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Morphological, physiological and biochemical response of *Chrysanthemum* to thiamine and salicylic acid

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Abstract

Chrysanthemum (*Chrysanthemum morifolium*) is a major ornamental plant with high economic importance. The effect of various rates of salicylic acid (SA) (50, 100, 150 and 200 mg l^{-1}) and thiamine (100 and 150 mg l^{-1}) was studied on some morphological, physiological and biochemical traits of cut chrysanthemums in an experiment on the basis of a randomized complete block design with three replications. The results showed that the highest stem diameter, stem length, flower diameter, flower number, cut flower number, carotenoid, shoot fresh weight and root uptake were related to thiamine rate of 100 mg l^{-1}. Thiamine rate of 150 mg l^{-1} was associated with the highest vase life, chlorophyll *a* and *b* and total chlorophyll. The highest reduced sugar and the lowest flowering time were observed in flowers treated with 150 mg l^{-1} SA. Also, the highest peroxidase was related to SA rate of 200 mg l^{-1}. In total, it was found that thiamine and SA play an important role in improving morphological, physiological and biochemical traits of cut chrysanthemums. However, different rates of these two compounds entailed various impacts.

Keywords: Biochemical traits; Chrysanthemums; Flower; Morphological traits; Physiological traits; Thiamine

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Introduction

Chrysanthemum (*Chrysanthemum morifolium*) is a major ornamental plant with high economic importance so that it is the second highest produced and consumed flower after roses (Da Silva 2004). The increased value of chrysanthemum production is rooted in its diversified applications in everyday life (Roein *et al*. 2015). Therefore, it is imperative to apply materials that can improve its growth and development.

Salicylic acid (SA), or ortho-hydroxybenzoic acid, is a plant phenol that is found in leaves and reproductive system of plants and impacts some plant activities like flowering, heat generation and resistance to pathogenic agents (Fathi and Esmaeelpour 2010). As a natural compound that is harmless to human, SA has high potential to reduce ethylene generation (Leslie and Romani 1988; Romani *et al*. 1989; Roustan *et al*. 1989) and extend crop longevity (Zhang *et al*. 2003). Foliar treatment of roses with SA and citric acid extended vase life and some qualitative traits (Hajireza *et al*. 2013). Martin-Mex *et al*. (2015) reported that SA advanced the flower initiation in *Gloxinia*. Anwar *et al*. (2014) found that SA accelerated the flowering of cut sage flowers, but it reduced their stem length and leaf area.

Thiamine is necessary for the biosynthesis of thiamine pyrophosphate coenzyme and plays a major role in carbohydrate metabolism (Bidwell 1979). It is synthesized in plant leaves, is mobilized into roots and controls the growth (Kawasaki

1992). Abd El-Aziz *et al*. (2007) reported that thiamine increased leaf number, plant height and plant fresh weight of *Syngonium*.

The objective of the present work was to examine the impact of SA and thiamine on morphological, physiological and biochemical traits of the cut flowers of spray chrysanthemum.

Materials and Methods

The study was carried out in a research greenhouse of Gorgan University of Agricultural Sciences and Natural Resources in May 2016 at day/night temperature of 28 and 16 degrees Celsius and relative humidity of 75 ± 5%. The rooted cuts of spray chrysanthemum were placed in pots with 15-cm diameter filled with soil and sand (1:2). Cuttings were placed in each pot and 25 days after planting the cuttings, all plants were pruned at the same length. Thirty days later, the first phase of the foliar application was carried out and then, it was repeated three times in 12-day intervals (Salehi *et al*. 2016). The applied treatments included salicylic acid (50, 100, 150, and 200 mg l^{-1}) and thiamine (100 and 150 mg l^{-1}). Distilled water was used as the control.

The measured traits included stem diameter, stem length, flower diameter, flower number, branch number, flowering time (days from planting to flowering), reducing sugar, peroxidase enzyme, carotenoid, chlorophyll, shoot fresh weight, vase life and water uptake. All traits (except vase life) were measured after flower harvesting and only for one time.

