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Some characteristics of tuberose as affected by pre-harvest application of calcium chloride and gibberellic acid

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Key words: bulbous plant, cut flower, ethylene, longevity, postharvest.

Abstract: In the present study the effect of gibberellic acid (GA_3) and calcium chloride ($CaCl_2$) sprays (0, 150, 300 and 450 ml L^{-1}), applied 25 and 15 days before harvesting, on physiological and morphological characteristics of tuberose 'Pearl Double' was studied. Cut flowers were harvested and transported to the laboratory where they were placed in distilled water. The experiment considered some parameters for evaluation, such as relative water content of leaves and petals, water intake, percentage of open florets, electrolyte leakage, ethylene production, chlorophyll and carotenoid content. Results indicate that the best treatment was the combination of 150 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} GA_3 for most of parameters.

1. Introduction

Tuberose (*Polianthes tuberose* L.) is one of the most important cut flowers in tropical and subtropical areas and as cut flowers they are among the most important for flower bouquets, baskets and wreaths (Kendirli and Cakmak, 2007). The florets have a very sweet fragrance and are widely cultivated in India and France as a source of essential oils for the perfume industry. *Polianthes* is also a common garden plant in the spring and it flowers during the summer and early autumn (De Hertogh and Le Nard, 1993). Two major cultivars, white-colored 'Single' and 'Double', are for commercial production (Shen *et al*., 2003). In tuberose, fewer than 50% of the buds normally open after harvest and florets and buds usually drop off after a few days in vase. Postharvest performance is worse in tuberose which has been shipped to distant markets (Waithaka *et al*., 2001). Keeping quality of spikes is only three days for florets and vase life of flowers is only a few days. Since it has delicate flowers and sellers and customers are keen to extend its vase life, it is necessary to improve its postharvest life (Anjum *et al*., 2001). Senescence in cut flowers is affected by three main parameters: the water balance, the supply of carbohydrates, and susceptibility to ethylene (Cortes *et al*., 2011). Treatment with gibberellic acid has also been shown to enhance postharvest life and quality of gerbera cut flowers. Using GA_3 at different concentrations improved membrane stability index, leading to better flower vase life of gerbera cut flowers (Emongor, 2004). Similar effects on membrane stability index have been reported in gladiolus with BA and GA_3 (Singh *et al*., 2008). GA_3 treatment of *sandersonia* flowers delays the senescence-associated increase in protease activity, which by implication delays the breakdown of senescence-associated proteins (Eason, 2002). Calcium (Ca) is an important element which is found in 3% of the earth's crust. It is essential to living organisms and to plant growth and development. Some of these benefits include stronger cell walls, increased postharvest life of flowering plants, and increased disease resistance (Robichaux, 2005). Calcium spray increased the life of rose petals by increasing the relative water content (RWC), maintaining turgidity of leaf cells, avoiding cell wall deformation, and decreasing electrolyte leakage from cells of cut flowers by increasing cell

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wall integrity and stability (Mortazavi *et al*., 2007). Floret abscission and short vase life of tuberose are common problems, thus the aim of this study was to determine the effect of foliar application of gibberellic acid and $CaCl_2$ treatments on some morpho-physical parameters that affect the vase life of cut tuberose.

2. Materials and Methods

This experiment was conducted in summer 2012 at the commercial tuberose (double cultivar) farm located in Tarom county of Zanjan province, Iran; latitude 36° 57′ North and longitude 48° 54′ East. About 500 m^2 of this farm is used for experimental treatments. The plants received as foliar spray $CaCl_2$ (from Merck), with molar mass 147.01 g M^{-1}, and gibberellic acid (from Merck), with molar mass 346.37 g M^{-1}, 15 and 25 days before harvest of flowers. $CaCl_2$ (0, 150, 300 and 450 ml L^{-1}) (0, 1, 2 and 3 mM) and gibberellic acid (0, 150, 300 and 450 ml L^{-1}) (0, 0.4, 0.8 and 1.2 mM) were used. Before application, leaves of plants sprayed with distill water, one hour after distil water spired, $CaCl_2$ treatment (four liters) and two hours after that gibberellic acid treatments (four liters) were done until runoff. When most of the two or three lowest inflorescence florets opened, flowers were harvested and immediately transferred to the postharvest laboratory at the Department of Horticulture, Faculty of Agriculture, Zanjan University. Flowering stems then were cut to 65 cm, weighted (fresh weight) and placed in 400 ml distilled water. All experiments were performed in a postharvest room equipped with a controlled environment maintained at 22±1°C, 45±5% relative humidity and light intensity for 12 h/day by cool-white fluorescent lamps.

