting the DNA concentration to 25µg ml^{-1}. The method of Williams et al. (1990) was followed using decamer primers, OPA 01- OPA 05 and OPB 01- OPB 05 (Operon Tech., Alameda, USA). The reaction mixture consisted of 1X buffer, 0.2 mM dATP, dTTP, dCTP, dGTP, 2 mM MgCl2, 0.2µm primer, 100ng of template DNA and 1 unit of Taq DNA polymerase (Roche). A thermal cycler (Eppendorf, Germany) was used for the amplification of DNA with an initial denaturation temperature of 94º C for two minutes. The reaction continued for 45 cycles, the temperature profile for each of the cycle was- denaturation at 94º C for one minute, annealing at 35º C for one minute and extension at 72º C for two minutes. The reaction was followed by five minutes hold at 72º C for ensuring complete extension of the primers.

Statistical analysis

RBD-ANOVA test was done in order to check whether any significant difference was present in the number of multiple shoots in solid media or in root lengths of the plantlets maintained in agar, coir and luffa medium. Test of significance was carried out by ‘Student’s t-test’ method (Datta, 2006).

RESULTS AND DISCUSSION

Reduction of the cost of proliferation media

The proliferated plantlets responded readily in culture medium and a considerable number of adventitious shoots emerged from each of the responding explants. In the propagation media 100% supplementation of sucrose by commercial sugar was found to be successful. The multiplication rate was almost similar in both supplemented media and control. No significant difference in proliferation rate was observed between the control and treatment (RBD-ANOVA Test, at level

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Culture Media</th>
<th>Sucrose concentration in media (%)</th>
<th>Sugar supplementation (%)</th>
<th>Commercial sugar concentration in media (%)</th>
<th>Cost reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PA-0</td>
<td>3.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>PA-0.5</td>
<td>2.50</td>
<td>16.67</td>
<td>0.50</td>
<td>2.34</td>
</tr>
<tr>
<td>3</td>
<td>PA-1.0</td>
<td>2.00</td>
<td>33.00</td>
<td>1.00</td>
<td>4.69</td>
</tr>
<tr>
<td>4</td>
<td>PA-1.5</td>
<td>1.50</td>
<td>50.00</td>
<td>1.50</td>
<td>7.03</td>
</tr>
<tr>
<td>5</td>
<td>PA-2.0</td>
<td>1.00</td>
<td>66.00</td>
<td>2.00</td>
<td>10.41</td>
</tr>
<tr>
<td>6</td>
<td>PA-3.0</td>
<td>0.00</td>
<td>100.00</td>
<td>3.00</td>
<td>15.12</td>
</tr>
</tbody>
</table>

#Control
Reduction of chemical cost was achieved by 15% (Table 1), giving its immense importance from industrial point of view.

Reduction of the cost of rooting media
Sing alternative matrix in liquid media
During rooting stage, maximum root lengths were observed in liquid media (no sucrose supplementation) using Luffa as supporting matrix, followed by coir and agar (Table 2). Cost reduction was maximum in coir matrix (62.83%), followed by luffa (52.5%) with respect to conventional agar media (Figure 1B).

Liquid media has many advantages over solid media such as efficient nutrient uptake, lower cost and dilution of excreted material (Smith and Spoomer, 1995; Aitcken-Christie et al., 1995). When the liquid media is supported by solid, biodegradable, fibrous matrices, the nutrients can diffuse easily through it and vitrification can be prevented (Gangopadhyay et al., 2002). In the present investigation, symptoms of vitrification were not observed.

RBD-ANOVA test revealed significant difference among the root lengths of the explants in three different supporting matrices (at p=0.001, calculated value of ‘f’(13.79) is greater than its Table value (~8.56)). The value of the treatment mean showed that the mean root length of agar media was lowest (1.045 cm) and media with luffa was highest (2.423 cm). Also, the fact that the observed ‘t’ value (3.2933) was higher than its Table value (at p= 0.01, ttable =2.82 ) indicated that the difference in root length between the highest and the lowest was highly significant.

Replacing sucrose with commercial sugar
When replacing sucrose with commercial sugar in rooting media significant root lengths were observed up to 66% supplementation (again better results were observed with Luffa). Reduction of cost due to the usage of Luffa matrix and 66% sucrose supplementation and is 61.92% (Fig. 1B).

From ANOVA test it was confirmed that supplementation of sucrose by commercial sugar is possible up to 66%, as no significant difference (at level p=0.001) results between the mean root lengths of control and treatment. But with 100% supplementation, there was significant difference (at level p=0.001) between the treatment and the control.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Culture Media</th>
<th>Agar (mean root length) ± SE</th>
<th>Luffa (mean root length) ± SE</th>
<th>Coir (mean root length) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>#PA-0</td>
<td>1.045±0.125</td>
<td>2.423±0.265</td>
<td>1.360±0.161</td>
</tr>
<tr>
<td>2</td>
<td>PA-0.5</td>
<td>1.045±0.188</td>
<td>2.407±0.394</td>
<td>1.346±0.154</td>
</tr>
<tr>
<td>3</td>
<td>PA-1.0</td>
<td>1.042±0.093</td>
<td>2.400±0.261</td>
<td>1.290±0.176</td>
</tr>
<tr>
<td>4</td>
<td>PA-1.5</td>
<td>1.037±0.576</td>
<td>2.250±0.434</td>
<td>1.190±0.137</td>
</tr>
<tr>
<td>5</td>
<td>PA-2.0</td>
<td>1.020±0.112</td>
<td>2.200±0.356</td>
<td>1.090±0.108</td>
</tr>
<tr>
<td>6</td>
<td>PA-3.0</td>
<td>0.700±0.157</td>
<td>2.000±0.329</td>
<td>0.900±0.151</td>
</tr>
</tbody>
</table>

Fig. 1. A) Rooted plants grown on different matrices for rooting, just after transplantation. B) Relative cost reduction by sucrose supplementation (at different percentages) using different matrices in liquid media. C) Transplanted plants into the soilrite during hardening.
Clonal fidelity and hardening

Clonal fidelity is one of the most important factors that should be tested before commercialization. To test the clonal fidelity of micropropagated plant, RAPD was done taking randomly selected in vitro raised plantlets. Ten RAPD primers were used in this experiment (In vitro raised plantlets were selected randomly). The result of the respective profile of ten samples (Fig. 2) is being presented because of the identical profiles obtained in all the samples tested with each primer.

After adequate rooting, the in vitro grown plants were transplanted into the soilrite in a humidity tent for hardening (Figure 1C). The plants grown in Coir or Luffa were successfully transplanted in pots with 99% survivality over 78% successful survival rate of the plants grown in agar-gelled medium. It was found to be easier to take out the rooted plants from coir and luffa without damaging the plantlets.

CONCLUSION

Conventionally agar and sucrose is used in multiplication and rooting stages. The result of the present investigation clearly indicates that 100% sucrose supplementation during proliferation reduces the chemical cost by 15.12% without causing any significant difference among the number of multiple shoots. During rooting no significant difference was observed up to 66% sucrose supplementation, and liquid media can be used with Luffa as supporting matrix, which reduced the chemical cost by 61.92%. Thus present experiment ensure almost 42.5% of total chemical cost reduction and an efficient micropropagation protocol (Scheme: 1) of pineapple for ready and commercial use.

![Scheme 1: Presentation of the established cost-effective protocol of pineapple micropropagation.](image)
ACKNOWLEDGEMENTS

We thank Prof. B. C. Ghosh, IIT Kharagpur for providing the plant material for our project.

Literature Cited


United State Department of Agriculture. 2013. www.uga.edu/fruit/pineapple.htm
Comparative Evaluation of Growth, Yield and Quality Characteristics of Various Gerbera (Gerbera jamesonii L.) Cultivars under Protected Condition

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Ten gerbera cultivars (‘Labinel’, ‘Lilla’, ‘Alp’, ‘Alberino’, ‘Bonnie’, ‘Avemaria’, ‘Mammut’, ‘Lexus’, ‘Terramixa’ & ‘Sarolta’) were evaluated for their growth, yield and quality characteristics under protected conditions during 2011. Among the cultivars studied, there were highly significant variations observed for growth, yield and quality parameters. Longest stalk length (60.3 cm) was exhibited by the cultivar ‘Alberino’ followed by ‘Lexus’ (59.0) and ‘Mammut’ (54.0 cm). The same cultivar also produced flowers with maximum diameter. With respect to vegetative parameters like number of leaves per plant and plant spread were also more in the same cultivar. Maximum number of flowers 135 per square meters was recorded in cv. ‘Avemaria’ (135) followed by ‘Alberino’ (125). Maximum vase life was recorded in cultivars ‘Alberino’ and ‘Lexus’ (6.6) followed by ‘Mammut’ (5.6) and “Sarolta” (5.6). Excellent quality flowers were observed in cultivar ‘Alberino’ (4.8) followed by ‘Lexus’ (4.4). Cultivar ‘Alberino’ and ‘Lexus’ were found superior with respect to growth, yield and vase-life characteristics under protected conditions.

