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Research Paper

The Effect of Cut Stem Length Treatment on Vase Life and Water Relations of Roses (*Rosa hybrida*) cv. 'Bingo White'

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Abstract

One of the most important factors in controlling the quality of fresh flowers is stem length because it is a major determinant of cut stem productivity. The grading and marketing of most cut flowers, like roses, are generally based on stem length. The present study aimed to evaluate the effects of cut rose flower stem length on vase life and water balance of rose cv. 'Bingo White' in a factorial experiment based on a Completely Randomized Design (CRD). Cut stem length was used at four levels of 25, 35, 45, and 55 cm kept in 50 mg/L sucrose sodium hypochlorite solution at two levels of 0 and 2% as preservative solutions with three replications at 23±2°C with 65±5% relative humidity and 12µmol/m2 s of light intensity (supplied by cool white fluorescent lamps) for 12 hours up to the end of vase life. Some qualitative and quantitative characteristics including vase life, flower and leaf water potential, ion leakage, membrane stability index (MSI), and leaf stomatal conductance were recorded. The results showed that the longest vase life (17 days) was significantly ($P < 0.01$) observed at the lowest stem length (25 cm) and the shortest vase life (10 days) was related to the highest stem length (55 cm). The lowest rate of leaf and petal ion leakage was significantly ($P<0.05$) observed in the lowest stem length (25 cm). The stem lengths of 35 and 45 cm, however, did not significantly differ from the stem length of 25 cm. The lowest rate of leaf and petal membrane stability index was significantly ($P < 0.05$) related to 55 cm stem length. The highest rate of flower and leaf water potential was significantly ($P<0.01$) obtained from 35 cm stem length as compared with other treatments. Stomatal conductance decreased over time, but it was not affected by stem length treatments significantly. Generally, our results showed that the shorter cut stem length treatment (25 cm) with the sucrose solution (2%) not only had the greatest effect on extending vase life but it also improved the water status, decreased the ion leakage, and increased the flower membrane stability index.

Keywords: Carbohydrate supply, Longevity, Rose, Stem length, Water status.

INTRODUCTION

Rosa hybrida is one of the most popular flowers among cut flowers (Suntipabvivattana, 2012), so it is widely used for decorative purposes (Hussen and Yassin, 2013). In the meantime, the international trade of cut rose flowers is growing rapidly, drawing interests in studying the post-harvest physiology of cut flowers because the post-harvest behavior of roses is the result of the physiological processes in their leaves, stems, flower buds, peduncles without leaves, or the location of the buds to the stem (Bhattacharjee and De, 2005). In this regard, stem length is one of the main reasons for determining the quality and efficiency of roses because the supply of roses to the market and their grading are based on the length stem (Oki *et al*., 2006). In addition, the vase life of different cultivars of cut flowers is correlated with the diameter and strength of the stem, which is a specialized organ for transporting water and materials from photosynthetic and chemical products (Myburg *et al*., 2001; Nowak, 1990). Although stems have the largest and most number of lines of the xylem vessel, the conductance of peduncles is lower than that of stems, and the lowest conduction is observed at the end of peduncles. Therefore, various components of the cut flowers have different resistance to water flow (Darlington and Dixon, 1991).

Accordingly, water potential is the water status of plant tissues (van Doorn, 2012), or it can be defined as the tendency for water inlet and outlet from the cells of a plant (Nijsse *et al*., 2001). According to some studies, the transfer of water and sucrose in vase solution occurs through xylem vessels in the stem (Victoria *et al*., 2003). Plant stems have xylem vessels that allow water to flow into the plant (Myburg *et al*., 2001), thereby influencing the growth and development of the plant (Nijsse *et al*., 2001), which determines the transpiration rate and the capacity to preserve the water content of the flower as well as the amount of absorption and transfer of the solution and the water balance in the cut flowers (Torre *et al*., 2003). Therefore, it can be said that the desirable status of water in cut flowers is a very important factor in determining their postharvest life (Dixon *et al*., 1988).

