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Evaluation of the Mechanical Strength of Gerbera Flower Stem in Response to Silicon and Salicylic Acid Application

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Abstract

Stem and peduncle bending by the weight of the flower is one of the main postharvest problems in gerbera (*Gerbera jamesonii* Bolus.) and sometimes caused stem crushing or water loss. In this research, silicon and salicylic acid were applied to enhance the mechanical strength of inflorescence stem, and the results revealed that stem curving was decreased by silicon and salicylic acid treatments. Shear strength and lignin content were increased by SA treatments at both upper and lower parts of the stem. It seems that lignin content was associated with shear strength at upper part of stem. Additionally, silicon content of flower stem was significantly increased under silicon application and mechanical strength of the lower part of stem has correlation with silicon content in stem tissue. These results suggested that silicon and salicylic acid application could enhance the mechanical strength of inflorescence stem and improve the cut flower quality in gerbera.

Keywords: Flexural strength, Fracture strength, Lignin, Shear strength, Vase life.

INTRODUCTION

Silicon (Si) is a non-essential element with beneficial effects on growth, development, rigidity of plant, yield, and disease resistance in a wide variety of plant species, including several crops (Epstein, 1994; Ma, 2004). All soil grown plants, contain silicon in their tissues and plants silicon content varies greatly among species and genotypes (Zhu and Gong, 2014). In the Netherlands, silicon amounts in soilless greenhouse plants have been reported significantly lower than plants in soil cultivation which is related to silicon resource limitations in non-soil substrates. (Liqun *et al.,* 2006).

Si supplementation is recommended for the hydroponic production of crops like cucumber (*Cucumis sativus* L.) and roses (*Rosa* sp.) to avoid negative effects in Si excluded plants (De Kreij *et al.,* 1999).

Si addition to the nutrient solution of gerbera (Gerbera jamesonii Bolus) improved overall crop quality, with a higher percentage of superior flowers and thicker flower stems than plants grown using the standard nutrient solution (Savvas *et al*., 2002). Similarly, Si nutrition in a closed hydroponic system, improved stem quality and mostly negative effects of recirculating nutrient solution on cut rose production (Ehret *et al*., 2005).

Beneficial or detrimental effects of Si application is reported on peat-based grown zinnia (*Zinnia elegans* Jacq.) and ornamental sunflower (*Helianthus annuus* L.) which were depended on the form, source and concentration of Si supplementation (Kamenidou *et al.*, 2009).

Although, silicon often deposited in plant cell walls as amorphous silica oxide (SiO_2, nH_2O) form, (Pilon-Smits *et al*., 2009) but as same as carbon it is able to make four bonds with other molecules) Leatherwood and Mattson, 2010). A relationship between Si and cell wall macromolecules, as well as a resituating role of Si for cell wall organic components, has been suggested (Yamamoto *et al*., 2012). Intra or extracellular silica in plants is useful for improving mechanical strength and alleviating biotic and abiotic stress (He *et al*., 2013).

Recent studies have shown the effect of silicon on the cellulose and lignin content in the green parts of many different species. Silica affects cellulose and phenolic metabolism, productivity and mechanical strength balance and therefore the defense potential and stability of the plant (Schaller *et al*., 2012). Some forms of silicon involved in cell walls strength through cross-linking, but the exact type of chemical bonding silicon to plant cell wall polymers was not clear (He *et al.*, 2013). This form of silicon indicates its importance in the cell wall integrity and emphasis on the biochemical and structural role of silicon in very small quantities.

Stems have many aspects such as height, toughness, shape and branching development etc. Stem toughness or mechanical strength, not only affects plant survival, but also plays an important role in bending or breaking of inflorescence stem (Zhao *et al*., 2013). A large central stem cavity in gerbera extended from 5 cm above the crown till about 10 cm below the floral head at harvest time. (Perik *et al*., 2012). Gerbera flowers are more susceptive to bent neck because of 10-13 grams capitol weight and central stem cavity at harvest time (Ferrante *et al*., 2007).