Keywords: Cultivars, Gerbera, Growth, Protected conditions, Vase life.
INTRODUCTION

Gerbera (Gerbera jamesonii L.) also commonly known as Transvaal Daisy is an important cut flower grown throughout the world (Pattanashetti et al., 2012) scattered from Africa to Madagascar (Khosa et al., 2011) into tropical Asia and South America (Tjia and Joiner, 1984). Variety in color has made this flowering plant attractive for use in garden decorations, such as herbaceous borders, bedding, and pots and for cut flowers as it has a long vase life (Bose et al., 2003; Chung et al., 2005; Chauhan, 2005). It ranks fourth in the international cut flower market and a popular cut flower in Holland, Germany and USA (Choudhary and Prasad, 2000). Modern gerbera arose from Gerbera jamesonii hybridized with Gerbera viridifolia and possibly other species (Leffring, 1973).

It is difficult to get good quality cut flowers of gerbera under open-field conditions. To meet the qualitative and quantitative standards, hybrid cultivars have to be grown under protected conditions (Pattanashetti, 2009). Previously, in a performance study of gerbera varieties, Sankar et al. (2003), Singh and Ramachandran (2002), Singh and Mandhar (2002) and Kandpal et al. (2003) grew gerbera under protected conditions and observed better growth, yield and quality characteristics under protected. In protected conditions, gerbera grows faster and produces larger and greener leaves with high dry matter content. As a result, the yield of the flowers increases and more side shoots will be formed. Protected conditions provide favorable environment for the growth of the plants by protecting the crop from heavy winds, pests, diseases and other climatic conditions (Khan, 1995). Good drainage in the protected house is also essential for gerbera cultivation (Labeke and Dambre, 1999).

The market requirement for cut flowers is very specific and it can be met consistently, only when the crop is grown under protected conditions. In places where the natural weather remain considerably cooler for most parts of the year as in parts of USA, UK and Australia the crop is being grown under fully protected climate in controlled green houses. In places near equator, with warmer sunny climate, semi protected conditions are successfully employed to cultivate the crop. Performance of gerbera varies with the region, season and other growing conditions (Horn et al., 1974).

Gerbera as a cut flower has tremendous demand in domestic and international markets. Though, different cultivars of gerbera exist in Pakistan, none has been officially released till date. Lack of potential variety is one of the main constraints towards its production in Pakistan. Hence, it is needed to evaluate cultivars for their vegetative, yield and quality characters and finally to recommend the suitable variety for the agro-climatic conditions of Punjab, Pakistan. Considering the above facts, the present research work was undertaken to study the performance of different cultivars of gerbera under protected conditions.

MATERIALS AND METHODS


The data on stalk length (cm), number of leaves/plant, plant spread (cm), number of stalks plant-1, flower diameter, number of flowers/m, vase life and quality of flowers were recorded. Flower quality was determined with the help of scale ranging from 1 to 5 very poor, poor, satisfactory, good, excellent respectively (Khosa et al., 2011). A panel of five judges was asked to perform sensory assessment using the above mentioned scale. Stalk length of the flowers was measured from the point of origin of stalk to the point just below the flower head and the average stalk length of flowers was recorded and expressed in centimeter (cm). Number of leaves/plant was recorded
from the tagged plants by counting the number of leaves and average number of leaves produced per plant was worked out. Diameter of flower was recorded at full bloom stage from the flowers harvested at peak flowering. The readings were taken from the tagged plants and average was measured and expressed in centimeters.

Gerbera flowers for vase life evaluation were harvested when all the florets opened fully and were perpendicular to the stalk. The flowers were harvested early in the morning and were immediately placed in fresh water. Later these flower stalks were cut to have uniform stalk length. After that flowers were kept individually in flask containing tap water. Flowers were observed daily till they were found unfit for containing in vase. The vase life was expressed in terms of days from the date of harvesting to final observation.

The climatic data consisting of daily observations of average temperature and relative humidity were also recorded during the whole study period by weather station (VENTUS, W831, Denmark) (Fig. 2).

The physiological characteristics of soil were also determined. The pH value of soil was 7.4 with electrical conductivity 2.0 dS m⁻¹. The organic matter of the soil was 0.9%. Available phosphorus, calcium and potassium contents of the soil were 55, 220 and 120 mg kg⁻¹ of dry soil, respectively. The soil was mixed with NPK as recommended doses to increase production (Dufault et al., 1990). Weeding and irrigation were done when it was necessary.

The data were subjected to analysis of variance (ANOVA) using Genstat (release 31.1; Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK) by using one-way ANOVA. The effects of various treatments were assessed within ANOVA and Fisher’s least significant differences were calculated following a significant (P≤0.05) F test (Steel et al., 1997). All the assumptions of analysis were checked to ensure validity of statistical analysis.

RESULTS AND DISCUSSION

Stalk length

Among the ten cultivars of gerbera, maximum stalk length was recorded in ‘Alberino’ (60.3 cm), followed by ‘Lexus’ (59 cm), ‘Mammut’ (54.0 cm) and ‘Terramixa’ (49.6 cm) while it was minimum in ‘Avenaria’ (40.6 cm), ‘Labinel’ (41.3 cm) and ‘Bonnie’ (43.6 cm) under protected conditions (Table 1). In a performance study of five gerbera cultivars, Sankar et al. (2003) observed almost same stalk length in gerbera cultivar ‘Yanara’ as observed by us in ‘Alberino’. A similar variation in plant height among gerbera cultivars was observed by Reddy et al. (2003). The stalk length is a genetic factor therefore it is expected to vary among the cultivars as earlier observed by Sarkar and Ghimaray (2004). Stalk length is a very important factor for a cut flower, especially for gerbera flower. It decides the quality cut flowers. As there will be more stalk length more reserved food will be stored in the stalk which will later be available to the flower for longer time period.
Number of leaves/plant

There was significant difference among the cultivars of gerbera for number of leaves per plant. With respect to cultivars, maximum number of leaves were recorded in ‘Alberino’ (28.6) followed by ‘Lexus’ (25), ‘Avemaria’ (23.6) and ‘Terramixa’ (21.3) while it was minimum in ‘Sarolta’ (17.6) followed by ‘Alp’ (18.3) and ‘Avemaria’ (19.3) (Table 1). Similar variation of number of leaves/plant of different gerbera cultivars was also reported previously in gerbera by Mahanta and Paswan (2003).

Plant spread

Significant difference was also observed among cultivars for plant spread. With respect to cultivars, maximum plant spread was recorded in ‘Alberino’ (60.0 cm) followed by ‘Lexus’ (55.0 cm), ‘Lilla’ (52 cm) and ‘Avemaria’ (50 cm) while it was least in ‘Terramixa’ (40 cm) followed by ‘Labinel’ and Bonnie which are statistically at par (Table 1). This difference among the cultivars may be due to bigger sized leaves produced by respective cultivars. The results are in accordance with the findings of Singh and Ramachandran (2002) and Thomas et al. (2004).

Flower diameter

Maximum flower diameter was recorded in variety ‘Alberino’ (9.6 cm), followed by ‘Lilla’ and ‘Avemaria’ (9.0 cm and 8.3 cm, respectively) while it was minimum in the variety ‘Sarolta’ (5.3 cm) (Table 2). The size of these flowers may be due to bigger ray florets which are in con-
formity with the findings of Singh and Ramchandran (2002) in gerbera. The bigger diameter of ‘Alberino’ might be due to the inherent characters of individual cultivars. These findings are also in accordance with the results of Gotz (1983), who also reported large differences in the flower diameter of different gerbera cultivars under greenhouse conditions.

**Number of flowers/plant**

Flower yield and its quality parameter decide the significance of the particular variety, which are suitable for commercial cultivation. Number of flower/plant was significantly varied within the cultivars. Maximum number (24.6/plant) of flower was recorded from the variety ‘Alberino’ followed by ‘Lexus’ (22.0), ‘Bonnie’ (20.0) and ‘Sarolta’ (19.0) while minimum (14.3/plant) was recorded from variety ‘Alp’ (Table 2). Maximum number of flowers per plant observed in variety ‘Alberino’ might be attributed to the greater leaf area and more number of leaves per plant as well as plant spread would have resulted in production and accumulation of maximum photosynthesis, resulting the production of more number of flowers with bigger size. The results are in accordance with the findings of Nair and Medhi (2002) in gerbera under protected conditions.

**Number of flowers/m²**

Maximum number of flowers per square meter were recorded in variety ‘Alberino’ (135), followed by ‘Avemaria’ (125), while it was minimum in ‘Terramixa’ (100) (Fig. 1). This appreciably good yield might be due favorable conditions under protected conditions.

**Vase life**

There was significant difference among the cultivars of gerbera regarding vase-life. Maximum vase-life was recorded in cultivars ‘Lexus’ (6.6) and Alberino (6.6), followed by variety ‘Mammut’ (5.6) while it was minimum in variety ‘Terramixa’ (3.0) (Table 2). The vase-life of the cut blooms terminated when the flower heads started drooping, which was followed by discoloration and fall of petals, which represented the end of effective vase-life of cut flowers. Variation in vase life among cultivars may be attributed to variations in their genetical make up. Jong (1985), Anuradha and Narayanagouda (1999) reported the similar results as that of the present investigation. Wankhede and Gajbhiye (2012) observed that among all the thirteen cultivars studied vino has more vase life.

