Impact of potassium on the yield of lily (Lilium longifolium × Asiatic cv. Termoli) and antioxidant enzyme activities under drought stress

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Abstract:

This study was conducted in two phases: 1) investigation of the effect of drought stress on relative water content and proline of leaf and 2) evaluate of the effect of drought stress and potassium concentrations in nutrient solution on the lily (Lilium longifolium × Asiatic cv. Termoli). Based on a completely randomized design in mixture of sand and perlite medium and in three levels of potassium (0, 6 and 12 mM), the growth indices, vase life of flowers, potassium concentration and antioxidant enzymes activity were measured. The results showed that the proline concentration increased about 7 times in the medium containing the moisture content of one third of field capacity (FC) compared to the FC treatment after 3 months in plant leaves. Lily vase life at 6 mM potassium increased 5.7 days relative to potassium-free conditions. Peroxidase activity in the nutrient solution with 12 mM potassium was significantly lower than the potassium-free conditions.

Keywords: Ascorbate peroxidase, Perlite, Peroxidase, Vase life, Superoxide dismutase.

Introduction:

The lack of water in Iran has always been a limiting factor in the cultivation and growth of crops and orchards. Drought stress is a limiting factor in different stages of growth especially flowering and seeding stages (Mohammadi Torkashvand, and Toofighi Alikhani, 2015). Lack of moisture causes the plant to respond to various morphological reactions such as reduction of leaf area, reduced growth rate, lignification, reduction of air system, increasing root growth, premature abscission, stoma closure and accumulation of antioxidants and soluble matters (Hughes et al., 1989). Drought causes many plant constraints due to decreasing the content of plant water, reduction of leaf water potential, stoma closure and increasing photorespiration and photosynthetic products (Nayyar and Gupta, 2006).

Among the various kinds of geophyte plants, lily is uniquely beautiful and bears colorful flowers and it high prices and is grown as cut flowers or pot plants (Sajid et al., 2009). This plant is ranked fourth in prices after rose, carnation and chrysanthemums. Every year, in Netherland flower auction market, this plant sales about 150 million cut flowers (Burchi et al., 2010). One of the most important factors affecting the quality of the flowers is appropriate nutrition. Nutritional studies on bulbous flowers are difficult, because the nutrients are in storage form in the bulb (Naseri and Ebrahim Gheravi, 1998). Hence, to overcome nutritional problems mentioned above, cultivation experiments on mediums without nutrients along with nutrient solution are recommended (Naseri and Ibrihimi Gheravi, 1998). Potassium element has considerable importance in lily nutrient. This element leads to optimal improvement in plant growth and increases flowers vase life because of its role in protein synthesis process, neutralizing anions and adjusting osmotic potential (Pardo et al., 2006). In the case of potassium deficiency, the activity of some enzymes along with the uptake and transport of some nutrients have been studied (Kanai et al., 2007). Morgan (1992) and Ma et al., (2004) reported that lines of rapeseed and mustard that showed high osmotic adjustment and had high concentration of potassium in their tissues.

Potassium ions catalyze the transfer of materials from photosynthesis. Photosynthetic electron transport system is the major source of reactive oxygen production in plant tissues that have the potential to produce single oxygen and superoxide (Asada, 1994). Drought stress is one of the most important environmental stresses that can lead to reduced plant growth and yield production (Sharma and Dubey, 2005). Drought stress leads to the production of reactive oxygen species. Appearance and the presence of these

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compounds known as oxidative stress is the main reason for reduced performance and its outbreak damage that eventually cause plant death (Ghorbani and Ladan Moghadam, 2005). Production of reactive oxygen species increases the capacity of the antioxidant defense system (Gressel and Salun, 1994). To counter oxidant stress from reactive oxygen radicals Plants have enzymatic and non-enzymatic antioxidant mechanisms. Non-enzymatic antioxidants are glutathione and ascorbic acid and antioxidant enzymes include superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase and peroxidase (Hsu and Kao, 2003). The present study was designed to investigate the effect of different concentrations of potassium in nutrient solution under conditions of drought stress on production yield of lily.