In addition, cut rose flowers are picked up at the maturity or bud stage, so they require many soluble carbohydrates to open. Treatment with sugars, such as sucrose and glucose in combination with antibacterial compounds, can increase the vase life of many cut flowers and can affect the production of ethylene and regulate the accumulation of carbohydrates in organs (Liao *et al*., 2000). Therefore, the shortening of the stems or the application of preservatives in the postharvest period will delay the occurrence of events caused by vascular occlusion. The severity of vascular occlusion in the stems of cut flowers varies with plant species (Spinarova and Hendriks, 2003). In other words, the short vase life of flowers is usually due to the undesirable water conductance in their stems, which leads to incomplete flower opening, petal wilting, and neck bent (Jin *et al*., 2006). Shorter stems (corresponding a shorter water transport path) or fewer leaves (corresponding to lower water loss from cut flower stems) will decrease the probability of expanding a negative water balance, leading to longer vase life of cut flowers (Fanourakis *et al*., 2013).

The purpose of this research was to study the effects of cut stem length treated with sucrose solution on the vase life and water balance of cut rose cv. 'Bingo White' and determining the most suitable cut stem length for this cultivar.

MATERIALS AND METHODS

Plant material preparation

In this experiment, the cut flowers of *Rosa hybrida* L. cv. 'Bingo White' were purchased from a commercial greenhouse producing rose flowers located in Tabriz, Iran. The flowers were harvested at the natural maturity stage (when the sepals began to bend) and were quickly transferred to the post-harvest biology lab of the Department of Horticulture, Faculty of Agriculture, Tabriz University. The roses were placed immediately in a bin containing distilled water. Then, three upper leaves close to the flower were kept on each stem. After that, all stems were cut to a length of 25, 35, 45, or 55 cm, and they were immediately placed in vase preservative solutions. Sucrose (0 and 2%) with 50 mg/L sodium hypochlorite solution was used as the vase solution. Vase preservative solutions were as follows:

1. Solution of 50 mg/L sodium hypochlorite without sucrose (S 0%)
2. Solution of 50 mg/L sodium hypochlorite + sucrose (S 2%)

The experiment was carried out at 23 ± 3°C, a relative humidity of 60 ± 5%, and light intensity of 12 µmol m^{-2} s^{-1} (cool white fluorescent lamps) for 12 hours per day.

Measurements

Vase life

The vase life was assessed from the time when the flowers were placed in the solution until the time when 50% of the petals showed wilting symptoms, dryness, or bent neck and were evaluated at the end of vase life (Rafi and Ramezanian, 2013).

Ion leakage and membrane stability index

Petal and leaf samples were prepared from the cut flowers by weighing 1 g from each sample. Then, the samples were washed three times with distilled water to remove contamination and shredded in small pieces. They were placed in test tubes containing 20 ml of twice-sterilized distilled water. The first group of test tubes was placed in a bain-marie at 40° C for 30 minutes, and the second group was placed in the bain-marie at 100°C for 10 minutes. The initial electrical conductance (EC1) for the first group and EC2 for the second group were measured by an EC meter device (Metrohm Model-712 Condutometer, Switzerland) and the ion leakage was calculated from the formula EL= (EC1/EC2) ×100. The membrane stability index was, then, calculated from the formula MSI= [1-(EL)] ×100 (Chakrabarty *et al*., 2009).

Water potential

A pressure chamber instrument (Model 615- I207 Bar/3000 PSI, USA) was used to measure leaf and flower water potential three times (2, 4, and 6 days of vase life period). Leaf samples with their petiole and flowers with its pedicel were cut off with a sharp and sterile razor. The samples were immediately placed in the pressure chamber instrument and the pressure control valve was gradually and slowly opened while the drain valve was closed. Then, the tips of the petiole and pedicel were observed with a magnifying glass while the pressure inside the pressure chamber was increasing. As soon as the phloem sap emerged at the site of the cut surfaces of the petiole and pedicel, the control valve was closed, and immediately the corresponding number was read at the pressure gauge. The negative leaf water potential was the actual pressure: $\psi = -P$ (Scholander *et al*., 1965; Scholander *et al*., 1964).