Stem diameter (middle of the top, middle and end of the stem), stem length and flower diameter were measured by a caliper. The time from planting to bud emergence was regarded as the flowering time. Reducing sugar was estimated by the method of Somogy (1952) in which phosphomolybdic acid and copper sulfate were applied. At first, 2 ml of the extracts taken from the treatments were poured into separate test tubes and were added with 2 ml copper sulfate solution. Then, they were placed in a water bath and when their color was changed into terra cotta and the tubes were cooled down, 2 ml phosphomolybdic acid was applied. The solutions were turned into blue in a few moments. The absorption rates of the solutions were read at 600 nm with a refractometer (model HRN32, Kruss, Germany). Peroxidase was estimated using 45 mmol guaiacol buffer and 225 mmol hydrogen peroxide buffer in which 475 µl of each buffer was mixed with 50 µl enzymatic extract taken from fresh petals and pre-prepared sulfate potassium (Gapinska *et al*. 2008) and was read at 470 nm with a spectrophotometer (Bestwick *et al*. 1998). Enzymatic activity was measured in µmol g^{-1} FW min^{-1}. Carotenoid and chlorophyll *a*, *b* and *a* + *b* were read using dimethyl sulfoxide at 645, 510, 480 and 663 nm, respectively, with a spectrophotometer (Barnes *et al*. 1992).

Vase life was considered as the time from the treatment initiation until flower senescence signaled with the wilting of petals and the discoloration of the leaves. Water uptake rate was measured by a graduated cylinder. Furthermore, the soil was analyzed whose results are presented in Table 1.

The study was conducted as randomized complete bock design with three replications. After analysis of variance, means were compared by the LSD test. Data were analyzed using SAS software.

Table 1. Analysis of the soil used in the experiment.

Location	Texture	Sand	Silt	Clay	OC (%)	TNV (%)	SP	EC*10	pH
Gorgan, Iran	Si-L	16	66	18	1.8	9.18	15.30	1.55	7.94
	N	K	P	Fe	Zn	Cu	Mn	Mg	Depth
	0.18	296	7.8	9.2	3.3	1.8	9.2	4	0-30

Results and Discussion

Results of analysis of variance revealed the significant impact of the treatments on stem diameter, stem length, branch number, flower number, flowering time, vase life, water uptake, peroxidase, carotenoid, chlorophyll *a* and *b*, and total chlorophyll at 1% probability level and on shoot fresh weight at 5% probability level, but they did not cause significant changes in flower diameter and reducing sugar (Table 2).

Table 2. Analysis of variance of the effects of salicylic acid and thiamine treatments on the measured characteristics of chrysanthemum cut flower.

SOV	df	Stem diameter	Stem length	Flower diameter	Flower number	Branch number	Flowering time
Block	2	0.32ns	24.6ns	2.27ns	3.0ns	0.19ns	5.9ns
Treatment	6	2.10**	133.6**	7.58ns	57.0**	2.63**	81.0**
Error	12	0.36	14.3	9.65	4.0	0.30	14.1
CV (%)		10.1	8.184	7.793	10.69	13.41	2.65

Table 2 continued

SOV	df	Fresh weight	Vase life	Water uptake	Reducing sugar	Peroxidase	Carotenoids	Chlorophyll a	Chlorophyll b	Total chlorophyll
Block	2	286.9	2.05	8.3	0.018	0.286	0.0006	0.001	0.0004	0.003
Treatment	6	1230.6*	62.38**	759.2**	0.029ns	1.410**	0.0050**	0.146**	0.1410**	0.574**
Error	12	355.0	1.05	58.3	0.030	0.090	0.0008	0.010	0.0100	0.030
CV (%)		18.23	5.37	6.1	28.6	11.1	6.88	8.62	9.59	8.98

ns,*,**: not significant and significant at 5% and 1% probability levels, respectively.