**Flower quality**

Flower quality is an important parameter for evaluation of cut flower quality, for both domestic and export markets. Excellent quality flowers were found in gerbera variety ‘Alberino’ (4.8) followed by ‘Lexus’ (4.4) and ‘Sarolta’ (4.3) (Table 2). Flowers of cultivars ‘Lilla’ and ‘Mammut’ were of inferior quality (3.7). Similar results were also reported by Nair and Shiva (2003), Steinitz (1982) and Awad et al. (1986) in gerbera and zinnia, respectively. Among the eleven cvs. studied, Hemla Naik et al. (2006) observed best flower quality in cultivar ‘Lexus’ which is not in line with our results. Due to better vegetative growth cultivar ‘Alberino’ also has better flower quality.

**CONCLUSIONS**

The above mentioned findings indicated that considering the important characteristics, the ‘Alberino’ is the best variety having large stalk length, more number of leaves, plant spread, and yield per plant, Number of flowers/m², long vase life and better flower quality. While, ‘Lexus’ also exhibited acceptable physical and flowering quality characteristics, so it can also be cultivated under protected conditions. ‘Alp’ and ‘Lilla’ are completely rejected due to low yield and poor flower quality. Hence, ‘Alberino’ and ‘Lexus’ being better physical adaptation, high yield and excellent flower quality can be successfully cultivated under the protected conditions.
Literature Cited


Application Methods of *Thymus vulgaris* Essential Oil and Their Effect on Vase Life and Qualitative Traits of *Gladiolus grandiflorus* L. Cut Flowers

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In this study, combined effect of two essential oils, Thymol and Carvacrol, with different concentrations in two methods on *Gladiolous grandiflorous* L. was investigated based on a completely randomized design with 3 replicates and four flower in each replicate. Sansusi variety applied that was red. In short term method, different concentrations of Thymol and Carvacrol including 25+25, 50+50 and 75+75 ppm with sucrose 6% and distilled water (as control) were evaluated. Red flowers were treated in mentioned solutions for 24 hours and then they were taken to distilled water to the end of evaluation period. In standard method, different concentrations including 12.5+12.5, 25+25 and 37.5+37.5 ppm and sucrose 2% and distilled water as control were used. Flowers were kept in solutions from beginning experiment until the end of vase life. Every 48 hour, once, solutions were made to replace. Preserver solutions can use with wholesalers or retailers to protect flowers in order to sold users. Thymol and carvacrol as the most essential oil of Thymus vulgaris have strong antimicrobial and antioxidant effects. In short term method, combined treatment of thymol and carvacrol with 150 ppm had the most soluble sugar and petal aqueous contents and the least rate of blossoming. In addition, the most vase life in combined treatment of carvacrol and thymol was observed in 100 ppm with mean of 11.57days. Concentration of Anthocyanin was the most in short-term method in compared to standard in last days of experiment.

**Keywords:** Carvacrol, Essential oil, Postharvest, Short term, Standard, Thymol.
INTRODUCTION

Gladiola in scientific name *Gladiolus grandiflorus* belongs to family Iridaceae that is a cut flower and a valuable garden plant. Gladiola in Iran has first order in cultivating area and second order in production rate among cut flowers. Cut flower quality is depends on many factors. All physiological abnormalities, which affect on their appearance, also affect on their market choice and economic value. The most important problem of most decorative plants is flower and leaves senescence. Essential oil is used as a substitute material of synthetic fungicide (Wright and Kader, 1997). Due to antimicrobial and antifungal properties of essential oil, their production by plants effectively increases defense mechanism against pathogens and pests (Aggarwal *et al.*, 2002). Thymol and Carvacrol essences are phenolic complex that have very strong antibacterial and antifungal effects (Yahyazadeh *et al.*, 2008). Essences are hydrophobic to act as catalyst. This property enables essences to inter into wall cell membrane lipids and bacteria mytocondria and destroy their structure and more permeability. After this step occur ions permeation and other cell contents. Although it’s possible such complex permeation from bacteria cells does not cause losing their life power, but losing much cell contents and exiting important molecules and ions will cause bacteria death (Bird, 2004). Essences act mechanism is similar to other phenolic complex due to having phenolic complex such as Thymol and Carvacrol. Microbial bio control factors have high potential to substitute synthetic fungicides for decay after fruit and vegetable cultivating (wright and kader, 1997). Vessel blockage causes solution and water leading decreasing in stems. Lead ability decreasing creates due to stems and vessels cut level blockage (Damunupola *et al.*, 2008). Vessel blockage due to bacteria decreases water absorption and causes stem bend and break and petals decay in Gerbera flower (Solgi, 2009). Bacteria cause woden vessel blockage and therefore decreases roses succulence (Torjesuns) (Van Doorn and Do wite, 1994). The most life of Gerbera flowers observed in Mentha, pulegium, Rosmarinus and Indian grenadine essential oils treatment (Ziaei *et al.*, 2008). That thyme essences especially thymol and carvacrol have fungicide, antioxidant and antiseptic properties (Ozkan *et al.*, 2004). Also according to research in 2007, carvacrol into bags having inoculated grape grains with gray moulds prevented such mould grow (Martinz-Romrou *et al.*, 2007). Different carvacrol densities on Qiwi fruit and Melon decrease bacteria life and delay Qiwi and Melon decay (Roller *et al.*, 2002). Sing in 2002 by thyme essence could stop E.Coli Grow. Regarding to mentioned item, this research was conducted to study the effect of thymol and carvacrol on Gladiola flower qualitative properties.

MATERIALS AND METHODS

Plant materials

Plant cultivation acts from a commercial green house in Varamin conducted a day before transferring to laboratory when flourished one or two buds at early morning and before weather warming and transferred to laboratory in suitable paper package.

Different treatments:

Flowers removed from package after transferring to laboratory the flowers height was 90 cm. In short term method combined treatments of Thymol and Carvacrolin different concentrations of 25+25, 50+50 and 75+75 ppm with sucrose 6% and distilled water as control were evaluated. Flowers were treated in mentioned solutions for 24 hours and after they were taken to distilled water to the end of evaluation period. In standard Method treatments with different concentrations including 12.5+12.5, 25+25 and 37.5+37.5 ppm and sucrose 2% and distilled water as control were used.

Evaluation room environmental condition

Examination place temperature was 20-22°C and rational humidity was almost 75 percent.
Light density was 18.9 mol/m²/s that satisfied by white floscent Light in 12 hour light and 12 hour darkness. In mentioned room, it was evaluated all factors and properties and vase life post harvest.

**Measuring properties**

**Vase life**

Vase life considered on the basis of time distance from harvest to when droopy buds were more than flourished buds.

**Flourishing speed**

To determine flourishing buds percent, it was calculated floret buds and total buds ratio and multiplied 100 and calculated in days 0, 2, 4, 6, 8, 10 and 12.

**Petal’s anthocyanin**

The amount of 0.5 g fresh petal crushed by liquid nitrogen. 10 cc acidic methanol solution (including methanol and clorodric acid 1%) was added to each sample. Samples were centrifuged and read solution absorbption rate by spectrophotometer in wave lengths 657 and 530 nm (Sankhla et al., 2005).

\[
\text{Anthocyanin} = \frac{D_{530}}{0.24} - D_{657}.
\]

**Petal aqueous contents**

To measure this property was used phenol-sulphoricacid method and dextroseas standard. 0.1 g dried samples crushed in pounder. To extract sugar added 10 cc ethanol 70%. Then 1 ml phenol 5% added and 5 ml concentrated sulphoric acid to each sample. Finally read absorbtion rate byspectrophotometerinwave length 485 mm (Stewart,1989).

Examination was investigated on the basis of completely randomized design with 3 replicates and four flower in each replicate. To analysis data statistically used SAS software. Means comparison were done by Duncan multiampplitude test on 5% Level.

**RESULTS**

**Petal anthocyanin:** Results showed that the, interaction effect of method and time was signifcant at 1% Level (Table 1).

Comparing interaction effect of method and time on Petal anthocyanin indicated that on third day flowers in standarad method had anthocyanin more than short term method. On day sixth, anthocyanin of treated flowers in short term method was more than standarad method. On day ninth, decreased in both methods traeetments. Tottaly in short term method, anthocyanin rate was better (Fig.1).

**Petal’s soluble sugar:** The interaction effect of method and treatment on soluble sugar at 1% level and the interaction effect of three factors (method, treatment and time) on soluble sugar was significant at 5% level (Table 1).