Phenylalanine ammonia lyase (PAL) is the first enzyme of the phenylpropanoid pathway that leads to the production of lignin. This enzyme is a biochemical marker of secondary metabolism in response to different types of stress or to plant growth regulators such as SA (Rodas-Junco *et al.*, 2013). Proposed a signaling model in which SA is perceived by receptors in the plasma membrane. This signal perception results in a stimulation of the enzymatic activity of PAL. Phospholipids such may be involved in the regulation of PAL activity as they are important signaling molecules in regulating diverse cellular processes in plants. Pretreatment with fluridone prevents the SA-induced rapid production of H_2O_2, activation of peroxidase and PAL involved in the formation of lignin in the cell walls of the roots. Pre-treated wheat seedlings with SA, in contrast to untreated plants, have an additional acceleration of deposition of lignin in the cell walls of roots, contributing to the strengthening of their barrier properties (Shakirova *et al*., 2013).

The purpose of this experiment was evaluation of mechanical strength of gerbera flower

stems in response to silicon and salicylic acid as two factors affecting lignin biosynthesis and strength of the tissue to improve the vase life of gerbera flower.

MATERIALS AND METHODS

Plants materials and treatments

The experiments were carried out using gerbera (*Gerbera jamesonii* cv. Tropic Blend) cultivated in National Institute of Ornamental Plants (NIOP), Iran (33°52'N, 50°25'E). Tissue culture propagated plants were transferred to 4L pots containing perlite and coco peat (1:1 V/V). Nutrient solution was set for pH=5.6 and EC=1.5^{-1}.8 dS m^{-1}. The plants grew well in hydroponic greenhouse with relative humidity of 64.8% day/81.6% night and natural day length during experiment period.

The average temperature was 26.2/18.6 °C day/night. Pots were irrigated 1-2 times per day for 2 min per irrigation cycle. Each pot was irrigated with 250 to 500 ml during the day depending on the season and temperature. Bed leaching was done weekly to prevent the salt accumulation.

Potassium silicate (Si) (0, 10 and 20 mM Si) and salicylic acid (SA) (0, 100 and 200 µM) were sprayed at 7 day intervals respectively from eight and two weeks before flower harvest. pH=6.8 was adjusted for potassium silicate solutions. All the sprays include the control surfactant (0.01% Tween-20). Four plants were randomly selected from each treatment and each replication for recording growth parameters.

Assessment of mechanical strength

Breaking and cutting strengths were measured immediately after harvest in 10 cm length segments at both upper and lower parts of the flower stem. Each segment was fixed on two backrests with 3 cm interval. The fracture strength was measured by the method of Burk *et al*., (2001) using a dynamometer (Facchini, FD110). The force was measured to stem breaking between the backrests or shearing by one blade on a rigid surface. Each evaluation involved 3 cut flowers.

Evaluation of stem flexural strength was taken with hanging 75g weight at the junction of the flowers to stem. Curving angle of the stem axis was measured from horizontal position.

Silicon content

Silicon content was determined by a modified Autoclave-induced digestion (AID) method (Elliot and Snyder, 1991). Briefly, crushed stem samples (0.1 g) were wetted with 3 ml of a 30% hydrogen peroxide solution in a polyethylene tubes. 4.5 g of NaOH and 6.0 ml of distilled water was added to each tube and gently mixed by vortex. The tubes were placed in an autoclave at 138 kPa for 1 h. Digested samples were brought to 50 ml with distilled water.

The Si concentration was determined by the colorimetric molybdenum blue method (Liang *et al.*, 2015). 1.0 ml of digested sample diluted by 10 ml of distilled water, followed by adding 0.25 ml of HCl : H2O (1 : 1), 0.5 ml of ammonium molybdate solution (10%, pH 7.0). Then 5 mL of 20 % tartaric acid and 1 mL reducing solution containing 8 g L^{-1} Na_2SO_3 , 1.6 g L^{-1} 1-amino-2-naphthol-4-sulfonic acid and 100 g L^{-1} $NaHSO_3$ were added immediately. After 30 min, the absorbance was measured at 650 nm with a spectrophotometer (Spectronic, genesis). A standard curve was prepared from Si standard solutions (Si1000, Kanto Chemical Co. Inc., Japan).