The following parameters were measured: fresh weight of flowers and numbers of open florets of inflorescences before being placed in distilled water, rate of opening and abscission of florets every three days, water uptake every four days, relative water content (RWC) of leaves and petals, cell membrane injury (CMI) of petals, chlorophyll and carotenoid content of plant tissue, and ethylene production of flowers. Vase life longevity was recorded when at least four open florets on the inflorescence were present.

To measure the RWC, 1 g of petal tissue (fresh weight, F.W.) was immersed in distilled water for 24 h and weighed again (turgid weight, T.W.), then dried at 80°C inside an oven for 48 h (dry weight, D.W.). RWC was calculated using the following equation (Turner, 1981):

$$RWC= (F.W.-D.W.)/(T.W.-D.W.)\times100$$

Cell membrane injury was measured following the Isacc and Urban (1995) method; after seeing the first flowers of inflorescence, measurements were done. One g of petal tissue of top open bud flowers weighted of each plant and washed with distilled water, then were immerged in glass container containing 10 ml of distilled water and were placed inside a benmary (Gemmy Ind. Corp., Taiwan) at 30°C for 60 min and EC was measured (EC_1), then were placed inside a autoclave at 120°C for 20 min, and EC was measured again (EC_2), and CMI was calculated using the following equation:

$$CMI =1-(EC_1/EC_2) \times100$$

Ethylene production of flowers 3, 6, 12 and 24 h after placement in 500 ml distilled water at 23°C was measured by an Ethylene biosynthesis bioconservation device model ICNA56. Treated flowering inflorescence was in distilled water in the laboratory with the same conditions inside the container and packaging (Volume 2.5 liters) were (Almost all the flowers were the same size). Flowers in the tank without meeting with any special hole for injection or sampling were used and thus ethylene concentrations were prepared by flowers. Chlorophyll and carotenoid content were determined by spectrophotometeric method (Arnon, 1949).

The present study was carried out in a complete randomized design with factorial arrangements including 16 treatments and three replications. Data were analyzed by MSTATC software and means were compared using LSD test at 5% level.

3. Results and Discussion

Effect of $CaCl_2$ and gibberellic acid on relative water content of tuberose leaves (RWCL) and florets (RWCF)

Results indicated that interaction of 450 ml L^{-1} gibberellic acid and 150 ml L^{-1} $CaCl_2$ had a significant effect at 5% level on the relative water content of florets compared with the control (Table 1). Our results agree with those of Cortes *et al*. (2011) on *Rosa x hybrid* cv. Grand Gala, Mortazavi *et al*. (2007) on *Rosa* x *hybrida* cv. Iliona and Abdolmaleki *et al*. (2015) on cut rose cv. Dolce Vita. Gibberellic acid reducing water loss via transpiration, increase water

uptake in plant tissues (Emongor, 2004). Calcium spraying increased the life of petals by increasing the RWC, maintaining leaf cell turgidity and avoiding cell wall deformation. Gibberellic acid application together with $CaCl_2$ might increase the efficiency of Ca use in plants.