Results showed that the sugar decreased in all treatment and carvacrol treatments in 50+50

<table>
<thead>
<tr>
<th>S.O.V.</th>
<th>df</th>
<th>Anthocyanin</th>
<th>Soluble suger</th>
<th>Petal aqueous contents</th>
<th>Flourishing speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>method*Traetment</td>
<td>5</td>
<td>0.07 ns</td>
<td>70.91**</td>
<td>5.25**</td>
<td>47.59**</td>
</tr>
<tr>
<td>method*time</td>
<td>3</td>
<td>3.08**</td>
<td>13.85 ns</td>
<td>20.17**</td>
<td>39.19 ns</td>
</tr>
<tr>
<td>method<em>Traetment</em>time</td>
<td>15</td>
<td>0.04 ns</td>
<td>35.48*</td>
<td>2.03*</td>
<td>7.93 ns</td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>0.2</td>
<td>59</td>
<td>4.88</td>
<td>25.58</td>
</tr>
</tbody>
</table>

**: Significant at the1%level of probability, *: Significant at the1%level of probability .ns: Not- significant.
and 75+75 ppm concentration in short term method at the end of time. In three treatments in thymol and carvacrol treatment on 50+50 ppm concentration, sugar rate was more than zero till day sixth but then decreased strongly and on day nine received to 22.77 mg/l. In 75+75 ppm concentration in short term method, suger rate during examination was almost fixed and on day ninth also did not decrease. Solution sugar rate on day ninth was more in standard method treatments and was higher than short term treatment. At least solution sugar rate in both method related to control (distilled water) (Table 2).

Flourishing speed: Results showed that the, interaction effect of method and treatments was significant at 1% Level (Table 1). In short term and standard method, control treatment (distilled water) had the most flourish speed. There was the least flourish speed in different short term method treatments but thymol and carvacrol treatment in 75+75 ppm concentration (in 52.71% speed) had the least speed. Therefore using short term method caused to increase Gladiola cut flowers flourish speed. (Table 3).

Petal aqueous contents: According to the variance analysis results, method and treatments, method and time interaction effect on petal water content was significant in 1% Level. Also method and treatments and time interaction effect in 5% become significant (Table 1).

Results of method, treatment and time interaction effect shows that in all treatments in both methods petal water content decreased but at 12.5 + 12.5 ppm thymol and carvacrol treatment the petal water content increased in third day. Thymol and carvacrol treatments in 75+75 and 50+50 ppm concentrations (5.27 and 4.82 g) in short term method had the most petal water

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Table 2. Method*Treatment*Time interaction effect on petal soluble sugar characteristics of *Gladiolus grandiflorus* L. Cut flowers

<table>
<thead>
<tr>
<th>Method/Treatment Time</th>
<th>zero day</th>
<th>Thirth day</th>
<th>sixth day</th>
<th>ninth day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short Term</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thym 25+Carv 25</td>
<td>30.31</td>
<td>33.77</td>
<td>29.7</td>
<td>18.33</td>
</tr>
<tr>
<td>Thym 50+Carv 50</td>
<td>30.31</td>
<td>34.68</td>
<td>33.21</td>
<td>22.77</td>
</tr>
<tr>
<td>Thym 75+Carv 75</td>
<td>30.31</td>
<td>33.53</td>
<td>33.13</td>
<td>30.57</td>
</tr>
<tr>
<td>Control (distilled water)</td>
<td>30.31</td>
<td>18.76</td>
<td>6.82</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thym12.5+Carv 12.5</td>
<td>30.31</td>
<td>41.17</td>
<td>33.62</td>
<td>27.82</td>
</tr>
<tr>
<td>Thym25+Carv 25</td>
<td>30.31</td>
<td>28.77</td>
<td>25.46</td>
<td>21.78</td>
</tr>
<tr>
<td>Thym37.5+Carv 37.5</td>
<td>30.31</td>
<td>31.41</td>
<td>26.09</td>
<td>21.37</td>
</tr>
<tr>
<td>Control (distilled water)</td>
<td>30.31</td>
<td>18.76</td>
<td>6.82</td>
<td></td>
</tr>
</tbody>
</table>

---

Fig. 1. Method*Treatment interaction effect on anthocyanin characteristics of *Gladiolus grandiflorus* L. Cut flowers

---

Table 2. Method*Treatment*Time interaction effect on petal soluble sugar characteristics of *Gladiolus grandiflorus* L. Cut flowers

<table>
<thead>
<tr>
<th>Method/Treatment Time</th>
<th>zero day</th>
<th>Thirth day</th>
<th>sixth day</th>
<th>ninth day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short Term</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thym 25+Carv 25</td>
<td>30.31</td>
<td>33.77</td>
<td>29.7</td>
<td>18.33</td>
</tr>
<tr>
<td>Thym 50+Carv 50</td>
<td>30.31</td>
<td>34.68</td>
<td>33.21</td>
<td>22.77</td>
</tr>
<tr>
<td>Thym 75+Carv 75</td>
<td>30.31</td>
<td>33.53</td>
<td>33.13</td>
<td>30.57</td>
</tr>
<tr>
<td>Control (distilled water)</td>
<td>30.31</td>
<td>18.76</td>
<td>6.82</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thym12.5+Carv 12.5</td>
<td>30.31</td>
<td>41.17</td>
<td>33.62</td>
<td>27.82</td>
</tr>
<tr>
<td>Thym25+Carv 25</td>
<td>30.31</td>
<td>28.77</td>
<td>25.46</td>
<td>21.78</td>
</tr>
<tr>
<td>Thym37.5+Carv 37.5</td>
<td>30.31</td>
<td>31.41</td>
<td>26.09</td>
<td>21.37</td>
</tr>
<tr>
<td>Control (distilled water)</td>
<td>30.31</td>
<td>18.76</td>
<td>6.82</td>
<td></td>
</tr>
</tbody>
</table>
content rate in ninth day. The least rate of water content in day ninth is related to control treatment (distilled water) (Table 4).

**Vase life time**: The interaction effect of method and treatment on vase life time was significant at 1% level (Table 5). Comparing thymol and carvacrol treatment mean in 50+50 ppm in short term method had the most vase life (11.67 days). The most vase life of vase in standard method of 25 + 25 ppm thymol and carvacrol treatment was 10.67 days. The least vase life was related to control treatment (distilled water). Therefore using short term method increased Vase life of Gladiola cut flowers (Table 6).

### DISCUSSION AND CONCLUSION

Because flowers in short term method were less time in solution, they could have the better results. In standard, method flowers due to continuous placing in solution did not cause properties improvement. Vase life decreased due to vessel blockage and due to blocking stems and vessels.

**Table 3. Method*Treatment interaction effect on Inflorescence speed characteristics of *Gladiolus grandiflorus* L. Cut flowers**

<table>
<thead>
<tr>
<th>Method</th>
<th>Treatment</th>
<th>LSmmean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short Term</td>
<td>Thym 25+Carv 25</td>
<td>54.94</td>
</tr>
<tr>
<td></td>
<td>Thym 50+Carv 50</td>
<td>57.17</td>
</tr>
<tr>
<td></td>
<td>Thym 75+Carv 75</td>
<td>52.71</td>
</tr>
<tr>
<td></td>
<td>Control (distilled water)</td>
<td>69.97</td>
</tr>
<tr>
<td>Standard</td>
<td>Thym12.5+Carv 12.5</td>
<td>67.02</td>
</tr>
<tr>
<td></td>
<td>Thym25+Carv 25</td>
<td>69.15</td>
</tr>
<tr>
<td></td>
<td>Thym37.5+Carv 37.5</td>
<td>65.96</td>
</tr>
<tr>
<td></td>
<td>Control (distilled water)</td>
<td>73.89</td>
</tr>
</tbody>
</table>

**Table 4. Method*Treatment*Time interaction effect on petal aqueous contents of *Gladiolus grandiflorus* L. Cut flowers**

<table>
<thead>
<tr>
<th>Method/Treatment Time</th>
<th>zero day</th>
<th>Thirth day</th>
<th>sixth day</th>
<th>ninth day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short Term</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thym 25+Carv 25</td>
<td>12.14</td>
<td>abc</td>
<td>10.49</td>
<td>bcde</td>
</tr>
<tr>
<td>Thym 50+Carv 50</td>
<td>12.14</td>
<td>abc</td>
<td>11.52</td>
<td>abcd</td>
</tr>
<tr>
<td>Thym 75+Carv 75</td>
<td>12.14</td>
<td>abc</td>
<td>12.29</td>
<td>abcd</td>
</tr>
<tr>
<td>Control (distilled water)</td>
<td>12.14</td>
<td>abc</td>
<td>7.50</td>
<td>efg hjik</td>
</tr>
<tr>
<td>Standard</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thym12.5+Carv 12.5</td>
<td>12.14</td>
<td>abc</td>
<td>14.00</td>
<td>a</td>
</tr>
<tr>
<td>Thym25+Carv 25</td>
<td>12.14</td>
<td>abc</td>
<td>9.81</td>
<td>bedf</td>
</tr>
<tr>
<td>Thym37.5+Carv 37.5</td>
<td>12.14</td>
<td>abc</td>
<td>9.99</td>
<td>bcdef</td>
</tr>
<tr>
<td>Control (distilled water)</td>
<td>12.14</td>
<td>abc</td>
<td>7.50</td>
<td>efg hjik</td>
</tr>
</tbody>
</table>