Stem diameter

The highest stem diameter was obtained from the 100 mg l^{-1} thiamine without showing significant difference to that obtained from the treatment of SA (100 and 150 mg l^{-1}). Also, the lowest stem diameter was observed for the control (Table 3). Nabigol *et al*. (2016) reported that SA increased stem diameter in dahlia. Higher stem diameter of the flowers treated with SA may be associated with their higher CO_2 absorption and higher photosynthesis rate (Khan *et al.* 2003).

Stem length

Comparison of means revealed that the highest stem length was related to plants treated with thiamine (100 mg l^{-1}), but this treatment did not exhibit significant difference from 150 mg l^{-1} of SA. The shortest stems were observed in the control treatment (Table 3). Flowering stem diameter is an important determinant of flower quality and marketability (Kheiry *et al*. 2011). Salicylic acid regulates cell division and enlargement (Vanacker *et al*. 2001). It was shown that 50 mg SA increased the length of the flowering stem of cut *Lilium* but as SA rate was increased, the enhancement of flowering stem length started to cease (Seyed Hajizadeh and Aliloo013). Also, Mansoori *et al.* (2015) stated that thiamine increased stem length of cut gerbera, which is consistent with our findings.

Table 3. Means of measured characteristics of chrysanthemum cut flower as affected by different levels of salicylic acid (S) and thiamine (T).

Treatment	Stem diameter (mm)	Stem length (cm)	Flower diameter (mm)	Flower number	Branch number	Flowering time (day)
S50^{+}	5.25	44.3	39.4	17.3	4.0	140.0
S100	6.36	47.9	39.7	17.3	4.7	137.0
S150	6.70	52.1	40.1	19.3	5.0	135.3
S200	5.96	45.0	39.5	21.0	4.3	141.7
T100	7.22	56.9	42.7	27.0	5.0	141.0
T150	5.26	39.3	40.4	14.0	3.0	150.7
Control (distilled water)	5.00	38.2	37.3	15.0	2.7	145.7
LSD 5%	1.067	6.73	5.53	3.56	0.97	6.68

Table 3 continued

Treatment	Reducing sugar (mg/g)	Peroxidase (kat mg^{-1} protein)	Carotenoids (mg.g^{-1} FW)	Chlorophyll a (mg.g^{-1} FW)	Chlorophyll b (mg.g^{-1} FW)	Total chlorophyll (mg.g^{-1} FW)
S50	0.48	1.97	0.35	0.82	0.66	1.48
S100	0.49	2.36	0.40	0.95	0.76	1.71
S150	0.72	2.95	0.43	1.23	1.04	2.27
S200	0.60	3.83	0.39	0.99	0.79	1.77
T100	0.61	2.27	0.47	1.25	1.09	2.34
T150	0.57	2.83	0.43	1.27	1.11	2.38
Control (distilled water)	0.43	1.78	0.34	0.73	0.57	1.30
LSD 5%	0.308	0.534	0.035	0.178	0.178	0.308

Table 3 continued

Treatment	Fresh weight (%)	Vase life (day)	Water uptake (ml g^{-1} FW)
S50	83.3	16.7	111.7
S100	95.0	17.3	128.3
S150	123.3	17.7	130.0
S200	106.7	17.0	113.3
T100	136.7	23.7	148.3
T150	95.0	27.0	140.0
Control (distilled water)	83.3	14.0	105.0
LSD 5%	33.52	1.82	13.59

$^{+}$mg l^{-1}

Flower diameter

Flower diameter plays a critical role in the marketing of cut flowers. Although the highest and lowest flower diameters were related to the treatments of thiamine (100 mg l^{-1}) and control, respectively, but the differences among treatments were not significant (Table 3). However, contrary to our findings, Mortazavi *et al*. (2015) reported that SA increased the floret diameter of Lilium. Baniasadi and Saffari (2015) found that thiamine alone and in combination with ascorbic acid and gibberellic acid resulted in significantly higher flower diameter in periwinkles.