Effect of $CaCl_2$ and gibberellic acid on water uptake of tuberose (WU)

Results indicated that 450 ml L^{-1} CaC_2 + 450 ml L^{-1} gibberellic acid gave maximum water uptake (Table 1). These results agree with results of Vijaya *et al*. (1999) on cut tuberose, Cortes and *et al*. (2011) regarding *Rosa hybrid* cv. Grand Gala, Dansheng (2003) on cut rose, and Sosa Nan (2007) on sunflower. Calcium interacts with polygalacturonic acid (PGA) groups, forming a structure known as an "Egg box", which causes the contraction of pectins in the pit borders, increasing the diameter and, consequently, water flow (Cortes *et al*., 2011). The increased reducing sugars in flower heads and stems of gerbera cut flowers may increase the osmotic potential of flower head and stem, thus improving their ability to absorb water and maintain their turgidity (Emongor, 2004). Water uptake improved in tuberose by foliar application of gibberellic acid and $CaCl_2$.

Effect of $CaCl_2$ and Gibberellic acid on opening florets of tuberose (OP)

Interaction of 150 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberellic acid yielded the maximum amount of opening florets (Table 1). This result concurred with Halevy *et al*. (2001) who reported that $CaCl_2$ treatment promoted bud opening and delayed senescence in rose cut flowers. The treated flowers stayed turgid and continued their initial postharvest growth for longer periods. Treatment with GA_3 is useful for improving the vase life of cut *N. tazetta* var. *chinensis* flowers (Ichimura and Goto, 2000). Spraying with 200 ml L^{-1} GA_3 increased plant height, early flowering, spike length and number, rachis length, flower weight and length, and total flower yield in a study carried out by Bharathi and Kumar (2009), which was the same as our results. Gibberellins increase hydrolysis of starch, fructans and sucrose into glucose and fructose which are utilized by the flowers for disc floret opening (Emongor, 2004).

Effect of $CaCl_2$ and Gibberellic acid on floret abscission of tuberose (AF)

The lowest percentage of floret abscission (9.83%) was found at 300 ml L^{-1} gibberellic acid while the highest (23.45%) was found at 150 ml L^{-1} $CaCl_2$ (Table 1). Our results were similar to those of Uthairatanakij (2005) who reported $CaCl_2$ significantly reduced the postharvest dropping of orchid buds flower compared to control. Calcium treatment probably increases the strength of cell walls. The abscission of leaves, flowers, and fruits is presumed to be brought about through the weakening of the cell walls in the abscission zone. This weakening may have two components: a solubilizing of the cell wall cementing sub-

Table 1 - Means of interaction of CaC_2 and gibberellic acid on relative water content (RWC) of leaves and florets (%), water uptake (mL), opening and abscission of florets, number of florets, fresh weight of flower (g), vase life (day), chlorophyll content a, b and total chlorophyll content (mg), and carotenoid content (µg) of cut tuberose (*Polianthes tuberosa* L.) after first flower opening of inflorescence