**Table 5. Analys of variance for vase life of *Gladiolus grandiflorus* L. Cut flowers**

<table>
<thead>
<tr>
<th>S.O.V.</th>
<th>df</th>
<th>Vase life</th>
</tr>
</thead>
<tbody>
<tr>
<td>method</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>16.38**</td>
</tr>
<tr>
<td>method*Treatment</td>
<td>5</td>
<td>1.06**</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>0.72</td>
</tr>
</tbody>
</table>

**: Significant at the1%level of probability

**Table 6. Method*Treatment interaction effect on vase life characteristics of *Gladiolus grandiflorus* L. Cut flowers**

<table>
<thead>
<tr>
<th>Method</th>
<th>Treatment</th>
<th>LS Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short Term</td>
<td>Thym 25+Carv 25</td>
<td>9.33</td>
</tr>
<tr>
<td></td>
<td>Thym 50+Carv 50</td>
<td>11.67</td>
</tr>
<tr>
<td></td>
<td>Thym 75+Carv 75</td>
<td>10.33</td>
</tr>
<tr>
<td></td>
<td>Control (distilled water)</td>
<td>7.67</td>
</tr>
<tr>
<td>Standard</td>
<td>Thym12.5+Carv 12.5</td>
<td>9.00</td>
</tr>
<tr>
<td></td>
<td>Thym25+Carv 25</td>
<td>10.67</td>
</tr>
<tr>
<td></td>
<td>Thym37.5+Carv 37.5</td>
<td>9.33</td>
</tr>
<tr>
<td></td>
<td>Control (distilled water)</td>
<td>6.33</td>
</tr>
</tbody>
</table>
cut area, solution lead to decrease in stems. Short term method by preventing vessel blockage and keeping flowers *torjescense* increased vase life relative to standard method (Damunupola *et al.*, 2008). Anthocyanine increased by buds flourishing. This study results related to keep anthocyanine nine days in petals that examined aminopurin benzyl on salvia (Setyadjit *et al.*, 2004). Also sugar concentrates in petal tissue, improve osmotic potential and increase carbohydrates rate in cut flowers to use in growth and respiration activities that this delays flowers senescence. A research indicated that queenolinhydroxy increases sugar concentration in *Sonia* rose cut flowers as an antimicrobial complex with succarose (Ichimura *et al.*, 2003). Slower florish speed is better. Essences by preventing bacteria entrance decrease water absorption and buds florish speed and buds in treatments flourished in longer time (DE *et al.*, 1996). Using short term thymol and carvacrol increased petal aqueous contents. This act cause the positive effect of thymol and carvacrol and succarose to keep flowers *torjescens* and improve flowers water relations in more short time. Possibly essences by creating negative pressure in cells help water absorption by flowers, that due to water potential decreasing, water entrance occurs quicker and causes cell expansion and sugar dilution in tissue. Experiment in 2004 to study giberlic acid effect on *Gerbera* cut flowers are similar to present study results (Emongor *et al.*, 2004). This research indicated that using thymol and carvacrol extracts in short time method increased vase life *Gladiolus* cut flowers. The most petal soluble sugar rate, petal aqueous contents and least florish speed in concentrations of 75+75 ppm observed in short time method. Therefore, short time method is recommend to increase vase life and keep flowers qualitative properties.

**ACKNOWLEDGEMENT**

The authors would like to thanks Islamic Azad University of Tehran, Science and Research Branch, Department of Horticultural, Specially Dr. Sepideh Kalatejari for supports.

**Literature Cited**


The Effect of Different Concentrations of Gibberellic Acid on Quantitative and Qualitative Characteristics of Three Cultivars Lacourtine, Yokohama and Red Favourite Tulip (*Tulipa gesneriana* L.)

Khani Shakarami¹, Rohangiz Naderi², Mesbah Babalar² and Zeinab Hamzehei¹*

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Tulip is one of the most important flowers to precocity. Treatment of tulip bulbs with gibberellic acid reduces the forcing period in the greenhouse resulting energy saving and reduces the tulip’s physiological disorders. This study carried out about three tulip cultivars includes ‘Lacourtine’, ‘Yokohama’ and ‘Red Favourite’. The results showed that gibberellic acid reduced significantly stem length, first internode length and duration of precocity period. Based on the results gibberellic acid at 250 and 500 ppm can be effective agent in tulip’s precocity.

**Keywords:** Cold treatment, Forcing period, Gibberellic acid, Tulip (*Tulipa gesneriana* L.).
INTRODUCTION

Tulip (Liliaceae) belongs to permanent bulbous plants. The initial diversity center of tulip genus is in Pamir and Tien-Shan the hillside of Central Asia and the second diversity is in Caucasus (Dole and Wilkins, 1999; Coskuncelebi et al., 2008). Tulip is one of the most important flowers to precocity. Different countries use tulip in different ways. In Western Europe most of tulips are used as cut flower and in the United States as a potted plant (De Hertogh, 1974). Treatment of tulip bulbs with gibberellic acid reduces the forcing period in the greenhouse. That resulting energy saving and reduces the tulip’s physiological disorders (Hanks, 1984). Regardless of cold treatments duration of tulip’s bulbs, gibberellin accelerates flower maturity and survivability. Tulip’s bulbs have dormancy periods which the most physiological changes occur during this period (Xu and Niimi, 2008). Increasing duration of cold treatment caused reduction of forcing duration in greenhouse and synchronicity tulip flower opening (Charles-Edwards and Rees, 1975). Terminal bud in addition to carbohydrate nutrient elements receives a range of hormones factors of plant. In major groups of plant hormones, gibberellins have an important role in plant flowering. Application of hormones for the manipulation flower growth is more common in comparison with other crops. Investment return are supplied the cost of employing this materials. One of the purposes of growth regulators substance application is control of flowering that is important economically. Growth regulators substances are used to balance internal hormones, depending on the time of application and concentration stimulate or inhibit flowering (Viemont and Crabbe, 2000). Several physiological mechanisms involved in the increased stem length of tulip flower. Studies indicate that cold treatment and gibberellin application accelerate the flowering, stimulate the tulip stem elongation and prevent the flower bud abortion (Hanks, 1984; Suh and Cho, 1997). The effect of gibberellin treatments may be due to increase nutrition stretching power by the flowers when the daughter bulbs compete to mother bulbs and photosynthesis materials. Many studies have been done on gibberellin applications to reduce the duration of cold treatment or potted tulips production. Flower stem length is a qualitative factor in tulip cut flower. Stem length is different after the gibberellin application based on cultivar, the development stage of flower bud before the cold treatment, duration of cold treatment, the differences gibberellin concentrations, and method and time of gibberellin application (Franssen and Voskens, 1997). Hormonal status of tulip bulbs has been studied during the dormancy. It has been reported that within cold treatment tulip’s bulbs increases free gibberellins (Hanks, 1984; Saniewski et al., 1999). Rebers et al. (1994) stated that GA4 is probably involved in the elongation of the stem tulip. Gibberellin application on tulip bulbs that were exposure cold treatment at 12 weeks resulted in increased stem length compared with the control treatment (12 weeks at 17 °C). However, gibberellin treatment reduces the length of last internode and stem in flower opening stage that may be due to the early development of flower. Overall, the effect of gibberellin treatment and cold treatment of bulb is related to the time and method of application (Suh and Cho, 1997). External application of gibberellin provides chilling requirement of tulip bulb partially and stimulates stem length and flowering. In Apeldoorn tulip gibberellin replaces just the part of chilling requirement. Tulips that have not received cold treatment or cold treatment were short time; gibberellin application does not influence in producing flowers with desirable quality. Tulips treated with gibberellin are shorter than those met chilling requirement completely. Plants provided their chilling requirement completely; gibberellin stimulates pistil development and production of auxin. These factors increase length of internodes (Jones and Hanks, 1984; Hanks, 1985; Rebers et al., 1994; Franssen and Voskens, 1997). Janowska and Jerzy (2004) reported that gibberellic acid increases the vase life of calla flowers. Saniewski et al. (1997) studied the effects of gibberellic acid on growth and flowering of some variety of Hyacinthus. They stated that application of gibberellic acid on Hyacinthus bulb before the cold treatment stimulates growth of inflorescence and leaves greatly and reduce the forcing duration. Gibberellin application reduced the forcing duration and stems length and increased the flower vase life. Followed by thermal treat-
ment at 2-20°C gibberellin caused the reduce forcing period 15-25% and increase the stem length, regardless of storage temperature compared with control. Also gibberellin treatment reduces the stem length, decreases the stem length is more at 5°C or less temperature (bulbs stored temperature). The results of gibberellin application during cold storage at 17°C before cold treatment, during cold treatment or after cold treatment at 5°C indicated that gibberellin application during cold treatment or at the end of the cold treatment was more effective on the traits measured (Hanks, 1984).

Our objective in this study was to evaluation of the influence of gibberellic acid on forcing period in greenhouse and some qualitative and quantitative characteristics of three cultivars of tulip.