Materials and Methods

In this research, a hybrid lily (Lilium longiflorum × Asiatic cv. Termoli) with pink flowers was studied. The imported premature bulbs of this flower with an approximate orientation of 18 to 20 cm were purchased from Eram Gol Shiraz Company, the official representative of Vanden Bass Company of the Netherlands in Iran. Premature bulbs of lily were transferred to the Islamic Azad University, Science and Research Branch, Guilan, Iran. The first phase of the study was investigation of the effect of drought stress on Relative Water Content (RWC) and proline of leaf. Interaction effect of drought stress and K concentration in nutrient solution was studied and evaluated in second phase.

First phase: A completely randomized design with three treatments in three replicates was carried out in a mixture of sand and perlite medium (50:50 v/v) to investigate moisture content of medium and the probable stress on plant in 2016. Perlite with a diameter of 1 to 2 mm (fine) was used and washed several times with double distilled water for fluoride reduction. River sand was washed several times to be free of any mud and then packed in cellophane bags and were disinfected using an autoclave (121°C for 15 min). The field capacity (FC) coefficient was measured at -33 kPa soil potential by pressure plate apparatus. This coefficient was determined to be 25.8% w/w. Treatments included the irradiation of media to achieve the moisture to contain at FC, ⅔FC and ⅓FC. The amounts of required water used were 270, 185 and 105 mL per pot, respectively. Every pot was irrigated by deionized water with nutrient solution once every three days. After one month, watering time was weekly shortened for 3 hours. Lily bulbs were incubated with Benomyl fungicide solution (0.1%) in ten liters of water for 15 minutes before planting and placed on paper without rinsing and then completely dried by air blow. Pots made with a height of 15 cm and two liters volume were disinfected with 1% sodium hypochlorite. Bulbs were planted after disinfection, materials penetrated into the pot at the depth of 10 cm and then were placed in a greenhouse spacing of 20 × 20 cm. Sampling was done around midday from top fully emerged young leaves to control RWC and proline concentration. The RWC was measured by following the method of Barrs and Weatherly (1962). Proline was extracted from a sample of 0.5 g fresh leaf in 3% (w/v) aqueous sulphasalycylic acid samples and estimated using the ninhydrin reagent according to the method of Bates et al. (1973). The absorbance of fraction with toluene aspirated from liquid phase was read at a wave length of 520 nm. Proline concentration was determined using a calibration curve and expressed as μmol proline g−1 FW.

Second phase: With regard to the results obtained in first phase; an irrigation regime was planned, so that the cultivation bed between two consecutive irrigation times was always dry. Treatments were selected at three levels of potassium concentrations including; 0 (without potassium), 6 mM potassium and 12 mM potassium in Hoagland nutrient solution (Hoagland, 1950). Every treatment was designed in three replicates and three pots in every replication.

The nutrient solution was used once every three days as an open system that contained nutrient solution with 130 mL of irrigation water. The status to maintain moisture in sand and perlite medium was in such a way that medium was encountered with drought and the plant faced with drought stress during both irrigations. To record the flowering time, the number of days from bulbs planted in pots to the first appearance of bud observation was counted. Sampling from top fully emerged young petal tissue was done around midday to measure membrane stability, H$_2$O$_2$ level and antioxidant defense components. Quantifying of H$_2$O$_2$ and membrane stability were performed immediately after sampling of the tissues for antioxidant enzyme assays, the petal tissues were ground, weighed 0.2 g in replicates, frozen in liquid nitrogen and stored at -80°C. Three replicates were maintained for all the measurements.