$CaCl_2$ (ml L^{-1})	Gibberellic acid (ml L^{-1})	RWC of least (%)	RWC of floret (%)	Water uptake (ml)	Opening florets (%)	Abscission of florets (%)	Number of florets	Fresh weight of flower (g)	Vase life longevity (day)	Chlorophyll content b (mg)	Total chlorophyll content (mg)	Carotenoid content (µg)
0	0	82.48 g*	76.12 de	111.7 bc	20.77 g	12.24 fgh	35.33 ab	78.46 ef	8.50 h	0.04600 bcdef	0.03267 efg	0.01300 g
	150	92.67 b	70.54 fg	105.6 bcdef	29.43 def	11.14 fgh	32.67 bcd	78.28 ef	9.30 g	0.03767 cdef	0.04867 cde	0.02333 cd
	300	87.51 def	76.66 de	98.89 f	30.15 def	09.83 h	36.33 a	88.27 ab	9.77 def	0.05400 bcdef	0.03567 defg	0.01300 g
	450	94.91 a	91.58 b	112.2 b	37.66 ab	10.70 gh	32.67 bcd	82.46 cde	11.37 b	0.07333 bc	0.01037 a	0.01900 de
150	0	79.39 h	85.82 c	110.6 bcd	31.02 def	23.45 a	33.00 bcd	78.64 def	10.80 c	0.03133 def	0.01733 g	0.01000 h
	150	96.66 a	70.73 fg	103.9 cdef	36.27 abc	18.75 bc	34.00 abc	79.74 def	9.43 fg	0.02100 f	0.02367 fg	0.01267 g
	300	82.27 g	73.75 ef	110.6 bcd	34.50 bcd	16.70 cde	32.00 cd	88.75 a	9.57 efg	0.04600 bcdef	0.03500 defg	0.01833 e
	450	73.99 i	96.10 a	113.3 b	40.74 a	13.72 efg	35.33 ab	82.01 cde	11.83 a	0.16260 a	0.05933 c	0.02233 ab
300	0	86.55 ef	79.45 d	103.9 cdef	30.74 def	20.92 ab	35.00 ab	80.92 def	10.13 d	0.06767 bcd	0.03333 efg	0.01067 h
	150	85.58 f	77.06 de	100.0 ef	27.94 ef	20.22 b	34.67 ab	83.57 bcd	9.90 de	0.03300 def	0.04700 cdef	0.01967 de
	300	89.02 cd	73.29 ef	110.0 bcd	29.82 def	14.23 def	36.00 a	77.50 ef	9.53 efg	0.04233 bcdef	0.05700 cd	0.02133 bc
	450	90.27 c	86.30 c	113.3 b	32.40 cde	13.75 efg	31.00 d	77.77 ef	11.00 bc	0.08000 b	0.08100 b	0.02367 a
450	0	86.48 ef	74.44 ef	103.3 def	29.83 def	17.16 cd	31.00 d	79.24 def	9.47 fg	0.06700 bcde	0.01700 g	0.01333 g
	150	88.33 cde	68.17 g	106.1 bcdef	26.56 f	16.61 cde	35.00 ab	75.88 f	8.70 h	0.02933 ef	0.02533 efg	0.01300 g
	300	87.68 def	71.28 fg	107.8 bcde	30.32 def	12.33 fgh	36.33 a	86.15 abc	9.30 g	0.04333 bcdef	0.03167 efg	0.01667 f
	450	89.49 cd	90.72 b	123.3 a	40.70 a	11.54 fgh	33.67 abc	86.35 abc	11.30 b	0.04833 bcdef	0.03433 defg	0.01633 f

Means in the same column followed by the same letter are not significantly different using LSD test level 5%.

stances, and a hydrolysis of the structural components of the wall. A major part of the cementing properties of walls is presumed to be through the binding of pectic substances by double salt formation with Ca ions (Poovaiah and Leopold, 1973). Gibberellic acid delays flower abscission by decreasing the amount of dry matter (Khan and Chaudhry, 2006).

Effect of $CaCl_2$ and gibberellic acid on number of tuberose florets (NF)

Our results indicate that the interaction of 300 ml L^{-1} gibberellic acid and 450 ml L^{-1} $CaCl_2$ yielded the maximum number of florets, while the interaction of 300 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberllic acid gave the fewest (Table 1). Our results were similar to those found by Parmar *et al*. (2009) on spider lily, Mukhopadhyay and Bankar (1983) on tuberose, and Singh *et al.* (1991) on African marigold (*Tagetes erecta* L.), who reported an increase in number of florets because of role of gibberellic acid on cell elongation and division.

Effect of $CaCl_2$ and gibberellic acid on fresh weight of tuberose florets (FW)

Our results show that the interaction of 150 ml L^{-1} $CaCl_2$ and 300 ml L^{-1} gibberellic acid led to the maximum fresh weight of florets (Table 1), findings which are in agreement with those of Cortes *et al*. (2011) and Dansheng (2003) regarding rose, Sosa Nan (2007) working on sunflower, and Vijaya *et al*. (1999) tuberose. The effect of gibberellic acid on the fresh weight of florets may be a result of its role on increasing cell division (Arun *et al*., 2000).