Stomatal conductance

Stomatal conductance of the leaves was measured at the lower part of the leaf using a porometer (AP4 Delta-T Devices Cambridge, UK) in fully developed mid-leaflet of the leaf under the flower.

Statistical analysis

This study was conducted as a factorial experiment based on a completely randomized design (CRD) with three replications for each treatment. The factors were four different levels of stem length (25, 35, 45, and 55 cm) and two levels of sucrose (0 and 2%). Each replication consisted of four cut flower stems. Data analysis was performed by using SAS software version 9.4

with the GLM method and means were compared by Duncan's multiple range test at the 5% level.

RESULTS

Vase life

The results showed that the effects of stem length and sucrose alone were significant ($P<0.05$ and $P<0.01$, respectively; Table 1) on the vase life of the cut rose flowers (Fig. 1).

According to Fig. 1a, there was a significant difference between 25 cm stem length and the other levels in extending vase life. Also, the treatment of sucrose 2% significantly increased the vase life of the flowers compared with sucrose 0% (Fig. 1b). There was a significant negative correlation (r = -0.534) between vase life and stem length (Table 3). However, a significant positive correlation (r = 0.465) was observed between vase life and sucrose. These results show that the vase life was increased by reducing stem length and by using sucrose (Table 3).

Table 1. Analysis of variation of the effect of stem length, sucrose and time on some qualitative characteristics of cut rose flowers cv. 'Bingo White'.

S.o.V	df	MS							
		Vase life	Stomatal conductance	Leaf ion leakage	Petal ion leakage	Leaf water potential	Flower water potential	Membrane stability leaf	Membrane stability petal
Stem length (SL)	3	61.579*	0.212**	80.76*	98.43**	92.65*	81.08**	80.77*	108.21**
Sucrose (S)	1	108.75**	0.085^{ns}	39.20^{ns}	38.01^{ns}	109.76**	34.92^{ns}	39.20^{ns}	50.45^{ns}
Time (T)	1	-	0.054^{ns}	89.46*	88.4*	17.85^{ns}	28.82^{ns}	89.5*	34.92^{ns}
SL×S	3	50.15^{ns}	0.064^{ns}	32.21^{ns}	41.07^{ns}	41.71^{ns}	39.28^{ns}	31.21^{ns}	41.76^{ns}
SL×T	1	-	0.012^{ns}	32.06^{ns}	37.4^{ns}	30.73^{ns}	45.22^{ns}	51.75^{ns}	30.57^{ns}
S×T	3	-	0.010^{ns}	42.96^{ns}	48.01^{ns}	37.98^{ns}	23.78^{ns}	41.76^{ns}	48.87^{ns}
SL×S×T	3	-	0.050^{ns}	40.34^{ns}	52.86^{ns}	53.76^{ns}	19.34^{ns}	40.80^{ns}	45.87^{ns}
Error	16	11.95	0.023	21.31	27.01	14.08	9.76	23.34	21.87
CV (%)	-	21	16	18.8	23.7	19.65	15.5	25.5	27.4

*, ** and ^{ns}: Significant at $P<0.05$, $P<0.01$ and insignificant, respectively.

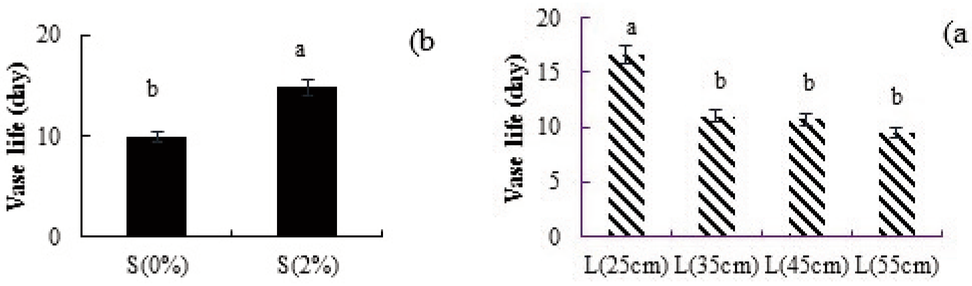

Fig. 1. Means comparison for the effect of stem length (a) and sucrose (b) treatments on vase life of cut rose flower cv. 'Bingo White'.