MATERIALS AND METHODS

This experiment was carried out in Jan 2009 at the Department of Horticulture, University College of Agriculture and Natural Resources of University of Tehran, Iran. Bulbs (three cultivars include ‘Lacourtin’, ‘Yokohama’ and ‘Red favourite’) were obtained from Mahde Laleha, Institute of Gachsar. To prevent fungal diseases the bulbs were disinfected with benomyl 2/1000 for 20 minutes and then were exposed to air an hour. In order to develop internal organs of flower, the bulb stored at 17-20°C for 35 days. During this period the bulbs were sampled weekly (each sample consisted of 5 bulbs), then the bulbs were analyzed. The pistil of bulbs are reached to G stage (at this stage, pistil has 3 lobes and anthers are specified). Moist cold treatment of bulbs was at 5°C for 12 weeks. After the cold treatment the bulbs were soaked at (0, 125, 250 and 500 ppm) concentrations of gibberellic acid for 24 hour then were cultured in perlite medium in greenhouse at 18-24°C. During the forcing period plants were fed with the Quick nutrient solution. The measured characteristics in this experiment included number of leaves, length and width of the lowest leaf, length of flower stem (Plant height), flower durability on the plant, flower number, flower diameter before the opening, length of upper internode, length of flower bud, stem diameter, mean of leaf area, shoot dry weight, duration of forcing period, percentage of flowering plants and percentage of shoot calcium. Two factor studied (Cultivar and hormone concentration) in different levels. However experiment was Factorial (Table 1). Each bulb was planted in a pot and each experimental unit was consisted five pots. The data obtained from pot measurements and laboratory observations were subjected to an analysis of variance using SPSS software and the Duncan mean separation test procedure was applied.

RESULTS

Results showed that plant height, length of upper internode and duration of forcing period

<table>
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<td>A</td>
<td>3</td>
<td>102.63 **</td>
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<td>B</td>
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<td>7.38 ns</td>
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<td>4.03</td>
<td>1.59</td>
<td>3618680.8</td>
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<td>14.82</td>
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**,** and ns: Significant in 0.01, 0.05 and non-significant, respectively
A: gibberellic acid B: tulip cultivars
PH: Plant height LUI: Length of upper internode MLA: mean of leaf area
DFP: duration of forcing period PSC: percentage of shoot calcium SD: stem diameter PPF: percentage of plants flowering
were affected significantly by different concentrations of gibberellic acid (Table 1). Mean of leaf area and percentage of shoot calcium were not significant in the analysis of variance table, however, with Duncan’s multiple range tests showed significant differences at 5%. According to the results the highest plant was the control (29.72 cm) and the lowest plants were those treated by gibberellic acid at 500 ppm (22.66 cm). There were no significant differences between 0 and 125 ppm concentration of gibberellic acid. The longest upper internode was belonging to control (10.61 cm) and the shortest upper internode was belonging to gibberellic acid at 500 ppm (7.33 cm) (Fig. 3). The highest and lowest average duration of the forcing period was related to control (21.77 days) and gibberellic acid at 500 ppm (13.44 days), respectively (Fig. 5). Results indicated that cold treatments and gibberellic acid accelerates flowering, stimulates stem elongation and prevent to flower bud abortion. The results of mean comparison showed that different concentrations of gibberellic acid had a significant effect on mean of leaf area and percentage of shoot calcium. Maximum mean of leaf area related to control (16636 mm²) and minimum mean of leaf area related to gibberellic acid at 500 ppm (14643 mm²). Also maximum and minimum mean of shoot calcium percentage belonging to gibberellic acid at 250 ppm (1.54%) and gibberellic acid at 500 ppm.
(1.3%), respectively (Fig. 4 and 6). Analysis of Variance shows that the effect of cultivar was significant on tulip stem diameter. The highest mean of stem diameter and the lowest mean of stem diameter were observed in Yokohama (8.7 mm) and Red favourite (8.02 mm) respectively (Fig. 1). The highest mean of plant flowering was related to Lacourtin (83.3%) and the lowest mean of plant flowering belonged to Yokohama (71.3%) (Fig. 7).

**DISCUSSION**

Means comparison showed that high concentration of gibberellic acid reduces the plant height. Miller and Kim (2008) found that application of GA4+7 and BA in color bud stage (to increase the flower vase life) had a low effect on plant height. The results of this study correspond to finding to Hanks (1985) and were inconsistent to finding to Miller and Kim (2008). Shoub and Hertogh (1974) reported that application of Ansymidol, GA3 and GA4+7 at premature stage in greenhouse reduce the tulip height. Contradiction of results may be due to differences in concentration and time of application of plant growth regulators. Effects of gibberellic acid on flowering and reduction of flower bud damage during storage at 20 °C probably may be due to stimulate of intracellular biosynthesis of indole acetic acid (IAA) (Xu and Niimi, 2007). The effect of gibberellin treatments on flowering, stimulation of stem elongation and prevent to flower bud abortion may be due to increase nutrition stretching power by the flowers when the daughter bulbs compete to mother bulbs and photosynthesis materials (Hanks, 1984; Suh and Cho, 1997). Saniewski (1989) reported that gibberellin is produced during the bulbs cooling plays an important role in the development of flower buds. Also other gibberellins synthesized during the stem growth control tulip stem elongation with auxin. On the other side, ethylene stops growth elongation of aerial parts of most of plants. It seems that growth elongation by the ethylene could be due to inhibiting of polar auxin transport. Van Bragt and Van Ast (1976) found that the upper internode length of plants treated with gibberellic acid reduced compared to control at the beginning of flowering. Jones and Hanks (1985) treated bulb of Apeldoorn tulip for 2-48 hours with gibberellic acid at 250 to 500 ppm prior to planting to reduce the storage period in greenhouse (duration of forcing period) after cooling treatments at 5 °C which gibberellic acid had the greatest effect on reducing of duration of forcing period for 7-10 days rather than control. Gibberellic acid cannot increase the growth of tulip upper internode because of may be before increase the growth upper internode converted to the less active or inactive substance. One of the symptoms of calcium deficiency is bending of neck of tulip flower (Topple). Calcium deficiency occurs during the forcing period in hydroponic method and at high relative humidity. In most of plants there are large amounts of calcium in the leaves. Older leaves have the more calcium than the younger leaves, unlike the phosphorus and potassium. Calcium content of lower leaves is more than the upper leaves in tulip. This process there is also in 42% and 82% relative humidity. So that the calcium amount of dry weight in 42% is more than 82% relative humidity. Because of the low relative humidity the more transpiration is occurring which causes to calcium absorb from the roots to shoots. Calcium addition to cell division in meristematic regions is involved in activating of some enzyme such as amylase (Nelson et al., 2003). Increasing of calcium dry weight may be due to the role of gibberellic in activation of some enzymes. Using histochemical method determined that gibberellin affected on activity of α-glucosidase enzyme in potato. This enzyme breaks down the starch (Alexopoulos et al., 2008). Considering the different cold range of varieties of tulip (13 to 20 weeks) adequate cold treatment is effective in flowering stage. Increasing treatment duration at low temperature reduces duration forcing period in greenhouse and flowers are open simultaneously. Also storing the bulbs in low
temperature causes the proper growth of stem, improves of flower quality and decreases physiological disorders during forcing period (Charles - Edward and Reese, 1975; Dole and Wilkins, 1999; Dole, 2003). Although Yokohama is an early crop but the present results it appears that reduction of flowering is due to genetical agents or thermal stress.

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Effect of Antibiotics and Essential Oils on Postharvest Life and Quality Characteristics of *Chrysanthemum* Cut Flower

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Chrysanthemum cut flower is not sensitive to ethylene and its vase life depends on vascular blockage. In order to evaluating of effect of antibiotics and essential oils on the vase life and quality characteristics of chrysanthemum cut flower an experiment carried out based on a RCD with 10 treatments: *Artemisia* at 3 levels (10, 30 and 50 %), amoxicillin at 3 levels (100, 200 and 300 mg l\(^{-1}\)), Rifampin at 3 levels (100, 200 and 300 mg l\(^{-1}\)) and the control plants in 3 replications. Analysis of variance showed that effect of treatments on measured traits was significant at \(p \leq 0.05\) or \(p \leq 0.01\). Mean comparisons also revealed that 30% of *Artemisia* oil, 200 mg l\(^{-1}\) amoxicillin and 200 mg l\(^{-1}\) rifampin caused the longest vase life, the highest preservative solution uptake, petal's soluble protein contents, leaf chlorophyll and maximum fresh weight.