Membrane stability was estimated by measuring the leakage of electrolytes (conductivity) due to damaged cell membrane according to Shanahan et al. (1990). One gram of leaf material (about 10 mm × 10 mm pieces) was taken in 10 mL of double distilled water in glass vials and kept at 10°C for 24 hrs. with shaking. The initial conductivity (EC$_1$) was recorded after bringing sample to 25°C by using conductivity meter. The samples were then autoclaved at 0.1 MPa for 10 mins., cooled to 25°C and final conductivity (EC$_2$) was recorded. Membrane stability was calculated as:

$$\text{MSI} = \left[1 - \left(\text{EC}_2/\text{EC}_1\right)\right] \times 100.$$  

Lilies stem end height, stem diameter, reproductive height (distance between the lowest pedicels to tip of the longest bud) and shoot dry weight were measured. To measure the durability of the cut flowers, cut flowers were placed in water at room temperature and under natural conditions, and the number of days from harvesting cut flowers until 50% of petals falling from each sample were counted.
To measure potassium, 0.3 g of oven dried sample with 2.3 mL mixture of sulfuric and salicylic acids were soaked for 24 hours. Then, the samples were heated to 180°C and the solution was colorless which was added intermittently and low hydrogen peroxide. Then, the solution was brought to the related volume with distilled water and filtered (Emami, 1996).

**Determination of H$_2$O$_2$ concentration:** For the measurement of H$_2$O$_2$ content, leaf samples (0.2 g) were homogenized with liquid nitrogen and suspended in chilled 5% trichloroacetic acid and centrifuged at 30,000 rpm for 10 mins. H$_2$O$_2$ was measured after passing the supernatant through a Dowex anion exchange resin to remove the colored compounds (Warm and Laties 1982). The reaction mixture contained 50 µl of test solution, 50 µl luminol in 0.2 M NH$_4$OH (pH 9.5) and 800 µl 0.2 M NH$_4$OH in 1.0 ml test tube, which were placed in Luminoskan TL Plus luminometer. Chemiluminescence was initiated by adding 100 µl 0.5 mM K$_3$Fe(CN)$_6$ in 0.2 M NH$_4$OH and photons emitted were counted over 5 s. H$_2$O$_2$ content was determined using a calibration curve.

**Determination of chlorophyll content:** Chlorophyll content was determined using Arnon (1949) method. To measure chlorophyll content, 0.5 g of green leaves in liquid nitrogen in a porcelain mortar in ice container without light with 0.5 g magnesium carbonate was ground and gradually was added about 10 mL of 80% acetone. One mL of the prepared extract after centrifugation was placed in a spectrophotometer cell and the amount of light absorbed by chlorophylls a and b was read at 645 and 663 nm wavelengths, respectively.

**Extraction and determination of antioxidant enzymes:** Petal tissues were ground in a mortar containing liquid nitrogen. Then, enzymatic extraction was carried out using Sairam et al. (1998) method. To extract superoxide dismutase enzyme, 0.5 g powder in 10 mL of phosphate buffer 0.1 M cold (pH 7.5) containing 0.5 mM EDTA was stirred. To extract ascorbate peroxidase, 0.5 g powder in 10 mL of phosphate buffer 0.1 M cold (pH 7) containing 0.5 mM ascorbic acid was stirred. The above mixture was filtered (with a soft cloth) and the filtrate was transferred to microtubes containers special for refrigerated centrifuges (all steps were performed in the refrigerator). The solution was centrifuged for 15 mins. at 4°C with 20000xg force and supernatant was used for enzyme activity. Ascorbate peroxidase activity (1, 11, 1, 11 EC, APX) was measured using Nakano and Asada (1987) method. Measurement of peroxidase activity (7, 1, 11, 1 EC, POX) took place using Hemeda and Kelin (1990) method. Dhindsa et al. (1981) method was used to measure superoxide dismutase (1, 1, 15, 1 EC, SOD) by measuring its ability to inhibit light restoration of nitro blue tetrazolium chloride (NBT).

Data were analyzed using SPSS software and ANOVA and mean comparison was carried out using Tukey test (p≤0.05) and EXCEL software was used for drawing shape results.