Number of flowers

Based on comparison of means, the highest and lowest number of flowers was obtained from thiamine concentration of 100 and 150 mg l^{-1}, respectively. Also, among various SA levels, the highest number of flowers was obtained from 200 mg l^{-1} SA (Table 3). Ascorbic acid is a vitamin that impacts flowering and number of flowers in plants (Hosseini *et al*. 2015). One reason for the effectiveness of ascorbic acid on flowers is its role in the process of signaling of the plant hormones during transition from the vegetative to reproductive phase (Barth *et al*. 2006). The same explanation may be true for the role of thiamine as a vitamin in increasing the number of flowers. Mansoori *et al*. (2015) revealed that thiamine increased the number of flowers in gerbera plants.

Martin-Mex *et al*. (2005) observed that lower concentration of SA induces flowering and increases the number of flower buds in African violet. Higher number of flowers has been reported in bachelor button plants treated with SA (Kamali *et al*. 2012). On the other hand, Seyed Hajizadeh and Aliloo (2013) stated that floret number decreased in cut Lilium when SA was applied, which is in disagreement with our results.

Number of branches

The highest number of branches was related to the treatments of 100 mg l^{-1} thiamine and 150 mg l^{-1} SA. Also, the lowest number was obtained from the control treatment (Table 3). The increase in branch number of spray chrysanthemum is important and desirable because it contributes to the plant's higher economic value. Since thiamine is synthesized in leaves and mobilizes into roots to control their growth (Kawasaki 1992), it can be said that thiamine increases the number of flowers by the same mechanism. Mahghoub *et al*. (2011) found that thiamine improved the number of flowering stems in dahlia plants significantly. Also, Nabigol *et al*. (2016) reported that SA increased the number of branches in tagetes.

Flowering time

Comparison of means indicated that the highest and lowest number of days to flowering was related to the treatments of 150 mg l^{-1} thiamine and 150 mg l^{-1} SA, respectively (Table 3). The impact of SA as an indigenous flowering regulator has been proven in some plant species (Hayat *et al.* 2007). It is likely that SA encourages flowering by inducing the uptake of more nutrients (Martin-Mex *et al.* 2015). Furthermore, Corina Vlot *et al.* (2009) stated that the strengthening impact of SA on flowering can be associated with its indirect influence on the synthesis of other signaling pathways of plant hormones including ethylene, auxin and jasmonic acid. In a study, SA application advanced flowering in African violets (Jabbarzadeh *et al*. 2009). However, Mansoori *et al.* (2015) in an investigation on gerbera, found that plants treated with thiamine did not show significant differences with the control in flowering time.

Reducing sugar

Although the highest and lowest reducing sugar content was obtained from SA (150 mg l^{-1}) and control, respectively, but the differences among treatments were not significant (Table 3). However, sugar content is a determinant of cut flowers' life. According to other findings the vase life of flowers is increased if more carbohydrates are stored in plants (Dyer *et al*. 1990). The increase in reducing sugar may improve photosynthesizing pigments significantly, resulting in higher carbohydrate content (Salehi *et al*. 2016).

Peroxidase

According to Table 3, the highest and lowest peroxidase content was observed in the treatment of 200 mg l^{-1} SA and control, respectively. Mansoori *et al*. (2015) reported that SA and thiamine increased peroxidase, which is in agreement with our investigation. Higher activity of antioxidant enzymes postpones the senescence of the plants (Hassanpour Asil *et al*. 2014). Activation of these enzymes inhibits ethylene biosynthesis and avoids the losses by external factors, resulting in the reduction of active oxygen species generated by the dissolution of hydrogen peroxide via activating antioxidants. Since active oxygen species induced by the dissolution of hydrogen peroxide are important factors in early aging of the petals and peroxide is a type of antioxidant that neutralizes the toxic effect of hydrogen peroxide free oxygen, then these enzymes hinder petal aging by this mechanism (Mortazavi *et al*. 2007). Salicylates postpone cell senescence in flower by boosting the activities of antioxidant enzymes and enhancing antioxidant system (Armitage and Laushman 2003).