Lignin content analysis

Insoluble acid lignin was quantified using TAPPI T 222 om-83 method. This method determined Klason lignin in stem tissue. Briefly, 0.5 g of crushed dry tissue was added to 45 ml acetone and after 8 hours was extracted. Then, 0.3 g was homogenized by sulfuric acid 72% (w / w), for 3 hours at 30 °C. Finally, the mixture was diluted with distilled water up to 4% by volume of sulfuric acid and was autoclaved for 1 hour at 121 °C. Hydrolyzed sample after leaching with 200 ml of distilled water and vacuum filtration was dried at 105 °C (Rogers *et al*., 2005).

Histochemical study

For histochemical study, phloroglucinol staining of hand cross-section of flower stems were carried out in which samples of three sections at upper, middle and lower parts of the flower stem were placed in a 1% phloroglucinol-HCl solution for 10 min, and mounted in 50% glycerol, 6N HCl (Rogers *et al*., 2005). Bright-field illuminated hand cross-sections were taken using a microscope (Leica, Wetzlar) and stereo microscope (Axiom diagnostic).

Assessment of vase life

Flowers were harvested at commercial maturity, when the two outer whorls of florets in the floral head showed mature stamens. After harvest, the dry flowers immediately were transported to the laboratory. Cut Flowers were placed in 20 cm height polyethylene bottles. The bottles contained approx. 500 mL of demineralized water. Each evaluation involved five cut flowers. In order to clean up pollutions, bottom of the stems were disinfected for 10 seconds in 10% sodium hypochlorite solution. The cut flowers were placed in a climate-controlled room at 20±2° C and 60±5 % RH. The photosynthetic flux density at the floral head level was 15 µmol m^{-2} s^{-1} (from cool white fluorescent tubes), using a 12 h photoperiod. Vase life was considered as finished when two or more of the outer floret rows had rolled or wilted slightly, and the pedicels displayed the characteristic 'bent-neck'.

Statistical analysis

Results were based on three replications in each experiment. Analysis of variance and correlation analyses were performed and treatment means were compared with Duncan's multiple range test ($P<0.05$) using SAS (Version 9.1; SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Our treatments peculiarly changed mechanical properties at different parts of the stem. The results showed that the stem curving was diminished by silicon application ($P<0.001$) and salicylic acid treatments ($P<0.01$) (Table1). Silicon and lignin content in stem tissue significantly affected flexural resistance. Curving angle had negative correlation with lignin ($P<0.05$) and silicon ($P<0.001$) content in stem (Table 2). The absence of a sclerenchyma cylinder in the upper part of the flower stem is one of the main reasons for less mechanical strength in gerbera (Perik *et al.*, 2012). Ferrante *et al.* (2007) also believed that non lignified areas may decrease withstand for

Table 1. Effects of preharvest Si and SA treatments on vase life, Si content, shear and flexural strength of the gerbera cut flowers