**Results and Discussion**

**First phase:** Table 1 shows the effect of watering treatment on relative water content (RWC) and proline on leaves. The RWC decreased in the treatments of ⅓FC and ⅔FC as compared to the FC, therefore, it decreased to 34% in ⅔FC than in FC applied at the second months of growth. RWC decreased in third months in comparison to the second months. The decrease of RWC at third months at ⅔FC treatment was more severe than second months (40.7%).

Proline increased about 7 times more in ⅔FC than in the FC treatment after 3 months of growth. The accumulation of osmolytes during stress was well documented. Recent studies (Molinari et al., 2004, Zhu et al., 2005) demonstrated that biosynthesis of low-molecular-weight metabolites, such as proline improved plant tolerance to drought and salinity in a number of crops. Accumulation of proline has been advocated as a parameter of selection for stress tolerance (Yancy et al., 1982, Jaleel et al., 2007). Results denote to severe stress of drought at the media irrigated under ⅔FC condition. Therefore, suggestion in considering an intermediate moisture condition between ⅔FC and ⅔FC in order to make drought stress in the growth media at second phase was recommended and utilized.

**Second phase:** Table 2 shows the effect of treatment on the growth indices of lily. The highest shoot (fresh/dry) weight was related to 6 mM of potassium with 158.3 g and 15.4 g, respectively. Shoot fresh weight in 12 mM potassium showed significant reduction compared to other treatments. It seemed that in 6 mM potassium treatment, this element had been adequately furnished and plants with potassium stock lost less water. As a result, water conservation increased shoot fresh weight. Tisdale et al. (1985) has also stated that, due to K$^+$ role in the growth and development of plant cells and making cell turgor and opening and closing of stomata, potassium could maintain water in the plant and this phenomenon has greatly increased plant fresh weight. It seemed that shoot fresh weight reduction in treatment of 12 mM potassium compare to 6 mM K was due to an antagonistic effect of this element and other nutrients. These findings were corresponded and similar to Barra-aguilar et al. (2012) in lily and Wang (2007) in the orchid flowers research investigation.

Significant differences were found for most measured characteristics treated with different concentrations of K (Table 3). There was no significant difference between the time of bud coloring in K-free and 6 mM potassium treatment (Table 2). Stem diameter in 12 mM potassium treatments were significantly lower compared to K-free treatment. According to the results shown in Table 2, the reduction in diameter of open flowers in 12 mM potassium concentration compared to the non-potassium treatment was found. Barrera-Aguilar et al. (2012) in study on
Table 1: The effect of different moisture regimes of media on Relative Water Content (RWC) and proline in leaves.

<table>
<thead>
<tr>
<th>Property</th>
<th>Time (month)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>RWC (%)</td>
<td>2</td>
<td>FC</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>⅔FC</td>
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<tr>
<td></td>
<td></td>
<td>⅔FC</td>
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<tr>
<td>Proline (µM/g FW)</td>
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Table 2: The effect of different moisture regimes of media on Relative Water Content (RWC) and proline in leaves.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>RWC (%)</td>
<td>2</td>
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<td>Proline (µM/g FW)</td>
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Table 3: Analysis of variance of the effect of different concentrations of K on the growth indices of lily.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean of square</th>
</tr>
</thead>
<tbody>
<tr>
<td>K concentration (B)</td>
<td>2</td>
<td>26446.8 **</td>
</tr>
<tr>
<td>A × B</td>
<td>4</td>
<td>11421.7 **</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>1832.5</td>
</tr>
</tbody>
</table>
| Time to appearance flower bud
| K concentration (B) | 2  | 248.4 *        |
| A × B               | 4  | 237 *          |
| Error               | 16 | 61             |
| Primary flower bud number
| K concentration (B) | 2  | 20.7 **        |
| A × B               | 4  | 5.84 *         |
| Error               | 16 | 1.55           |
| Opened flower diameter
| K concentration (B) | 2  | 10188 *        |
| A × B               | 4  | 9888.9 *       |
| Error               | 16 | 2452           |