Ion leakage and membrane stability index

The results indicated that stem length treatment had a significant ($P < 0.05$) effect on decreasing stem leaf ion leakage (Table 1) as the highest level of ion leakage (19.303%) was observed in the leaves of 55 cm stem length compared with the other levels of stem length (Table 2). Also, ion leakage of flower petals was significantly ($P < 0.01$) the highest in 55 cm stem length (49.936%) in comparison to the other levels of stem length treatment (Table 2). Membrane stability index of the leaf and flower petal samples were significantly ($P <0.05$ and $P < 0.01$, respectively) decreased by increasing stem length as the lowest value was observed in the stem length of 55 cm (80.769 and 45.11%, respectively; Table 2). However, there was no significant difference among cut stem lengths and evaluation times in leaf and flower ion leakage content (Fig. 3). Pearson correlation coefficient values among treatments and variables showed that there was a significant and positive correlation between ion leakage of leaves and petals and stem length as $r=0.322$ and $r=0.273$, respectively (Table 3). These values for correlation relationship between stem length and membrane stability index of the leaf and petal samples were significant and negative ($r = -0.322$ and $r = -0.335$, respectively; Table 3).

Leaf stomatal conductance

Leaf stomatal conductance was decreased significantly ($P < 0.01$) by increasing stem length (Table 1) so that the highest value (0.283 mmol $m^{-2} s^{-1}$) was observed at stem length of 25 cm and the lowest (0.187 mmol $m^{-2} s^{-1}$) at stem length of 55 cm (Table 2). There was a non-significant negative correlation ($r=-0.06$) between stem length and stomatal conductance of stem leaves (Table 3).

Water potential

Leaf and flower water potential was influenced by the treatments of stem length and sucrose during the vase life of cut rose flower cv. ‘Bingo White’. The results showed significant effects of stem length and leaf and flower water potential at the $P <0.05$ and $P <0.01$ levels, respectively (Table 1). As shown in Fig. 2a, the lowest value of leaf water potential (-9.078 bars) was observed at the maximum stem length (55 cm), which significantly differed from the other levels of stem length by the end of vase life. The highest leaf water potential (-3.67 bars) was related to the stem length of 35 cm although there was no significant difference among the stem lengths of 35, 25, and 45 cm. Also, the treatment of sucrose 2% showed higher water potential (-3.88 bars) than sucrose 0% (-7.43 bars), indicating a significant difference between the levels of sucrose treatments on the leaf water potential (Fig. 2c). According to Fig. 2b, the stem length of 55 cm showed the lowest flower water potential (-10.97 bars) by the end of vase life, which significantly differed from the other levels of stem lengths although there was no significant difference among the other treatment levels of stem lengths by the end of vase life (Fig. 4). According to Table 3, there was a negative significant correlation ($r=-0.558$) between stem length and flower water potential and a positive significant correlation ($r=0.441$) between leaf water potential and sucrose treatment so that they showed a better flower water potential in shorter lengths and the presence of sucrose improved the leaf water potential.

Table 2. Mean comparison of the effect of stem length treatment on some of traits of cut rose flower cv. ‘Bingo White’.

Stem length(cm)	**Leaf ion leakage (%)**	**Flower petal ion leakage (%)**	**Stomatal conductance (mmol m^{-2} s^{-1})**	**Leaf membrane stability (%)**	**Petal membrane stability (%)**
25	^{b} 13.547	^{b} 38.075	^{a} 0.283	^{a} 86.465	^{a} 61.915
35	^{b} 14.639	^{b} 37.888	^{b} 0.236	^{a} 85.361	^{a} 62.112
45	^{b} 14.415	^{b} 33.853	^{a} 0.273	^{a} 85.583	^{a} 66.148
55	^{a} 19.303	^{a} 49.936	^{c} 0.187	^{b} 80.697	^{b} 45.111

In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan’s multiple range test.

Table 3. Pearson correlation coefficients of stem length and sucrose treatments with the recorded traits.