Potassium silicate (mM Si)	Salicylic acid (µM)	Vase life (day)	Si in stem tissue (mg g^{-1} DW)	Stem curving angle (degree)	Shear strength at down of stem (Kg)
0	0	20.62 e ± 0.63	2.36 f ± 0.18	20.00 a ± 2.50	4.43 d ± 0.48
	100	24.30 c ± 0.52	1.34 g ± 0.05	12.50 b ± 1.44	4.23 d ± 0.38
	200	22.53 d ± 1.11	1.05 h ± 0.18	14.17 b ± 0.83	6.07 c ± 0.26
10	0	25.80 b ± 0.40	3.08 e ± 0.21	13.33 b ± 1.67	5.02 cd ± 0.39
	100	23.40 cd ± 0.78	3.87 d ± 0.14	8.44 c ± 0.87	5.69 c ± 0.51
	200	24.57 c ± 0.55	3.71 d ± 0.19	10.44 bc ± 1.44	6.99 b ± 0.43
20	0	24.90 c ± 0.79	8.44 a ± 0.31	7.11 c ± 1.50	5.92 c ± 0.50
	100	24.03 c ± 0.82	5.51 c ± 0.19	5.33 d ± 0.33	7.83 a ± 0.30
	200	27.10 a ± 0.53	6.21 b ± 0.26	5.55 cd ± 0.55	5.87 c ± 0.29
S.o.V	df				
Si	2	***	***	***	***
SA	2	ns	***	**	**
Si × SA	4	**	***	ns	***
CV (%)		5.181	8.751	22.966	10.860

, *, ns = Significance respectively at 0.01 and 0.001 levels and non-significance

Table 2. Pearson correlation coefficients of Si and lignin content with mechanical strength indices

	Dry matter	Stem curving	Fracture strength at top of stem	Fracture strength at down of stem	Shear strength at top of stem	Shear strength at down of stem
Si content	-0.501**	-0.68***	0.191ns	0.439*	0.258ns	0.399*
Lignin content	0.448*	-0.404*	0.181ns	0.083ns	0.440*	0.263ns

*, **, ***, ns = Significance respectively at 0.05, 0.01 and 0.001 levels and non-significance

flower weight in gerbera stem. Sclerenchyma and xylem cells both contain high levels of lignin in their secondary walls but no sclerification reported in upper parts of gerbera flower stem. Therefore, bending can be the result of lack of mechanical support or enough cell walls thickness, especially in xylem (Perik *et al.*, 2012).

In lignin free primary cell walls, silicon compounds cross-linked with the cell wall components such as pectin and polyphenols. These cross links could increase the elasticity of cells walls during the growth. Attached Si to the cell wall, is present probably in the form of a semi-ester compound of silicic acid (R_1-O-Si-O-R_2). These substances act as bridges in polyuronides structure (Broadley *et al.*, 2012).

SA treatments affected the shear strength at the upper ($P<0.05$) (Fig. 1) and lower ($P<0.01$) parts of the stem (Table 1). Salicylic acid treatments also increased the lignin content ($P<0.001$) in the stem tissue (Fig. 3). It seems that lignin content was associated with shear strength. There was a significant correlation ($P<0.05$) between the lignin and the shear strength at upper part of stem (Table 2). Although, SA treatments also increased dry matter percentage ($P<0.01$) in stem tissue (Fig. 2), but dry matter did not have significant correlation with mechanical properties of stem (data not shown). Si application significantly enhanced the vase life of cut flowers. Although, we were not able to found if salicylic acid extended the longevity of cut flowers but it had interaction with Si (Table 1).

SA is a weak acid, ubiquitously produced by various plants in different organs that reduces the cytosolic pH, thereby causing multiple effects (Friedman *et al.*, 2003).

Salicylic acid is a key agent in growth signals and influxes monolignol biosynthesis pathway for secondary cell wall formation (Gallego-Giraldo *et al.*, 2011). Phenylpropanoid metabolic pathway for lignin production, begins from monolignol. Monolignols transfer to apoplastic and polymerized to lignin production (Fleck *et al.*, 2011).