**Keywords:** Chrysanthemum, *Artemisia* essential oil, Antibiotic, Vase life, Preservative solution uptake, Petals soluble protein content.
INTRODUCTION

Chrysanthemum (*Dendranthema grandiflorum* L.) belongs to Asteraceae family which has been planted since thousands years ago, and now is the most important cut flower in the world (Khoshkhui, 2010; Shiravand and Rostami, 2009). Chrysanthemum belongs to non-climacteric flowers and its senescence is in response to changes that occur in the carbohydrate content, and water relations. Moreover, the quality of cut chrysanthemum reduced due to the formation of air embolism inside the vessels, this can be occurred both in the stems kept in vases (directly in water) and in stems stored in wet storages (Bartoli et al., 1996; Adachi et al., 1999). Another reason for the decline in the cut flowers quality is leaves yellowing, it occurs due to the chlorophyll degradation during senescence, so using disinfectant compounds can be overcome this occasion (Edrisi, 2009; Halevy and Mayak, 1981). Di (2008) in his study on cut gerbera flowers found that use of tetracycline and penicillin caused delay in protein degradation and also increased POD and SOD activity. Oraee et al. (2011) studied on *Gerbera jamesonii* and found that thymus oil improved vase life and reduced microbial contamination in stem end and vase solution.

The aim of this study is investigation on effect of antibiotics and essential oils on vase life and postharvest quality of cut chrysanthemum cv. White.

MATERIALS AND METHODS

Cut chrysanthemum flowers cv. ‘White’ was purchased from a commercial producer in Tehran province and immediately were transferred into the postharvest laboratory under standard conditions. All cut flowers were cut in the height (60 cm) and recutted and placed into vases containing determined concentrations of Artemisia essential oil and antibiotics for 24 hour pulse treatment.

This study carried out based on randomized complete design with 3 levels of Artemisia essential oil (10, 30 and 50 mg l⁻¹), rifampin and amoxicillin in 3 concentrations (100, 200 and 300 mg l⁻¹), and the control flowers, at 3 replications and 30 plots. After 24h pulse treatment, cut flowers were transferred to 500 ml preservative solution containing 8-hydroxyquinoline sulphate (250 mg l⁻¹) and sucrose 3% (Fig. 1).

Vase life, preservative solution uptake, petal’s protein content, leaves chlorophyll content and fresh weight loss were measured. Petal’s protein content was measured according to Bradford (1976) method and chlorophyll was measured based on Mazumdar and Majumdar (2003) method. Fresh weight was measured by digital scale (0.01g) and fresh weight loss and water uptake calculated by followed formula:

\[
\text{Fresh weight loss} = \text{fresh weight in 1st day} - (\text{fresh weight in final day} + \text{recuts weight})
\]

\[
\text{Solution uptake (ml g}^{-1} \text{F.W.)} = 500 - (\text{Amount of\ vase solution in final day} + \text{Amount of room evaporation})/\text{Fresh weight in first day.}
\]

For determination of vase life, leaf yellowing and petal wilting were evaluated in the end of flower longevity (Nabigol et al., 2005).

Data analysis was performed using SPSS and MSTATC soft ware and mean comparisons was done by LSD test.

RESULTS AND DISCUSSION

Vase life

Effect of different treatments on vase life was statistically significant at 5% probability level. Mean comparisons also revealed that 200 mg l⁻¹ rifampin had the highest vase life (11.33 days) as compared to the control (6 days) (Fig. 2).
Increasing of vase life with these treatments may be due to the antimicrobial properties that could prevent vascular blockage and improvement of water uptake, which causes water relations enhancement, so it prevents water stress and wilting of petals (Jalili Marandi et al., 2011, Di, 2008). Our results were agreement by Figueroa et al. (2005), Kiamohammadi et al. (2011), and Mohmmadi Ostad Kalayeh et al. (2011) on other cut flowers. Oraee et al. (2011) studied on Gerbera jamesonii and found that thymus oil improved vase life and reduced microbial contamination in stem end and vase solution. Al-Humaid (2008) in his study on penicillin and streptomycin showed that these antibiotics could enhance postharvest quality of Gladiolus hybridus.

Water uptake

Effect of different treatments on water uptake was significant at the 5% probability level. Mean comparisons also revealed that among all treatments, 30% Artemisia essential oil by 1.09 ml g⁻¹ F.W. water uptake had the most absorption as compared to other treatments. Effect of amoxicillin (200 mg l⁻¹) on water absorption was significant (1.06 ml g⁻¹ F.W). Effect of rifampicin on water uptake showed that 200 mg l⁻¹ antibiotic had greater uptake as compared to the control (0.99 and 0.71 ml g⁻¹ F.W, respectively) (Fig. 3). This superiority may be due to improved water relations and hydraulic conductivity in cut flower which prevents vascular blockage in addition to water
uptake in the vessels that ultimately increases water absorption (Monshizadeh et al., 2011; Figueroa et al., 2005). Another reason for these results could be defined as control the activity of microorganisms (such as bacteria and fungi) which prevents vascular blockage, these results are in consistent with Jalili Marandi et al. (2011) and Burt (2004).

**Fresh Weight Loss**

Fresh weight loss affected by different treatments ($p \leq 0.05$). Mean comparisons also revealed that 30% Artemisia essential oil with 4.09 g and 200 mg l$^{-1}$ rifampin with 3.79 g in fresh weight loss were the best treatment compared to other treatments (Fig. 4).

Petridou et al. (2001) evaluated the effect of antimicrobial and anti-ethylene compounds on quantitative and qualitative properties of cut chrysanthemum and found that using these compounds prevents vascular blockage and fresh weight loss which finally increased vase life, our findings also confirms these results. Al-Humaid (2008) in his study on penicillin and streptomycin showed that these antibiotics could enhance postharvest quality of Gladiolus hybridus. These results also confirms by Mohammadi Ostad Kalayeh et al. (2011).

**Petal's Protein Content**

Effect of different treatments on petal's protein content was statistically significant at 1% probability level. Mean comparisons indicated that 30% Artemisia essential oil with 32.76% and 200 mg l$^{-1}$ rifampin with 32.85% protein content were the best treatments (Fig. 5).

Increasing of petals protein may be due to reduction of proteolytic enzymes activity. Improvement of water uptake ultimately leads to membrane stability and prevents protein degradation.
Kazemi et al. (2011) showed that vase life extending compounds by maintenance membrane stability caused protein content enhancement and prevent protein degradation. Also, it is observed that cell membrane stability is reduced with senescence progressed (Ezhilmathi et al., 2007). Hashemabadi (2011) found that the use of antimicrobial agents is effective to maintain of cell membrane in cut carnation cv ‘Tempo’. Zamani et al. (2011) studied on the impact of vase life extending compounds on MDA activity and cell membrane stability of cut chrysanthemum and stated that these compounds increased membrane stability about 25 to 40% more than the control which is in consistent with present results. Di (2008) in his study on cut gerbera found that use of tetracycline and penicillin caused a delay in protein degradation and also increased POD and SOD activity.

**Total Chlorophyll Content**

Results showed that 200 mg l⁻¹ rifampcin with 5.63 mg g⁻¹ F.W had highest chlorophyll among all applied concentrations (Fig. 6).

The superiority of all treatment composed to control may be due to antimicrobial properties of essential oils and antibiotics as vase life extending compounds which prevents senescence with controlling vascular blockage and water absorption enhancement (Elgimabi and Ahmed, 2009; Edrisi, 2009). Also, controlling the activity of chlorophyll degradation enzymes is another reasons for chlorophyll content enhancement with these treatments that is in accordance with Ferrante et al. (2002). Mousavi Bazzaz and Tehranifar (2011) evaluated the effects of herbal essential oils on *Alstroemeria* cut flower and found that the use of these compounds is effective to increase chlorophyll content. As well, similar results has been reported by Hashemabadi (2011) and Basiri and Zarei (2011) which confirm our findings.

**CONCLUSION**

According to all findings, it could be resulted that 30% *Artemisia* essential oil and 200 mg l⁻¹ rifampin had the most efficiency and enhancing impact on postharvest quality of cut chrysanthemum cv. White, so using these compounds with determined concentrations are recommended.

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تأثیر آنتی بیوتیک‌ها و اسانس‌های گیاهی بر عمر پس از برداشت و خصوصیات کیفی گل بریده داوودی

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5 باشگاه پژوهشگران جوان و نخبگان

درصد آماری 1: 10 اثر تیمارها بر صفای اندازه‌گیری شده در سطح 1 بر روی گل بریده داوودی آزمایشی بر پایه طرح کاملاً تصادفی با 10 تیمار: اسانس درمنه در سطح 300 میلی گرم در لیتر (0.100 و 0.200 و 0.300 میلی گرم در لیتر) به همراه شاهد در تکرار انجام شد. تجزیه واریانس داده‌ها نشان داد که اثر تیمارها بر صفای اندازه‌گیری شده در سطح 5 درصد آماری معنی‌دار بود. مقایسه مانگیک‌ها همچنین نشان داد که 30 درصد اسانس درمنه در سطح 2 میلی گرم در لیتر آمکسی سیلیزن و 0.2 میلی گرم در لیتر ریفامپسین بالاترین عمر گل‌گاجی، بیشترین جذب محلول پروتئین گل‌برگ و کلروفیل و بیش‌ترین وزن‌تر را به خود اختصاص دادند.

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اثر غلظت‌های مختلف اسید چیپرلیک بر خصوصیات کمی و کیفی لاله (Tulipa gesneriana L.)