	Stem length	**Sucrose**	**Vase life**	**Leaf Stomatal conductance**	**Leaf ion leakage**	**Petal ion leakage**	**Leaf water potential**	**Flower water potential**	**Leaf membrane stability**	**Petal membrane stability**
Stem length	1									
Sucrose	0.00^{ns}	1								
Vase life	**0.534-	*0.465	1							
Stomatal conductance	^{ns} 0.060-	-0.222^{ns}	^{ns} -0.154	1						
Leaf ion leakage	*0.322	0.152^{ns}	^{ns} 0.226	-0.109 ^{ns}	1					
Petal ion leakage	*0.273	^{ns} 0.190	^{ns} -0.142	-0.261 ^{ns}	***0.578	1				
Leaf water potential	^{ns} -0.287	**0.401	^{ns} 0.377	0.079 ^{ns}	0.175 ^{ns}	-0.019 ^{ns}	1			
Flower water potential	**-0.558	^{ns} 0.050	^{ns} 0.295	-0.137 ^{ns}	0.044^{ns}	-0.201 ^{ns}	***0.591	1		
Leaf membrane stability	*-0.322	^{ns} -0.152	^{ns} -0.226	0.109 ^{ns}	***-1.00	***-0.578	0.176 ^{ns}	-0.044 ^{ns}	1	
Petal membrane stability	*-0.335	^{ns} -0.078	^{ns} 0.281	0.099 ^{ns}	**-0.450	***-0.836	0.127 ^{ns}	*0.367	**0.450	1

† ^{ns} not significant, * Significant at $P < 0.05$, ** Significant at $P < 0.01$ and *** Significant at $P < 0.001$

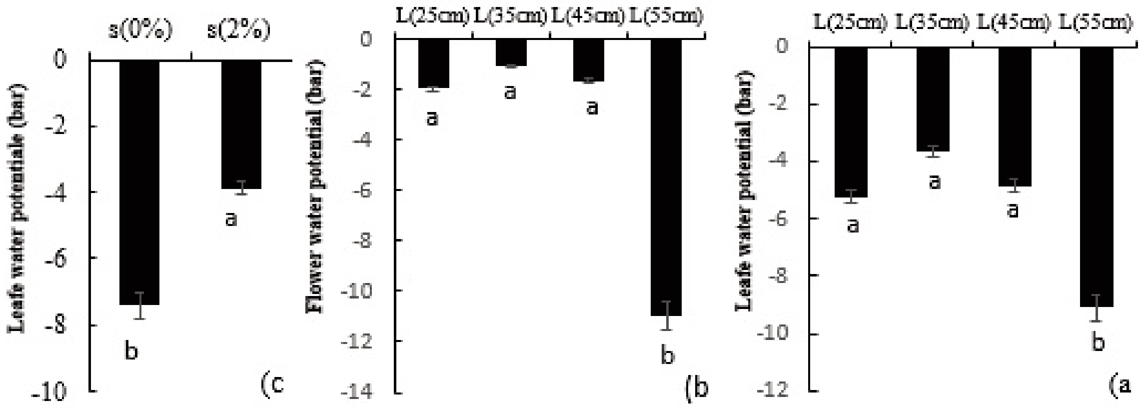

Fig. 2. The mean effect of stem length (cm) on the leaf water potential (a), flower (b), and the effect of sucrose (%) on leaf water potential (c) in rose cv. ‘Bingo White’.