Lilium in Mexico, examined the effect of 4 potassium levels (0, 5, 10, 20 mM) in Hoagland solution on Lilium growth that were grown in acidic peat and photosynthesis process. The results showed that at concentrations of 5 to 10 mM K, flower diameter, plant height and plant dry weight increased; however, higher concentrations of potassium had adverse effect on the listed traits. Wang (2007) examined the impact of different levels of potassium (50, 100, 200, 300, 400, 500 ppm) on the Phalaenopsis orchid. His results demonstrated that the largest and tallest inflorescence regardless of the medium was obtained at level of 300 ppm.
Impact of potassium on the yield of lily (Lilium longifolium × …

33

Fig. 1 - Effect of K concentration in nutrient solution on vase life of lily. Means with different letters are significantly different at $P \leq 0.05$ by LSD test.

Fig. 2 - Effect of K concentration in nutrient solution on K concentration of leaf. Means with different letters are significantly different at $P \leq 0.05$ by LSD test.

ml/l. The higher amount had an inverse effect on all traits.

Postharvest and potassium concentration of leaf: Lily postharvest life at the concentration of 6 mM potassium increased 5.7 days more than in the potassium-free treatment (Fig. 1); however there was no major significant difference between postharvest life in 12 mM potassium in nutrient solution and potassium-free conditions.

The reason for reduction of flowers postharvest life of 12 mM potassium in compared to 6 mM of potassium was due to high concentration of potassium in the root environment which prevented the uptake of calcium and magnesium in the plant. It is worth mentioning that among other elements, calcium and magnesium play the most important role in increasing the postharvest life of cut flowers. The effect of calcium on the lily postharvest longevity (Seyedi et al., 2011), Robichaux (2008) in Poinsettia and Sosanan (2007) in sunflower has been reported. The reason for no significant difference in the lily postharvest of 12 mM potassium level in comparison to potassium-free is that the lilies are bulbous plants and nutrients stored in its bulb (Naseri and Ebrahim Gheravi, 1998). According to the results shown in Fig. 2, the increase of potassium in nutrient solution increased potassium concentrations in shoots. Increasing potassium uptake can be a reason for the increase of vase life. The positive impact of potassium causes the increasing growth of root and consequently provide the further uptake of nutrients and water by plant and thereby decreasing transpiration too (Umar, 2006).

Chlorophyll content: The results of Fig. 3 shows that chlorophyll a, b and total chlorophyll content in nutrient solution without K was lower than in nutrient solution containing 6 mM potassium. Pessarakli (1999) revealed that the durability of photosynthesis and maintaining chlorophyll under stress are indicative of the physiological resistance to stress. In drought conditions, chlorophyll concentration increases due to reduced leaf surface area, and plant loses more water due to more transpiration. Therefore, it reduces relative water content and consequently lower photosynthesis (Zadeh Bagheri et al., 2014). It has been reported that,
as a non-organic osmolite in osmotic adjustment, potassium has been effective to reduce the negative effects of drought stress (Ma et al., 2004 and 2006). Consequently it improves metabolic processes which include forming chlorophyll. Potassium has been involved in the synthesis of chlorophyll pigment precursor (Kumar and Kumar, 2008). This element plays a key role in protein synthesis, photosynthesis process and transported materials. In the case of potassium deficiency, the activity of some enzymes, the uptake and transport of some nutrients will be reduced (Kanai et al., 2007).

Morgan (1992) and Ma et al. (2004) have reported that lines of rapeseed and mustard that showed high osmotic adjustment had high concentration of potassium in their tissues, such that among the osmotic substances effective in osmoregulation, approximately up to 78% to minimum of 23% potassium were involved (Morgan, 1992, Ma et al., 2004). Kumar and Kumar (2008) reported that by increasing the consumption amount of potassium sulfate, an increase in the relative chlorophyll content was found. These authors stated that higher photosynthetic activity from an increase in the relative chlorophyll content in leaves could be due to potassium's role in the synthesis of chlorophyll pigments precursor.