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کلید واژگان: تیمار سرمای، دوره پیش‌رسی، اسید چیپرلیک، لاله (Tulipa gesneriana L.)
اثر کاربرد اسانس‌های *Thymus vulgaris* به عمر گلدان و صفات
*Gladiolus grandiflorus* L.

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*ایمیل نویسنده مسئول*

در این مطالعه، اثر ترکیبی دو اسانس تیمول و کارواکرول، با غلظت‌های مختلف بر پایه طرح کاماً تصادفی در *Gladiolus grandiflorus* L. در سه تکرار و چهار گل در هر تکرار بررسی شد. واریته "سنوسی" قرمز رنگ در آزمایش، ۲۵+۲۵ های مختلف تیمول و کارواکرول شامل به کار رفت. در روش کوتاه مدت، غلظت % و آب مقطر (به عنوان شاهد) ۷۵+۷۵ و ۵۰+۵۰ میلی گرم بر لیتر همراه ساکارز و آنتوسیانین در محلول شدند. هر ۲۴ ساعت تیمار شدند و آب مقطر قرار داده شدند. در روش استاندارد، غلظت‌های مختلف تیمول و کارواکرول شامل ها از % و آب مقطر (به عنوان شاهد) ازالیبی‌شدند. گل‌های قرمز رنگ در محلول‌های ذکر دهه بیشتر عمر شدند و تا پایان دوره آزمایش در آب مقطر قرار داده شدند. در روش استاندارد، غلظت‌های مختلف تیمول و کارواکرول شامل ها از % و آب مقطر (به عنوان شاهد) استفاده شدند. گل‌ها در محلول‌های شروع آزمایش تا پایان عمر گلدانی نگهداری شدند. در روش کوتاه مدت، محلول‌های مختلف تیمول و کارواکرول از محلول بیشترین استفاده شدند. اسانس تیمول و کارواکرول با ۱۵۰ میلی گرم بر کیلوگرم، بیشترین قند محلول و گلبرگ و کمترین شکوفایی نیز‌ها را دارا بود. علاوه بر این، بیشترین عمر گلدانی در غلظت ۱۰۰ میلی گرم در لیتر تیمول و کارواکرول با ۱۱/۷ روز مشاهده شد. غلظت آنتوسیانین در روش کوتاه‌مدت بیشتر از روش استاندارد بود.
ارزیابی رشد، عملکرد و خصوصیات کیفی ارقام مختلف زربرا (Gerbera jamesonii L.)

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ده رقم زربرا (Labine، Lilla، Alp، Alberino، Bonnie، Avemaria، Mammut) از نظر رشد، عملکرد و خصوصیات کیفی، در شرایط حفاظت‌شده در سال 2001 ارزیابی شدند. در بین ارقام مطالعه‌شده، سه رقم تغییرات خاصی مشخصی از نظر رشد، عملکرد و خصوصیات کیفی نشان دادند. لکسوس سبزی در رقم با 2/6 سانتی‌متر و بعد از آن، Alberino طول ترین ساقه در رقم 5/9 سانتی‌متر و 5/6 سانتی‌متر بودند. همین رقم گل‌هایی با بیشرتین قطر توپی کرد. با توجه به خصوصیات روشی مثل تعداد برگ در گیاه و کاهش گیاه، این خصوصیات در این رقم بیشتر بود. بیشرتین تعداد گل (Aberino) در رقم (۱۲۵) ملاحظه شد. بیشرتین عمر Alberino و سپس Avemaria (۱۳۵) در رقم Mammut (۵/6) و بعد Lexus (۵/6) و Alberino (۴/8)، Mammut (۵/6) و بعد Lexus (۵/6) و Alberino (۴/8) مشاهده شد. کیفیت عالی گل در رقمنام Lexus و Alberino و Alberino و Lexus در شرایط حفاظت‌شده برتر از ارقام دیگر بودند.
ارائه یک دستورالعمل مؤثر و اثر برخش در کشت درون شیشه‌ای (In Vitro) آناناس

اینسیتا دوانت، جوینیا بهاردرا، پریتیا ساها و سیراج داتا
گروه بیوتکنولوژی، موسسه تکنولوژی هالادیا، هالادیا، بنگال غربی، هندوستان

(Ananas comosus var. Queen) چامد مورا شیک و اسکوگ (MS) با 3 میلی‌گرم بر لیتر بنزیل آمینوپورین (BAP) و 50 میلی‌گرم بر لیتر آدنین (IAA) سولفات غنی شد. دو رویکرد برای کاهش هزینه محیط درصد در طی مرحله ریشه دهی به طور موفقیت آمیزی کارگرین ساکارز شد. مجدد در طی مرحله ریشه دهی، محیط مایع مورا شیک و اسکوگ جایگزین محیط جامد شد. مواد کوو، نیتروژن و لوفای قابل زیستی به عنوان ماده حمایتی استفاده شدند. از طریق سوخته‌گیری، رویکرد در محیط‌های ریشه‌زایی، موثر تشخیص داده شد. ممکن است کلیه گاه‌چه‌های ایجادشده در شرایط درون شیشه‌ای نوسط فن مارکر مولکولی زیست تایید شد.

کلیدواژگان: آناناس، ریز ازدیادی، شکر تجاری، Coir، Luffa
اثر اسید چیبرلیک و سرمادهی مرطوب بر جوانه زنی بذر آلستروموریا (Alstroemeria ligtu hybrid)

فرضیاتی تیم‌نشده Alstroemeria ligtu به دو روش درون شیشه‌ای و درون خاکی برای جوانه زنی بذر انجام شد. بذرها در مخلوط خاک (نسبت 1:1:1) با نیمی از غلظت MS محیط کشت و طول روز 21 درصد رشد اتفاق رشد 41 درصد آغاز شد و درصد جوانه‌زدن 70 درصد و 76 درصد درصد بزرگتر از درون شیشه و درون خاکی بود. سرمادهی مرطوب در شرایط سرد به مدت 400 میلی گرم بر لیتر با نیمی از غلظت MS می‌تواند خیس‌دانه برای جوانه زنی بذر آلستروموریا (Alstroemeria ligtu hybrid) باشد. سرماده‌ای درون شیشه‌ای بیشتر از درون خاکی ۷۰ درصد جوانه‌زدن باعث افزایش جوانه زنی شد. جوانه‌زدن به دلیل عناصر غذایی و یک درصد ساکرز، با نیمی از غلظت MS محیط کشت و طول روز 21 درصد رشد داشت. سرماده‌یی درون شیشه‌ای به معنی شدیدتر از درون خاکی بود و درصد جوانه‌زدن با پیشرفت درون خاکی کاهش یافت. بنابراین بذر آلستروموریا (Alstroemeria ligtu hybrid) در صورت وجود گرم اسید چیبرلیک همراه با سرماده‌یی درون شیشه‌ای می‌تواند خیس‌دانه برای جوانه زنی بذر آلستروموریا (Alstroemeria ligtu hybrid) باشد.
اثر سایه بر خصوصیات کیفی و مقدار کلروفیل گل های شاخه برده رز (Rosa hybrida cv. Avalanche)

شدت نور یک عامل محدود کننده در تولید گلخانه ای گل های رز است. هدف از این آزمایش بررسی تأثیر سایه (۰، ۳۰، ۶۰ و ۹۰ درصد سایه) بر کیفیت، و محتمات کلروفیل گل های شاخه برده رز (Rosa hybrida cv. Avalanche) تحت شرایط گلخانه ای است. این آزمایش در قالب طرح بلور مختل اجرا گردید. مطالعه به زبان گیاه‌شناسی گزارش شد. در صورت شرایط جوانه، وزن تر و خشک و قطر ساقه گل اثر گذاشت. به طوری که اولین ظهور جوانه، بیشترین قطر و وزن تر و خشک ساقه گل در شرایط بدون سایه مشاهده شد. با این حال، سایه اثر معنی داری بر طول ساقه گل دهی و سطح بزرگ نداشت. اما سطح ویژه برگ در ۶۰ درصد سایه افزایش یافت. نتایج نشان داد که محتمات کلروفیل گل کلروفیل و گل درصد سایه کاهش یافت. به طور کلی، سایه می‌تواند یکی از علل کیفیت پایین گل رز شاخه بریده باشد. بنابراین پرورش دهنده‌گان رزهای گلخانه‌ای با باید معمایی گلخانه را طوری در نظر بگیرند که عمق نفوذ نور به حداقل برسد.

کلید واژگان: خمیدگی، رزهای گلخانه‌ای، سایه دهی، کلروفیل، کیفیت بازاریابی.
اثر سیلیسیم بر رشد و صفات زینتی گل همیشه بهار (Calendula officinalis L.)

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چکیده
اثر سیلیسیم بر رشد و صفات زینتی گل همیشه بهار (Calendula officinalis L.) تحت تنش شوری

کلید واژگان: اسید هیومیک، پراکسیداسیون لیپیدی، نانو ذرات نقره، پروتئین گل، گلمریم، عمر
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