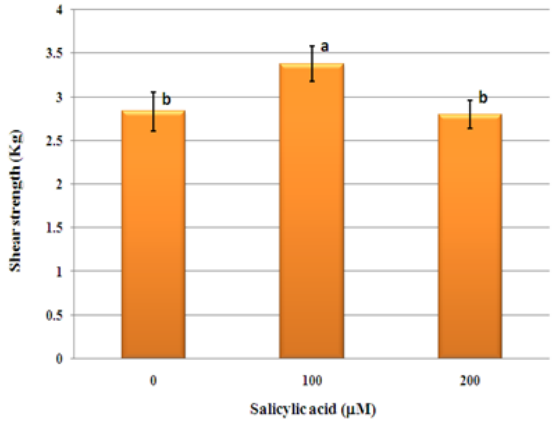

Fig. 1. Effects of preharvest SA treatments on shear strengthat upper 10 cm segment of gerbera flower stem. Data are mean values of n = 27 and the error bars represent standard errors. Different letters indicate significant differences using duncan's multiple range test at $P<0.05$ level.

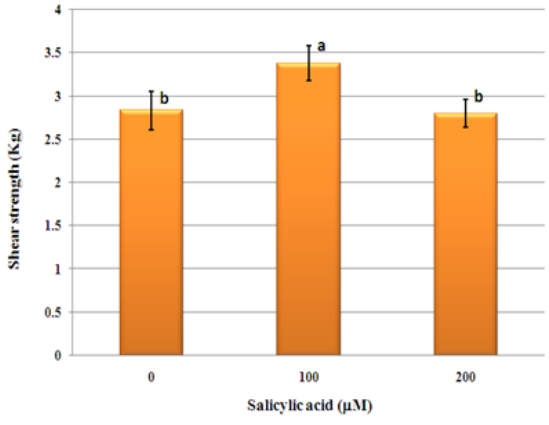

Fig. 2. Effects of preharvest SA treatment at 0, 100 and 200 µM on dry matter percent in gerbera flower stem. Data are mean values of n = 27 and the error bars represent standard errors. Different letters indicate significant differences using duncan's multiple range test at $P<0.05$ level.

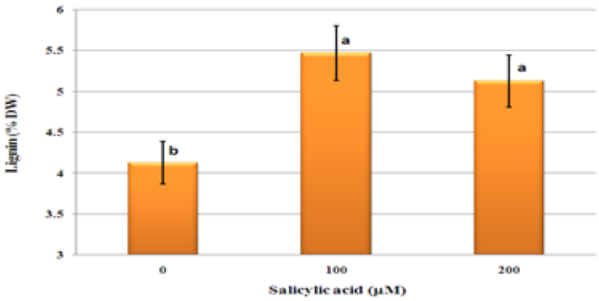

Fig. 3. Effects of preharvest SA treatment at 0, 100 and 200 µM on lignin content of gerbera flower stem. Data are mean values of n = 27 and the error bars represent standard errors. Different letters indicate significant differences using duncan's multiple range test at P< 0.05 level.

According to our histochemical study, salicylic acid improved flexural strength through increasing cell wall thickness around the vessels bundle and xylem in the stem (Fig. 4). The secondary cell wall mainly is made of cellulose, hemicellulose and lignin. The mechanical strength of secondary cell walls and hydrophobicity of the vascular system provided by phenylpropanoid-derived polymer lignin cross-links and both of them are essential to defense against biotic stress (Gallego-Giraldo *et al*., 2011). The ability of SA to stimulate H_2O_2 production and activation of peroxidase system may play an important role in biochemical processes associated with the biosynthesis of lignin and suberin, which are involved in the strengthening of barrier properties of cell walls (Shakirova, 2007).

Si supplementation, had significantly strengthened lower part of the stem, but had no effect on the top of the stem. Chemical analysis showed that silicon content in stem tissue is significantly increased by silicon application (P<0.001) (Table 1).

Zhao et al. (2013) has reported significant Si enhancement in peony inflorescence stem by silicon supplementation which was distributed mainly in the cortex and xylem tissues. Silicon treatments can increase the mechanical strength and quality of herbaceous inflorescence stalks in peony. They believe that Si treatment increased lignin content of inflorescence stems, which may be the result of collaboration lignin biosynthesis genes as well (Zhao *et al*., 2013). But silicon treatments in our experiment did not affect the lignin content in stem tissue. It seems silicon could improve the strength in the semi woody tissues (the lower part) of stem without affecting the lignin content. Silicon supplementation increased fracture strength (P<0.05) (Fig. 5) and shear strength (P<0.001) (Table 1) in the lower part of the flower stem. The mechanical strength of both shear and fracture strengths at the lower part of stem were associated with silicon content in the stem tissue.