Membrane stability index (MSI): Fig. 4 shows the effect of treatment on Membrane Stability Index (MSI). The MSI increased 16.8% in 6 mM K (89.2%) compared to the K-free treatment (72.4%). This can be the cause of less membrane lipid peroxidation (Selote et al., 2004) and the role of potassium in keeping turgor pressure and adjustment of stomata movement (Kant and Kafkafi, 2002). The MSI was found to be 81.3% in 12 mM K treatment.

H$_2$O$_2$ and antioxidant enzymes activity: Fig. 5 shows that the accumulation of H$_2$O$_2$ decreased 0.4 µmol/g DW in 6 mM K than with no K treatment (2.08 µmol/g DW). The H$_2$O$_2$ concentration increased in 12 mM (1.94 µmol/g DW) as compared to the 6 mM K, but
Impact of potassium on the yield of lily (Lilium longifolium × …)

Fig. 5 - Effect of K concentration in nutrient solution on activity of peroxidase and concentration of H$_2$O$_2$. Means with different letters are significantly different at $P \leq 0.05$ by LSD test.

Fig. 6 - Effect of K concentration in nutrient solution on the activity of ascorbate peroxidase and peroxidase. Means with different letters are significantly different at $P \leq 0.05$ by LSD test.

It was lower than free-K treatment. Potassium plays an important physiological role in unfavorable environmental conditions in cells and its high rate content increases the plant's tolerance (Cakmak, 2005; Vyas et al., 2001). Potassium is essential for CO$_2$ fixation in chloroplasts and biphosphate carboxylase ribulose activity. The reason for higher demand for potassium in plants under drought stress is that potassium is effective to protect photosynthetic CO$_2$ fixation. Drought stress by closing stomata, while decrease of CO$_2$ fixation induced production of reactive oxygen species that under lower availability of potassium, their formation is reinforced (Cakmak, 2005). Manivel et al. (1995) showed that plants of rapeseed that received potassium, the distribution and movement of matter accelerate and store carbon hydrates in roots was maintained and the effect of stress in leaves treated with potassium was minimal. Potassium protects cells against difficult conditions particularly during drought stress condition (Kant and Kafkafi, 2002).

Fig. 6 shows that peroxidase activity at 12 mM potassium in the nutrient solution was significantly lower than the K-free conditions. In K-free and 6 mM condition, more activity was seen in the peroxidase enzyme. Ascorbate peroxidase enzyme activity showed decrease in potassium treatments as compared to K-free conditions. Sharma and Kuhad (2006) in Brassica reported that the harmful effects of water stress were lowered significantly due to increased potassium intake. Criley et al. (2001) investigated the effect of potassium and reducing water stress in clove. They reported that in addition to lowering effects of water stress, potassium consumption reduced calyx splitting syndrome and increased the plant quality. Sween et al. (2003) concluded that the drought stress causes to change the rate of minerals uptake and pH of raw sap, this tends to accelerate the accumulation of nutrients such as...
potassium.

Conclusion
In nutrient solution without K in which the potassium is supported by the bulb alone, the resistance of plant against drought stress of the medium was less than in the concentration of 6 mM K. By increasing K concentration to 6 mM led to promotion of the growth and resistance to drought stress. This caused to reduce the activity of antioxidant enzymes and increased membrane stability; consequently the vase life of flowers increased, considerably. With increasing the concentration of potassium to 12 mM, the positive effect of K decreased against drought stress because of decrease in membrane stability and increase in H$_2$O$_2$ concentration. Although the concentration of 12 mM K proved that the growth of plant and vase life was better than the treatment with no potassium. It seems that the decrease in membrane stability and vase life in the higher concentration of K in nutrient solution can be due to the antagonism impact of potassium in the uptake of other particularly nutrient such as calcium that this needs to be considered for further investigations.

References:
Impact of potassium on the yield of lily (Lilium longifolium × …