Carotenoids

Comparison of means showed that thiamine application increased carotenoids content in chrysanthemum cut flower plants compared to the control (distilled water). Among various SA levels, the highest carotenoid was related to 150 mg l^{-1} SA, but it was not significantly different from the thiamine treatments (Table 3). Carotenoid and anthocyanin are two important pigments of flowers (Goodwin and Britton 1998; Grotewold 2006). Carotenoids are a large group of pigments in chloroplast and chromoplast (Lopez-Juez and Pyke 2005). SA is involved in photosynthesis via influencing stomatal factors, pigments, chloroplast structure and enzymes involved in various photosynthesis phases (Pasandipour *et al*. 2013). We observed the increase in petal carotenoid content in plants treated with SA in the present study, too. According to Samadi *et al*. (2016), artichoke plants showed an increase in carotenoid content when treated with SA. Mahgoub *et al.* (2011) reported that thiamine enhanced the photosynthesizing pigments in dahlia plants.

Chlorophyll

Means comparison showed that thiamine application increased chlorophyll a, chlorophyll b and total chlorophyll significantly when compared with the control treatments. Among SA concentrations, the highest chlorophyll content was observed in plants treated with 150 mg l^{-1} (Table 3). It has been reported that thiamine induces the growth in some plants lacking chlorophyll by re-synthesis of chlorophyll (Oertli 1987). Thiamine is likely to enhance the photosynthesis of chrysanthemum and increase its chlorophyll content. Also, it has been shown that ascorbic acid is involved in enzymatic reactions as a coenzyme (Price 1966) and improves photosynthesis (Golan-Goldhirsh *et al*. 1995). Bedour and Rawia (2011) stated that thiamine and ascorbic acid increased the chlorophyll content of cut dahlia flowers.

SA maintains pigment content, stomatal conductivity, chlorophyll content at the proper level, controls photosynthesis system and photosynthesis rate, and thereby helps better growth and development of the plants (Hayat *et al*. 2010). Effect of SA on pigments is related to its influence on the activity of ACC synthase or oxidase in the ethylene production process (Leslie and Romani 1988). SA at proper concentration hinders the generation of ethylene and thereby influences the production and longevity of pigment molecules (Lesli and Romani 1989). This mechanism is the possible explanation for the increased level of chlorophyll in chrysanthemums treated with SA in the present study.

Shoot fresh weight

The highest shoot fresh weight was related to thiamine (100 mg l^{-1}) and the lowest one to the control and SA rate of 50 mg l^{-1} (Table 3). Vitamins like ascorbic acid and thiamine improve plant fresh and dry weight (Abd El-Aziz *et al*. 2007). The increase in plant's fresh weight under the treatment of thiamine is probably due to the stimulation of cell division and the increase in water uptake. As mentioned before, Abd El-Aziz *et al*. (2007) also reported that thiamine increased shoot fresh weight of *Syngonium*. Alaey *et al*. (2011) showed that SA application resulted in the higher fresh weight of cut rose flowers.

Vase life

Means comparison indicated that the longest and shortest vase life was obtained from the application of 150 mg l^{-1} thiamine and from the control treatments, respectively. Also, the longest vase life among the treatments of SA was obtained from the plants treated with 150 mg l^{-1} SA (Table 3). One reason for the decrease in post-harvest longevity of cut flowers is the deficiency of carbohydrate reserve (Marissen 2001). The initiation of the hydrolysis of proteins and carbohydrates marks the initiation of the aging process in response to the lack of free sugars used in respiration (Weinstein 1951). Therefore, it may be said that thiamine plays an effective role in extending the longevity of flowers through the synthesis of carbohydrates. Hosseini *et al*. (2015) also reported that thiamine extended the vase life of marigold (*Tagetes erecta*) cut flowers.