Si is involved in the biosynthesis of cell wall components, therefore affects the mechanical properties of cell walls and consequently its permeability to water (Liang *et al.,* 2015). It seems silicon can affect concentration and metabolism of polyphenols in the xylem cell walls. So, not only silicon is important in cell walls and reinforce its strength, but it also may increase the elasticity of

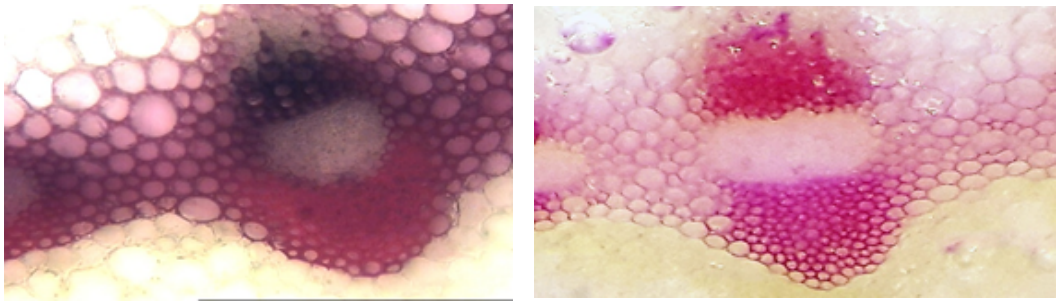

Fig. 4. Cell walls thickness of vascular bundles in the middle part of the stem
Control (right) and salicylic acid (left)

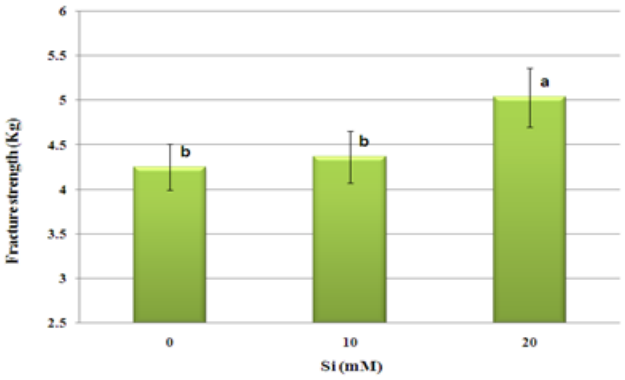

Fig. 5. Effects of preharvest Si treatments on fracture strength at lower 10 cm segment of gerbera flower stem. Data are mean values of n = 27 and the error bars represent standard errors. Different letters indicate significant differences using duncan's multiple range test at P<0.05 level

the wall during cell growth (Broadley *et al*., 2012). More recently, Si have reported in a hemicelluloses bound form in the cell walls of suspension-cultured rice cells, providing the potential to improve the mechanical properties of the cell walls against stresses (He *et al*., 2013; 2015). Silicic acid, such as boric acid, has a high affinity with phenols such as caffeic acid and related esters and forms mono, di and polymeric complexes of silicon with high stability and low solubility. Therefore, it is believed that silicon acts as an immobile ingredient in lignin and cell walls (Broadley *et al.*, 2012).

CONCLUSION

The youngest tissue in the upper part of the gerbera stem has less lignin and may be susceptible for bending. Silicon supplementation and increased Si in stem tissue improved the mechanical resistance at the lower part of the stem more. Flexural strength was more influenced by silicon application and increased Si level in stem tissue. SA treatments and induced lignification were less effective for flexural strength of the stems, but improved the shear strength at the upper segment of the stem. Since the Si application could not affect lignin content, it looks that tissue resistance increased by silicon associated cell wall components or modified metabolism of polyphenols.

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