Effect of $CaCl_2$ and gibberellic acid on vase life of tuberose (VL)

Application of 150 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberellic acid had significant effect on vase life parameter of tuberose (Table 1). Cortes *et al*. (2011) found that using $CaCl_2$ in the vase water of rose cv. Grand Gala gave maximum fresh weight. Loss of cell membrane integrity is characteristic of senescence in plants. Calcium protects the membranes from lipid degradation probably through several mechanisms. Calcium can stabilize the plasma lemma by binding to the negatively charged head groups of PL, which become less prone to degradation by lipolytic enzymes (Cheour *et al*., 1992). Analogous results were found by Uthairatanakij *et al*. (2005) regarding spraying $CaCl_2$ on Dendrobium orchid, and by Robichaux (2005) regarding the effect of calcium chloride, sulfate or nitrate spray on the vase life of rose and poinsettia. Gibberellic acid increases water absorption and relative water content, resulting in vase life longevity. Our results also agree with the findings of Su *et al*. (2001) on tuberose and Emongor (2004) on gerbera flower.

Effect of $CaCl_2$ and gibberellic acid on cell membrane injury of tuberose (CMI)

Results reveal that the minimum cell membrane injury was at 300 and 450 ml L^{-1} gibberellic acid and with interaction of 450 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberellic acid (Fig. 1). The enhancing effect of the application of Ca can be explained on the basis of its role in cell membrane structure. It may be noted that Ca stabilizes cell membranes by connecting various proteins and lipids at membrane surfaces, influences the pH of cells and prevents solute leakage from cytoplasm and increase shoot elongation (Al-Whaibi *et al*., 2010). If low Ca makes the membrane more permeable, it should follow that elevated concentrations make the membrane less permeable (Hepler, 2005).

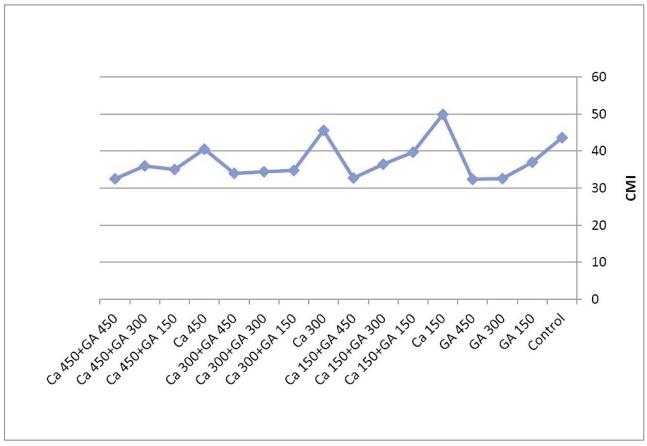

Fig. 1 - Means of interaction of $CaCl_2$ and gibberellic acid on cell membrane injury (CMI) of leaves of cut tuberose.

Effect of $CaCl_2$ and gibberellic acid on ethylene production of tuberose

The lowest values of ethylene production after 3 h were found in 450 ml L^{-1} gibberellic acid and in of 300 ml L^{-1} $CaCl_2$ + 450 ml L^{-1} gibberellic acid; these values were significant compared to control and most of the other treatments. Ethylene production after 6 h of treatment with 300 ml L^{-1} gibberellic acid and after 12 h with 300 ml L^{-1} $CaCl_2$ combined with 150, 300 and 450 ml L^{-1} gibberellic acid, respecitvely, showed the lowest values. After 24 h of treatment, the lowest levels were found with 450 ml L^{-1} $CaCl_2$ + 150, 300