Salicylates retard senescence in flowers by inducing the activities of antioxidants and strengthening cell antioxidant system (Ezhilmathi *et al.* 2007). It seems that SA uses the same mechanism to improve the longevity of chrysanthemums. Hegazi (2016) stated that SA priming extended the vase life of *Rudbeckia fulgida* cut flowers by 17 days.

Water uptake

Among the treatments the highest water uptake was obtained from the thiamine concentration of 100 mg l^{-1}. Although 150 mg l^{-1} of SA increased water uptake significantly as compared to the control treatment, but water uptake obtained by the thiamine concentration of 100 mg l^{-1} was significantly higher than the water uptake from this concentration of SA. Water balance has an important factor on quality and vase life of cut flowers. The capacity of water uptake and transpiration determines the balance between these two processes in cut flowers (Da Silva 2003). In a study, SA decreased cell membrane permeability and postponed the senescence in gladiolus cut flowers (Ezhilmanthi *et al.* 2007). Capdeville *et al.* (2003) showed that the application of SA in the preservative solution of rose cut flowers reduced the growth of *Botryotinia fuckeliana* considerably and extended their vase life.

Conclusion

The present study revealed that thiamine and SA play a significant role in improving some important characteristics of cut chrysanthemum. Various concentrations of these two compounds had different impacts on chrysanthemum. However, 100 mg l^{-1} of thiamine and 150 mg l^{-1} of SA showed better results than other concentrations for most traits. On the other hand, some traits like vase life proved to be the best at thiamine concentration of 150 mg l^{-1} and postponed flowering.

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واکنش فیزیولوژیکی، مورفولوژیکی و بیوشیمیایی گل بریده داودی به تیامین و اسید سالیسیلیک

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چکیده

گل داوودی با نام علمی *Chrysanthemum morifolium* یکی از مهم‌ترین گیاهان زینتی است که دارای اهمیت اقتصادی بالایی می‌باشد. به منظور بررسی تاثیر اسید سالیسیلیک (۵۰،۱۰۰،۱۵۰ و ۲۰۰ میلی‌گرم در لیتر) و تیامین (۱۰۰ و ۱۵۰میلی گرم در لیتر) بر برخی صفات مورفولوژیکی، فیزیولوژیکی و بیوشیمیایی گل شاخه بریده داودی اسپری، آزمایشی در قالب طرح کاملا تصادفی با سه تکرار انجام شد. بر اساس نتایج حاصل بیشترین قطر ساقه، طول ساقه، قطر گل، تعداد گل، تعداد شاخه گل، کارتنوئید، وزن تر اندام هوایی و جذب ریشه در غلظت۱۰۰ میلی‌گرم در لیتر تیامین به دست‌آمد. غلظت۱۵۰میلی‌گرم در لیتر تیامین، بیشترین عمر گلجایی و کلروفیل a، b و کل را ایجاد کرد. بیشترین قند احیا و کمترین زمان ظهور گل در گل‌های تیمارشده با اسید سالیسیلیک (۱۵۰ میلی‌گرم در لیتر) مشاهده شد. همچنین بیشترین میزان آنزیم پراکسیداز مربوط به اسید سالیسیلیک (۲۰۰ میلی‌گرم در لیتر) بود. به طور کلی نتایج پژوهش حاضر نشان داد که تیامین و اسید سالیسیلیک نقش بسزایی در بهبود صفات مورفولوژیکی، فیزیولوژیکی و بیوشیمیایی گل شاخه بریده داودی داشتند. در عین حال غلظت‌های مختلف هر دو ماده تاثیرات مختلفی داشتند.

واژه‌های کلیدی: اسید سالیسیلیک؛ تیامین؛ صفات بیوشیمیایی؛ صفات فیزیولوژیکی؛ صفات مورفولوژیکی؛ گل داودی