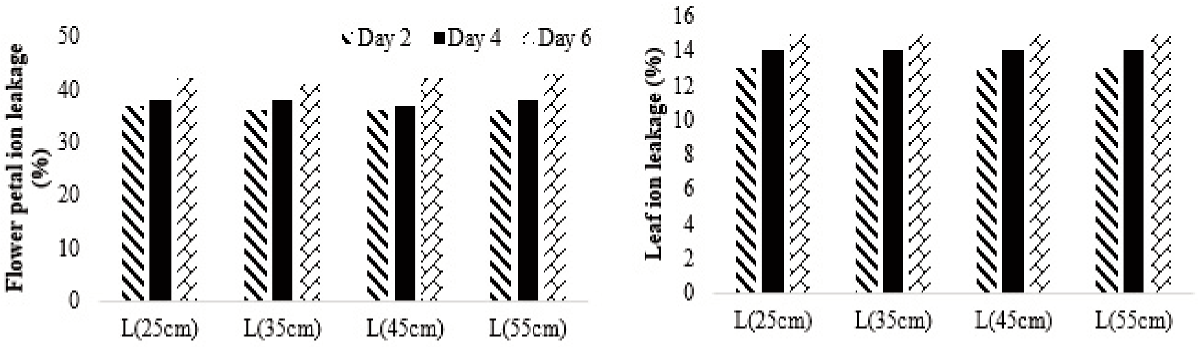

Fig. 3. The mean effect of stem length (cm) and evaluation time (days 2, 4 and 6) on flower petal ion leakage and leaf ion leakage in cut rose cv. ‘Bingo White’.

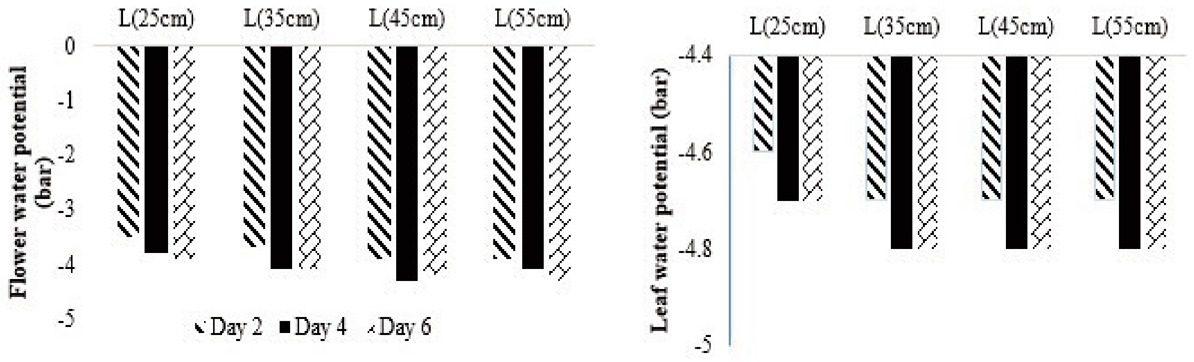

Fig. 4. The mean effect of stem length (cm) and evaluation time (days 2, 4 and 6) on flower petal water potential and leaf water potential in cut rose cv. ‘Bingo White’.

DISCUSSION

According to the results, the use of the stem length of 25 cm and sucrose solution increased the vase life and improved the water relationships of the cut flower of *Rosa hybrida* cv. 'Bingo White'. The movement of water in the plants carried of through the stem xylem vessels (Myburg *et al*., 2001). Also, the transfer of water and sucrose in the vase solution occurs through the xylem vessels in the stem (Victoria *et al*., 2003). Therefore, it can be said that the slight facilitation of water flow in the lower vessel elements of the stem hinders the vascular blockage in that part (Spinarova and Hendriks, 2003). As Mortensen and Gislerod (1999) pointed out, it is easier to compensate for water losses in shorter stalks. Also, in studies on different cultivars of cut flowers, it was concluded that in stems with the same number of leaves, longer stem length implies a longer water transfer path, which is negatively associated with the vase life. This depends on the cultivar (Särkkä, 2002). Särkkä (2005) stated that longer stems of cut rose flowers exhibited a higher rate of bent neck incidence, which was related to the vase life of the cut flowers. This is consistent with the results of this study.

The water potential of the xylem vessels is reduced because of the increase in transpiration or the decrease in water absorption, and consequently, the cavitation increases. The results of the present experiment are partly indicative of this issue, which has a better water supply in a shorter length. As a result, more water moves toward the flower and leaves, and the relative water content increases. On the other hand, the entry of air into the cut stems causes roses to be subject to water stress, which reduces the water conductance in the stems (Evans *et al*., 1995). In this case, since the transpiration rate is greater than the absorption rate, the water loss due to transpiration is not compensated by the amount of water absorption, and the leaf water potential is reduced. The water absorption rate during the post-harvest period was not sufficient to compensate for the increase in leaf transpiration, which led to the emergence of stress symptoms in the cut stems. Thus, the leaf water potential reduces (Fanourakis *et al*., 2012). A study on water relations in cut roses cv. 'Samanta' showed that cavitation in vessel elements started in stems at a water potential of -0.2 to -0.4 MPa. The cavitation rate was increased with decreasing water potential, which was related to the reduction of hydraulic conductivity in the stem. A vast majority of cavitation occurs in stems with water potential below -2.5 MPa and the vase life may, in some cases, be related to the percentage operation of the remaining xylem vessels in the stem (Dixon *et al*., 1988).