and 450 ml L^{-1} gibberellic acid. Highest ethylene production was found at 3 and 12 hours with treatments of 150 ml L^{-1} $CaCl_2$ + 150 ml L^{-1} gibberellic acid, at 6 hours with treatment 150 ml L^{-1} $CaCl_2$, and 150 ml L^{-1} $CaCl_2$ + 150 ml L^{-1} gibberellic acid at 24 hours (Fig. 2). Pre-harvest treatment of $CaCl_2$ decreased ethylene production, which agrees with the results of Uthairatanakij *et al*. (2005) on orchid and Cortes *et al*. (2011) on rose. Calcium decreased activity and effect of ethylene on cell walls and affected senescence with inhibition of cell membrane injury. The application of calcium spraying gave the result to improve the strength of plant cell wall and delayed the senescence processes by inhibition of ethylene synthesis. In addition, calcium ions also seem to affect ethylene action on cell membranes by inhibiting ion leakage and reducing the effect of ethylene on senescence (Asfanani *et al*., 2008). Gibberellic acid treatments decreased ethylene production compared with the control, findings that agree with the results of Ichimura and Goto (2000) on *Narcissus* and Lers *et al.* (1998) on parsley. Inhibition of ethylene production by gibberellic acid is related to the ethylene production enzyme. Gibberellic acid inhibited ACC enzyme activity and resulted in inhibition of ethylene production (Ben-Arie and Ferguson, 1991).

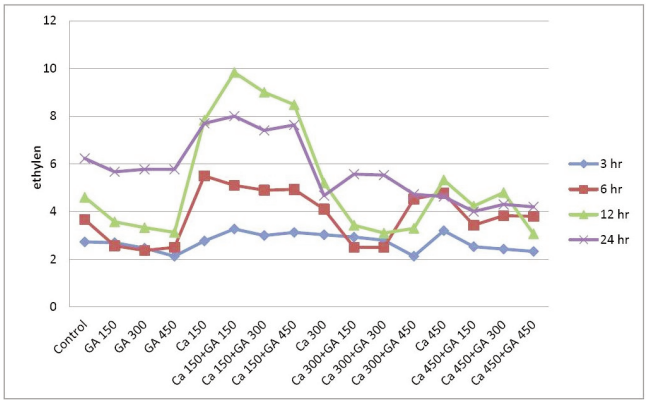

Fig. 2 - Means of interaction of $CaCl_2$ and gibberellic acid on ethylene production after 1, 2, 3 and 4 hours (nL g^{-1} h^{-1}) of cut tuberose.

Effect of $CaCl_2$ and gibberellic acid on chlorophyll and carotenoid of tuberose

Results of this study indicate that the interaction effect of 300 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberellic acid on chlorophyll a content was significant. Also 150 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberellic acid had a significant effect on chlorophyll b content. The lowest chlorophyll b content was found in treatment with 150 ml L^{-1} of gibberellic acid and $CaCl_2$. Total chlorophyll content was highest with 450 ml L^{-1} gibberellic acid. The maximum carotenoid content was recorded at 300 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberellic acid, with the lowest level was found at 150 and 300 ml L^{-1} $CaCl_2$ (Table 1). Calcium treatment caused the leaves to grow greener in color and the stems to grow more (Asfanani *et al.,* 2008). According to the results of Aharoni (1989), Lers *et al*. (1998), Ichimura and Goto (2000), Ferrante *et al*. (2002), and Khan and Chaudhry (2006), yellowing of leaves destruction and decrease of chlorophyll can be delayed by gibberellic acid treatments. Our results reveal that using $CaCl_2$ and gibberellic acid together was better at increasing chlorophyll a, b, and total content than using each one alone. The same results were observed for the content of chlorophyll a, b, and total of faba bean (*Vicia faba* L.) cv. Taraby (Al-Whaibi *et al.,* 2010).

4. Conclusions

Pre-harvest treatments with $CaCl_2$ and gibberellic acid improved some morphological and physiological parameters as well as vase life of cut tuberose. Combining $CaCl_2$ and gibberellic acid had significant effects on some parameters. Floret abscission and low vase life of tuberose are common problems that can be improved by using $CaCl_2$ and gibberellic acid before harvest.

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