External sucrose plays an important role in the quick preparation of a substrate for respiration and slowing down the destruction of proteins. Therefore, it delays aging (Suntipabvivattana, 2012). The accumulation of carbohydrates in petals decreases the water potential in petals, so the cells expand by the entry of water, which may lead to flower opening (Horibe *et al*., 2014). In the present study, the maximum length of the stem (55 cm) had the minimum water potential due to the probability of the occurrence of the cavity and the imbalance between the absorption and transpiration of the leaves in the cut flower. In addition, the presence of sucrose in solution, and its movement towards the leaf, reduced the water potential in the cells and increased water flow to the leaves. In this case, the water state of the flower is better in shorter stems and sucrose solutions for proportionally long stems and sucrose-free solutions. During the aging process, changes occur in the permeability of membranes and protein degradation increases (Kuiper *et al*., 1995). The inability to absorb water and high transpiration in cut flowers is one of the main symptoms of aging, which causes early wilting (Meeteren *et al*., 1999; van Ieperen *et al*., 1999). Physiological changes that lead to aging include changes in the lipid membrane, increased ethylene production, membrane permeability loss, ion leakage, and eventually weight loss due to the water loss. With the leakage of the cellular compositions outside, the strength and integrity of the membrane of the cell disappear (Bhattacharjee and Banerji, 2010). Regarding the relationship between the contents, it can be stated that the increase in ion leakage was tested over time in cut rose flowers and the ion leakage was more in petals and flower leaves on longer stems due to the water imbalance in the stem and the probability of vascular occlusion, which finally results in ion leakage in the petals and leaves at early aging stages. With the appearance of aging signs, cellular leakage occurs and cell membrane strength and integrity disappear. Consequential changes in flower physiology lead to aging. These changes in the lipid membrane increase ethylene production and the loss of overall permeability, which leads to ion leakage and eventually a decrease in flower weight, which leads to an increase in water loss. Cellular leakage leads to a loss of structural stability of the cell membrane (Bhattacharjee and Banerji, 2010). Therefore, it can be said that due to water imbalance in the stem and imbalance between absorption and transpiration in longer stems in the present study, early deterioration occurred in the flowers, which decreased the stability of the membrane in flower petals and leaves.

Stomatal conductance control is affected by water flow and water potential (Terfa *et al*., 2014). When a plant is exposed to water stress, its water potential decreases. In this case, the stomata are closed (Torre *et al*., 2003). On the other hand, one of the important factors in aging is the water status in the plant, which decreases water potential by losing water, resulting in vascular occlusion and bend neck of the flower (Suntipabvivattana, 2012). The abscisic acid in petals increases during aging, accelerates metabolic processes, and shortens the vase life of the flower (Borohov *et al*., 1976). Therefore, it can be said that water loss can occur due to the occlusion or the increase in stomatal transpiration in the stem over time, in which the amount of abscisic acid in the flower increases, and as a result, aging occurs in the flower, and stomatal conductance decreases over time in leaves.

CONCLUION

In general, the results showed that shortening (re-cutting) of cut rose flowers along with the use of a sucrose solution extended their vase life and improved leaf and flower water relations in cut stems. The 25 cm cut rose stems treated with sucrose 2% vase solution showed the longest vase life in the cut stem of rose cv. ‘Bingo White’. Our results revealed that short stems (i.e. short water transport path) would be less posed to the chance of developing a negative water balance leading to a longer vase life and higher membrane stability index in their leaves and flower petal cells which would delay of senescence in cut rose flowers.

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